Antibiotics Unearthed: Antibiotic Discovery and Citizen Science

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Abstract

Resistance to antibiotics is a natural phenomenon in bacteria; an unavoidable result of their evolutionary capabilities. This, coupled with their ability to transfer resistance genes from resistant to sensitive bacteria, fuels a constant arms race between antibiotic use and resistance. Currently, this race is being tipped in the bacteria's favour through human misuse of antibiotics.

I present a citizen science PhD project, part funded by the Microbiology Society, that melds a search for novel antibiotics with an analysis of different approaches used to engage members of the public in scientific discourse: Antibiotics Unearthed. I adapted and optimised a method for collecting soil samples in schools and colleges for use at public events.

This resulted in the long-term storage of 165 bacterial isolates exhibiting antagonistic activity against indicator bacterial strains such as *Bacillus subtilis*, *Salmonella enterica* serovar Typhimurium, *Salmonella enterica* serovar Typhimurium DT104 and *Staphylococcus epidermidis* as well as medically relevant pathogens including Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus faeci*um, *Klebsiella pneumoniae* and *Candida albicans*. From these, fifteen isolates with distinct morphological and antagonistic profiles were selected to be sent for whole genome sequencing. Phylogenetic tree and antiSMASH software allowed the identification of underexploited bacterial species and their biosynthetic gene clusters coding for antibiotic and secondary metabolite production.

Facebook metrics suggest that user engagement with this project was not affected by the types of content we released. However, the number of people who see any given content is driven by external events such as antibiotic awareness week.

Through the application of a coding schedule developed from key literature on the public discourse of antibiotics and antibiotic resistance, I identified key themes through interview data with participants. These key themes, when applied to long-term portfolios have been used to evidence discursive transformations of portfolio holders. This study has produced outcomes and impacts in the scientific, participant and socio-ecological and economic dimension.

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Abbreviations

µg	Microgram
µL	Microlitre
AC	All Culture
ALA	Atlas of Living Australia
AMCs	Antimicrobial Compounds
AMR	Antimicrobial Resistance
BGCs	Biosynthetic Gene Clusters
BHI	Brain Heart Infusion
bp	Base Pairs
CDC	Centre for Disease Control
CFU	Colony Forming Units
CRWA	Charles River Watershed Association
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
EARS-Net	European Resistance Surveillance Network
EEA	European Economic Area
ESI-MS	Electrospray-tandem-mass spectrometry
EU	Europe
g	Gram
gDNA	Genomic DNA
GP	General Practitioner
HAL	Human Altered Landscape
HIV	Human Immunodeficiency Virus
IASA	Invasive Alien Species Act
LB	Luria Bertani
MDR	Multi-drug resistant
MDROs	Multi-drug resistant organisms
MIC_{50}	Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms
mL	Millilitre
MOPS	3-(N-morpholino)propanesulfonic acid

NA	Nutrient Agar
NHS	National Health Service
NJ	Neighbour-Joining
NMR	Nuclear Magnetic Resonance
NRPS	Non-ribosomal peptide synthetase
P.S.I	Pounds per square inch
PBS	Phosphate-Buffered Saline
PhD	Doctor of Philosophy
PKS	Polyketide Synthase
R&D	Research and Development
RNA	Ribonucleic Acid
RPs	Ribosomal Peptides
rRNA	Ribosomal Ribonucleic Acid
SEM	Soil Extract Media
SFM	Soy Flour Mannitol
STEM	Science, Technology, Engineering and Mathematics
TA + $CaCl_2$	Tryptic Agar + Calcium Chloride
TB	Tuberculosis
TSA	Tryptic Soy Agar
U.K	United Kingdom
U.S.A	United States of America
UN	United Nations
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organisation
XDR	Extensively drug resistant

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Chapter 1 Introduction

1.1 Antibiotics and Antibiotic Resistance

1.1.1 The history of the word Antibiotic

The word ‘antibiotic’ was first used in 1860 to mean - opposed to a belief in the presence or possibility of life. Its adaptation to mean injurious to or destructive to living matter, especially microorganisms, began in 1890 (Oxford English Dictionary, 2018) when there were early attempts to use the ‘antibiotic effects’ of some microorganisms therapeutically. Pasteur and Joubert (1877) inoculated bacteria into animals with anthrax; Cantani (1885) introduced *Bacterium termo* into the lungs of tuberculous patients and Gasperini reported the antagonistic effects of certain actinomycetes against cultures of Pseudomonas (1899) (Foster and Raoult, 1974).

The word ‘antibiosis’ was used to mean the antagonistic effect of *Penicillium* on bacteria following an observation that preceded Fleming’s discovery by at least 60 years (Foster and Raoult, 1974). Antibiosis is still used to describe the antagonistic effect two microorganisms can have towards each other. However, as science began to purify the ‘antibiotic’ compounds away from the microorganisms, the word ‘antibiotic’ was used to describe streptomycin and several other antibiotics by Selman Waksman in the 1940s. In this case the word ‘antibiotic’ was interpreted as:

> *“a chemical substance, produced by micro-organisms, which has the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms” (Waksman, 1947).*

Research eventually led to the discovery, synthesis and production of synthetic compounds that had the same effect as the natural products produced by microbes. This led to the argument that the term ‘antibiotic’ should be redefined to denote any class of organic molecule that inhibits or kills microbes using bacterial targets, without any consideration of the source of the particular compound (Davies & Davies, 2010). A current definition of ‘antibiotic’ is

> *“Any substance that inhibits the growth and replication of a bacterium or kills it outright” (Microbiology Society, 2018).*

This change in definition relates to the effect a compound has on bacteria:

> *“Antibiotics are used to treat or prevent some types of bacterial infection” (NHS.UK, 2016).*

and:

> *“Antibiotics are medicines used to prevent and treat bacterial infections” (World Health Organisation, 2017).*

In scientific discourse, antibiotics are understood to relate to bacteria. However, outside of scientific discourse there is confusion (Mendelson *et al.*, 2017). This may be due to the changing definition of the word antibiotic, or the presence of words that sound the same but have nuanced meanings. To illustrate this, ‘antimicrobial’ is a word that refers to a broad group of medicinal products that kill or stop the growth of living microorganisms. These products

include antibiotics, antivirals, antifungals and antiparasital drugs (European Center for Disease Prevention and Control, 2012). It is noticeable that in each instance, the word anti- precedes the type of infectious agent that it is effective against, in all cases except for antibiotics (used to treat bacterial infections). Instead the word 'antibacterial' is used to describe products such as soaps, detergents, health and skincare products and household cleaners but not the medicines to treat humans or animals with infections. For this project I use the term 'antibiotic' to mean

> *"A substance that inhibits the growth and replication of a bacterium or kills it outright (O'Neill, 2016b).*

I now discuss antibiotics as a special category of antimicrobial drugs that underpin modern medicine as we know it.

1.1.2 Antibiotics revolutionised medicine

Antibiotics, substances that inhibit the growth and replication of or kill a bacterium, are arguably the single greatest health care advance in history. Medical care as we now know it is founded on the availability of effective antibiotics (Rice, 2008b). Antibiotics have transformed surgical operations, transplant medicine and childbirth into routine procedures with positive outcomes in most cases. Historically this has not been the case; previously microbial infections including bacterial infections caused significant mortality and morbidity.

Infectious diseases caused by pathogenic microorganisms such as bacteria, viruses, and fungi, were some of the Western world's largest killers before the discovery of antibiotics (World Health Organisation, 2019). The Centre for Disease Control (CDC) reported:

> *"in 1900, the three leading causes of death were pneumonia, tuberculosis (TB), and diarrhoea and enteritis, which (together with diphtheria) caused one third of all deaths" (Centers for Disease Control and Prevention, 1999a).*

Tuberculosis (TB), diphtheria and many pneumonias are caused by bacterial pathogens. Since the discovery of antibiotics, non-infectious (non-communicable) diseases, often age related, have replaced infectious diseases as the biggest killers (Dye, 2014):

> *"in 1997, heart disease and cancers accounted for 54.7% of all deaths" (Centers for Disease Control and Prevention, 1999a).*

In 1999, heart disease accounted for 268.2 deaths per 100,000 people in the United States of America (U.S.A) whilst the two most deadly modern day infectious diseases, pneumonia and influenza, accounted for just 34 (Centers for Disease Control and Prevention, 2000). Confidence that infections could be prevented or treated by antibiotics allowed major leaps forward in research into and treatment of non-communicable diseases such as cardiovascular diseases, cancer, respiratory diseases and diabetes that replaced infectious diseases as the leading cause of morbidity and mortality in the western world.

Perhaps the most well-known aspect of the history of the discovery of antibiotics occurred in 1928. Dr Alexander Fleming, upon returning from a summer vacation, noticed one of his agar plates was contaminated with a mould (fungus) that was preventing *Staphylococcus aureus*, a pathogenic bacterium, from growing close by. His investigations identified the fungus as *Penicillium notatum,* which was producing the penicillin that was inhibiting the growth of *S. aureus* (Bowater, 2017). This is credited as the first discovery of an antibiotic in the United Kingdom (U.K). However, it took another ten years before Howard Florey (1898-1968) and Ernst Chain (1906-1979) along with the lesser known Norman Heatley (1911-2004) successfully developed a small-scale industrial process for purifying penicillin from a *P. notatum* culture. In 1941, because of economic hardships in the U.K caused by the outbreak of the second world war, production moved to the U.S.A where penicillin was mass produced[1] and became a life-saving drug throughout the second world war and beyond (Bowater, 2017).

Thus began the golden era of antibiotic discovery (Aminov, 2010), a 20-year period, between 1950 and 1970 where many antibiotic compounds were discovered, natural products were adapted and new compounds were synthesised. Antibiotics were commonly referred to as magic bullets[2], a term coined by Paul Ehrlich (1854-1915) who proposed that there may be clinically relevant molecules that could target pathogenic bacteria without harming human cells (Bowater, 2017). In 1967, one of modern medicine's best-known quotations was used:

> *"It is time to close the book on infectious diseases and declare the war against pestilence won"*

This quote was attributed to William H. Stewart, the tenth Surgeon General of the U.S.A (1965-1969) (Spellberg & Taylor-Blake, 2013). This quote represents the general optimistic outlook of that era; that antibiotic discovery had effectively ended our fight with pathogenic bacteria. However, evidence from a reanalysis of William H. Stewart's speech indicated that he never actually said this but in fact, advocated for the opposite. He recognised infectious diseases had not been conquered (Spellberg & Taylor-Blake, 2013)

1.1.3 The predictable emergence of resistance

Predictably, the book on infectious diseases had not been closed. Infectious diseases are still a considerable cause of illness and death around the world. Two thirds of all deaths are caused by just 20 species of 1400 recognised human pathogens and parasites, and the majority of these are mainly bacteria and viruses (Dye, 2014). This problem is compounded by the ability of bacteria to become resistant to the antibiotics that we have been using to kill them.

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[1] *P. notatum* was swapped for *Penicillium chrysogenum,* due to its ability to produce more penicillin in culture.

[2] This term was borrowed from German folklore and was originally *magische Kugel.*

Antibiotics are among the most important tools in medicine, but their efficacy is threatened by the evolution of resistance (Baym et al., 2016). Antibiotic resistance can be defined as:

> *"Bacteria becoming increasingly resistant to the drugs that were previously effective against them" (Microbiology Society, 2019b).*

Antibiotic resistant bacteria nullify the standard, previously effective antibiotic treatments leading to the development and persistence of illnesses with significant morbidity and mortality. Perhaps even more troubling is that these hard to treat infections are spreading across the globe.

In order to understand how humans have contributed to antibiotic resistance, it is necessary to understand that antibiotic resistance in bacteria is a natural and unavoidable manifestation of their evolutionary capabilities (Peters *et al.*, 2008). There are two distinct evolutionary processes at play with the spread of antibiotic resistance namely genetic mutation and horizontal gene transfer.

Pray (2008) highlighted the rate at which a bacterial population can mutate. A single *Staphylococcus aureus* bacterium replicates once every 30 minutes and has 2.8 x 10^6 nucleotide base pairs (bp) in its genome. At the expected mutation rate of one mutation per 10^{10} bp, it takes just 30 hours for one, single bacterium to grow into a population in which every single bp has mutated 30 times. Ramaswamy & Musser (1998) identified that *Mycobacterium tuberculosis*, the causative agent of TB, also has spontaneous, predictable rates of chromosomally borne mutations that confer resistance to antibiotics.

Mutations alter cellular mechanisms of bacteria in four ways to confer antibiotic resistance (Table 1-1).

Table 1-1. Mechanisms of antibiotic resistance in bacteria. Adapted from (Hawkey, 1998)[1] and (Bowater, 2017)[2]

Mechanism of antibiotic resistance	Description
Antibiotic modification[1]	The resistant bacteria retain the same sensitive target as antibiotic sensitive strains, but the antibiotic is prevented from reaching it because it is modified or broken down.
The lock out[2]	Antibiotic resistant bacteria protect the antibiotic target by preventing the antibiotic from entering the cell or pumping it out faster than it can flow in.
Change the lock[2]	Alterations in the antibiotic target may mean that the antibiotic penetrates the cell and reaches the target site but does not inhibit the target because of structural changes in the target molecule.
Create a decoy[1]	Bacteria may protect themselves by producing an alternative target (usually an enzyme) that is resistant to inhibition by the antibiotic while continuing to produce the original sensitive target.

The ability of a bacterial genome to rapidly mutate inevitably leads to a substantial, naturally occurring pool of pre-existing resistance genes (Peters *et al.*, 2008). A genomic analysis of 480 bacteria (actinomycetes from the

genus *Streptomyces*) isolated from soil samples originating from diverse environments (urban, agricultural and forest) revealed resistance to 21 different antibiotics, including natural products, their semisynthetic derivatives, and completely new synthetic molecules. Concerningly, without exception, every strain in the library was found to be multi-drug resistant (MDR) to seven or eight antibiotics on average, with two strains being resistant to 15 of 21 drugs (D'Costa *et al.*, 2006).

The ability of a single bacterium to develop natural resistance to antibiotics is exacerbated by their second evolutionary capability, horizontal gene transfer. This is where antibiotic resistance genes can be transferred from a resistant bacterium to a susceptible one (Bowater, 2017). There are three main modes of genetic transfer between bacterial cells. The most common is conjugation, but the process can also occur through transformation and transduction (Table 1-2).

Table 1-2. The three main modes of genetic transfer between bacterial cells. Taken from Table 6.4 (Bowater, 2017)

Acquiring Resistance: Transferring DNA
Conjugation: Two bacterial cells adhere together, and plasmid[3] DNA is passed from the donor cell to the recipient cell through specific channels that can include resistance genes.
Transformation: When bacteria die and cells lyse, genetic material is released into the environment. Under certain conditions, this DNA is taken up by other bacteria in the surrounding area, including resistance genes.
Transduction: When a bacterium is infected with a phage[4], the phage can accidentally incorporate bacterial DNA into its own genome. When the phage lyses the bacterial cell, phage particles are released into the environment and can infect a new bacterium. The phage particles carrying the additional piece of bacterial DNA can insert into the new bacterial genome, taking the new DNA with it that can include resistance genes.

Whilst bacterial resistance to antibiotics is a natural process, the use and misuse of antibiotics increases the rate of emergence of these antibiotic resistance genes. Fleming himself, in his 1945 Nobel prize lecture, warned of the possibility that misuse of antibiotics could lead to a scenario where bacteria developed resistance:

[3] Separate to the single, dynamic double helical molecule of chromosomal DNA used by bacteria to house much of their genetic material, plasmids are small, circular (occasionally linear) pieces of DNA that can be copied independently of the chromosome. They contain a wide variety of genes that code for different proteins and enzymes that give the bacterial cells different properties (including antibiotic resistance).

[4] A phage is a virus, specifically a virus that infects bacteria, which contain a DNA or an RNA genome that contains the genetic information needed to make new phage (Bowater, 2017).

"…There is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant."(Fleming, 1945)

Selection pressure that increases the likelihood of a bacterium developing and maintaining an antibiotic resistance gene emerges if suboptimal doses of an antibiotic are used. Genetic diversity within populations, combined with rapid bacterial generation times, gives bacteria the ability to rapidly adapt to become resistant to or tolerant of antibiotics when the bacteria are not destroyed through the correct treatment regime (Peters *et al.*, 2008; Meek, Vyas and Piddock, 2015; Holmes *et al.*, 2016). Correct dosing of antibiotics limits the environmental pressure put on bacteria to develop or maintain resistance because the target bacteria are destroyed and cannot mutate or transfer resistance genes through horizontal gene transfer. As an example, prior to 1960, *Mycobacterium tuberculosis* was treated with an antibiotic regime that cured tuberculosis in nearly all patients. This treatment takes a long time to work and can cause unpleasant side effects; however treatment was highly successful because it was carried out in hospitals where compliance could be assured (Wood and Iseman, 1993). In the late 1960s, this antibiotic therapy began to take place in an outpatient setting. This shift to outpatient care reduced compliance and led to rising rates of treatment failure, relapse, and acquired drug resistance among *Mycobacterium tuberculosis* bacteria (Wood and Iseman, 1993).

Increasing use of antibiotics, particularly inappropriate use, is recognised as the main selection pressure leading to the decline in effectiveness of existing antibiotic drugs (Bronzwaer *et al*., 2002; Goossens *et al*., 2005; O'Neill, 2016b). An example of antibiotic use correlated with antibiotic resistance can also be found in the most common cause of community-acquired pneumonia, *Streptococcus pneumoniae* as shown in Figure 1-1. (Baquero *et al.,* 1991; O'Neill, 2016b).

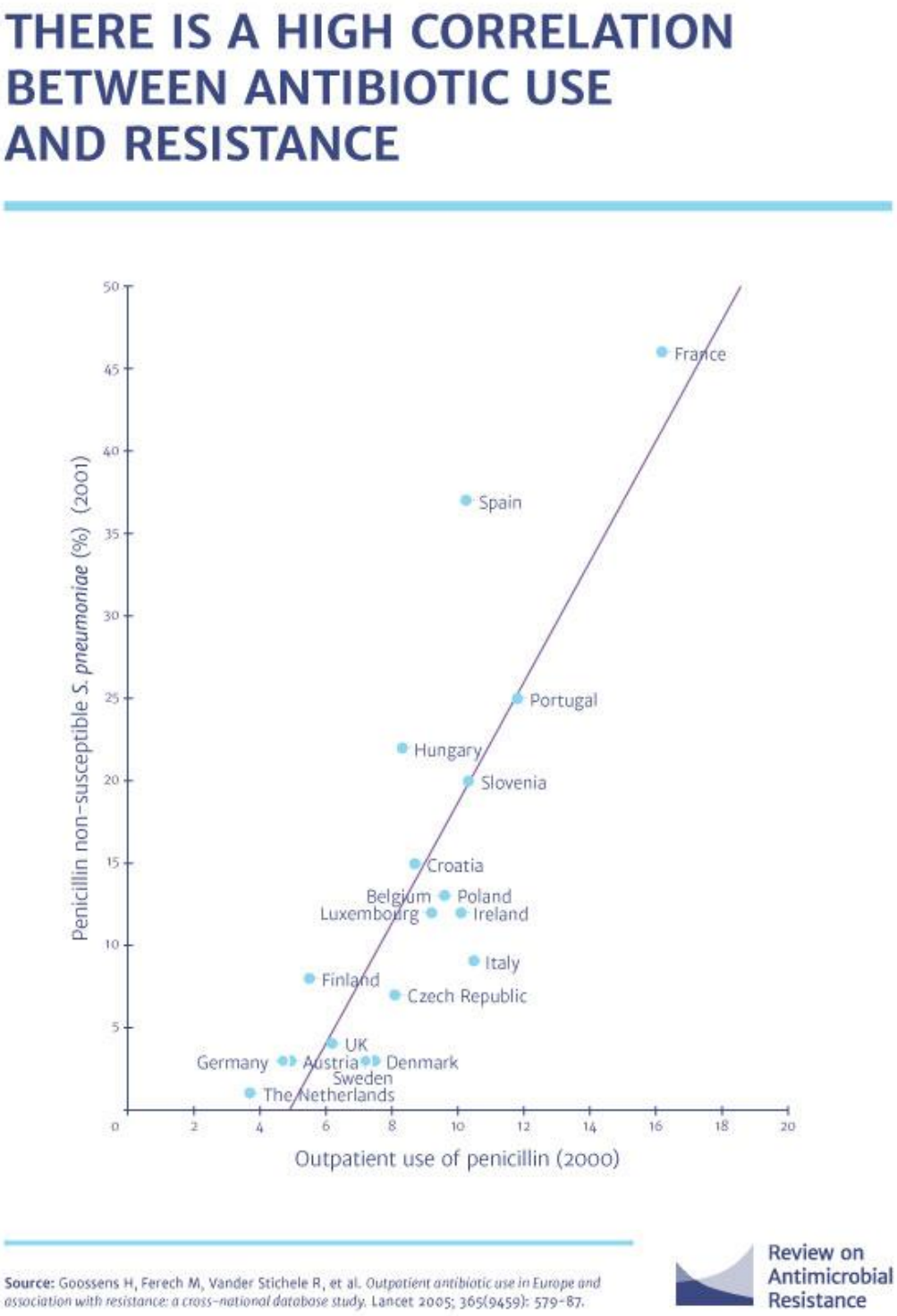

Figure 1-1. There is a correlation between antibiotic use and resistance. Taken from the Review on Antimicrobial Resistance: (O'Neill, 2016b). Outpatient use of penicillin in 2000 (DID - defined daily doses per 1000 inhabitants daily) is along the x axis, whilst the % of *S. pneumoniae* which are penicillin non-susceptible (2001) are along the y axis. Blue dots represent counties plotted against both axis. The source of the data for this figure is Goossens *et al* (2005).

Figure 1-1 highlights how the strength of selection pressure, in this instance the increased use of penicillin outside of a hospital setting, is intimately and positively associated with the rate of evolution of resistance. An increase in the occurrence of antibiotic resistant bacteria has the potential to have a devastating impact on public health and the provision of healthcare worldwide.

A serious warning was issued in an April 2014 World Health Organisation (WHO) report "*Antimicrobial Resistance: Global Report on surveillance*". It said that the post-antibiotic era, in which common infections and minor injuries can kill, is not an apocalyptic fantasy but a real possibility for the 21st century (World Health Organisation, 2014). A 2014 press release by the Wellcome trust quoted the U.K Chief Medical Officer, Professor Dame Sally Davies:

> *"We have reached a critical point and must act now on a global scale to slow down antimicrobial resistance" (Wellcome Trust, 2014).*

In the U.K, antimicrobial resistance (AMR) has been placed on the National Risk Register of Civil Emergencies (U.K Cabinet Office, 2015) emphasising the significance of this problem. Another review on AMR sponsored by the Wellcome Trust and the U.K Department of Health (O'Neill, 2016b) modelled the global consequences of AMR to the year 2050. The report issued a stark warning that AMR could kill 10 million people per year by 2050, a number greater than the present mortality of cancer, as well as amass a global cost of $100 trillion U.S Dollars. This sum is equivalent to the profit of the entire globe for one full year.

1.1.4 Incidence of antibiotic resistance infections

After estimating the risk of antibiotic resistant bacteria in the future, it is worth taking stock of the problem as it exists today. Over the last ten years in the U.S.A, the CDC has produced two reports highlighting the strains of antibiotic resistant bacteria that pose an urgent or serious threat to the general public and are responsible for significant levels of hospital acquired infections (nosocomial infections). Pathogenic bacteria with the hazard level 'urgent' are high-consequence antibiotic-resistant threats because they meet significant risks across several key criteria. These threats may not be widespread at the current time but have the potential to become so and they require urgent and aggressive action. Those with the hazard level 'serious' are significant antibiotic-resistant threats. For varying reasons (e.g. declining domestic incidence rate), they are not considered 'urgent', but these threats will worsen and may become 'urgent'. Therefore, they also require prompt and sustained action (Centers for Disease Control and Prevention, 2013b). Table 1-3 shows compiled data taken from the 2019 report identifying the 14 pathogenic bacteria (four urgent, ten serious) which pose the greatest threat to the U.S.A population but this is also true globally (Centers for Disease Control and Prevention, 2019). It also highlights whether each bacterium is Gram-positive or Gram-negative.

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Table 1-3. Antibiotic-resistant pathogenic bacteria which pose an urgent or serious threat to the general public, as reported by the U.S.A CDC (Centers for Disease Control and Prevention, 2019)**.** The pathogenic bacteria are listed with their threat level, details about their role in infection, number of cases, number of deaths and medical cost. Also included is whether each bacterium is Gram-positive or Gram-negative.

Bacteria	Threat Level	About	Cases	Deaths	Medical Cost	Organism
Carbapenem-resistant *Acinetobacter*	Urgent	Cause pneumonia, bloodstream and urinary tract infections.	8,500	700	$281 Million	Gram-negative bacteria
Clostridioides difficile	Urgent	Causes life threatening diarrhoea and colitis	223,900	12,800	$1 Billion	Gram-positive bacteria
Carbapenem-Resistant *Enterobacteriaceae* spp	Urgent	Major concern for patients in healthcare facilities. Some are resistant to nearly all antibiotics.	13,100	1,100	$130 Million	Gram-negative bacteria
Drug-resistant *Neisseria gonorrhoeae*	Urgent	Causes the sexually transmitted disease gonorrhoea resulting in ectopic pregnancy, infertility and increased risk of HIV infection.	550,000	N/A	$133.4 Million	Gram-negative bacteria
Drug-resistant *Campylobacter*	Serious	Causes diarrhoea, fever and abdominal cramps and can spread from animals to humans through contaminated food.	448,400	70	N/A	Gram-negative bacteria
ESBL-producing *Enterobacteriaceae* spp	Serious	Spread rapidly and cause of complicate infections in healthy people.	197,400	9,100	$1.2 Billion	Gram-negative bacteria
Vancomycin-resistant *Enterococcus* spp	Serious	Cause serious infections in healthcare settings including bloodstream, surgical site and urinary tract infections.	54,500	5,400	$539 Million	Gram-positive bacteria
Multi-drug-resistant *Pseudomonas aeruginosa*	Serious	Affects people with weakened immune systems. Particularly	32,600	2,700	$767 Million	Gram-negative bacteria

		dangerous for patients with chronic lung diseases.				
Drug-resistant non-typhoidal *Salmonella* spp	Serious	Can spread from animals to people through food. Causes diarrhoea, fever and abdominal cramps. If spreads to the blood can have life-threatening complications.	212,500	70	N/A	Gram-negative bacteria
Drug-resistant *Salmonella Typhi*	Serious	Causes typhoid fever which can be life-threatening.	4,100	<5	N/A	Gram-negative bacteria
Drug-resistant *Shigella* spp	Serious	Spreads in faeces through direct contact with contaminated surfaces, food or water. Causes diarrhoea, fever and abdominal cramps.	77,000	<5	N/A	Gram-negative bacteria
Methicillin-resistant *Staphylococcus aureus*	Serious	Causes difficult-to-treat staph infections including septicaemia.	323, 700	10,600	$1.7 Billion	Gram-positive bacteria
Drug-resistant *Streptococcus pneumoniae*	Serious	Causes pneumococcal disease, ranging from ear and sinus infections to pneumonia and bloodstream infections.	900,000	3,600	N/A	Gram-positive bacteria
Drug-resistant *Mycobacterium tuberculosis*	Serious	Causes TB, among most common infectious diseases and a frequent cause of death worldwide.	847	62	$164,000 per MDR case, $526,000 per XDR case	Acid-fast Gram-positive bacteria

The CDC proposed actions that must be taken to stop the spread of antibiotic resistance. These included changing the narrative of a future post-antibiotic era. Instead, they suggest that the narrative should change so that we acknowledge that an antibiotic resistance era is already here. The U.S.A currently has more than 2.8 million antibiotic-resistant infections each year with over 35,000 deaths (Centers for Disease Control and Prevention, 2019). Even after the 2013 report was published, the CDC realised the burden of antibiotic-resistance threats in the U.S.A was greater than they had initially thought. Improved infection prevention measures, improvements in antibiotic stewardship and prescribing practices, as well as the effective use of vaccines that emerged as a result of this report have seen the number of deaths caused by antibiotic resistance slowly decreasing (Centers for Disease Control and Prevention, 2019).

Concerns about antibiotic resistance are not restricted to the U.S.A. A 2019 study looked at 16 antibiotic resistant bacteria monitored by the European Resistance Surveillance Network (EARS-Net) throughout 2015. It identified 671,689 infections and 33,110 deaths attributable to antibiotic-resistant bacteria, as well as 874,541 disability adjusted life years, that is the number of years lost due to ill-health, disability or early death (Cassini *et al.*, 2019). The authors noted that the burden of infections with antibiotic-resistant bacteria in Europe (EU) and the European Economic Area (EEA) is substantial when compared with other infectious diseases, and it has been increasing since 2007.

In the U.K, a Public Health England 2019 report showed 60,788 antibiotic-resistant infections occurred in England during 2018, a 9% rise from the 55,812 antibiotic-resistant infections found in 2017 (Public Health England, 2019). Further, there was a 32% increase of antibiotic-resistant bloodstream infections from 12,972 in 2014 to 17,108 in 2018 (Public Health England, 2019).

Of the antibiotic-resistant bacteria causing illness and death, the ESKAPE pathogens are extraordinarily important. They cause many of the hospital acquired infections and represent paradigms of pathogenesis, transmission, and resistance (Rice, 2008). The ESKAPE pathogens, difficult to treat species of bacteria, are noted as creating a global burden with the development of drug-resistance (O'Neill, 2016a). Each letter of the ESKAPE pathogens represents a bacterial genus, detailed in Table 1-4.

Table 1-4. The bacterial genera, and where available species, that make up the ESKAPE pathogens.

Abbreviation	Bacteria
E	*Enterococcus faecium*
S	*Staphylococcus aureus*
K	*Klebsiella pneumoniae*
A	*Acinetobacter baumannii*
P	*Pseudomonas aeruginosa*
E	*Enterobacter spp.*

Of the 14 pathogenic bacteria listed in Table 1-3, six are ESKAPE pathogens. Whilst *Klebsiella* is not mentioned by name as part of the ESKAPE pathogens, it falls under the bracket of *Enterobacter* spp along with *Escherichia coli*.

However, the remaining eight pathogenic bacteria are also a global concern. For example, *Mycobacterium tuberculosis*, the pathogen associated with causing TB, is estimated to have infected 67 million children worldwide (Dodd, Sismanidis and Seddon, 2016). Almost 15 years ago in 2006, the CDC published the results of a disturbing survey of an international network of TB laboratories. This survey showed that of all the TB cases detected worldwide, 20% were MDR (Centers for Disease Control and Prevention, 2006), with half of these meeting the criteria for being Extensively drug resistant (XDR), showing resistance to first and second-line anti-TB drugs (Shah *et al.*, 2007). First-line drugs are those that are chosen to treat an infection in the first instance, often cheaper, easier to produce or offer less side-effects. Second-line drugs are those chosen if first-line drugs fail to treat the infection. In 2004, according to a WHO estimate, 424,203 cases of TB were MDR, 261,362 of which occurred in China, India or the Russian Federation, 62% of the estimated global burden (Zignol *et al.*, 2006). There is a financial, as well as a health incentive to tackle this problem, as treatment for MDR-TB patients requires use of second-line drugs for ≥24 months. These drugs are more costly, toxic, and less effective than first-line drugs used for routine treatment of TB (Wood and Iseman, 1993; Rajbhandary, Marks and Bock, 2004). More recently, of the 10 million cases of global TB estimated in 2017, around 458,000 case were MDR-TB (Park, Satta and Kon, 2019).

Currently, incidences of antibiotic-resistant bacterial infections are on the rise globally. Whilst some countries are getting better at preventing death caused by these infections, the number of deaths is still far too high. Further, the medical cost associated with these infections is placing a significant burden on healthcare services. The U.S.A CDC has stressed the need to stop believing that antibiotic resistance is a problem ‘over there’ when in fact, it is going on in our own backyard (Centers for Disease Control and Prevention, 2019).

1.1.5 Antibiotic resistance is underfunded and understudied

When it comes to AMR, the literature indicates that the global response does not match the severity of the threat. Antibiotic resistance research is underfunded. By 2050, inaction on AMR is estimated to cost the global economy $100 trillion USD (O’Neill, 2016b). In a UK report focussed on securing new drugs for future generations, commissioned by the UK Government, led by the economist Lord Jim O’Neill, a $2 billion USD innovation fund was suggested to jump start the discovery of new antibiotics and diagnostics. Furthermore, a $16 – $37 billion USD package was proposed to overhaul the antibiotics pipeline, providing funds for drug companies after new antibiotics are brought to market (O’Neill *et al.*, 2015). In comparison to the expected cost of inaction ($100 trillion USD), these funds appear to be good value for money. However, even with this incentive, the reality is that venture capitalists do not find antibiotic development attractive. Less than 5% of venture capital investment in pharmaceutical R&D ($37bn) between 2003 and 2013 was for antimicrobial development ($1.8bn) (O’Neill, 2016b). An

evaluation of the 2016 review on AMR showed there has been very little progress on the central and most expensive recommendations for transforming research and development (R&D) incentives (Clift, 2019). This may be a disappointment, but recent history should have indicated that this is not a total surprise.

Even government backed funding does not always reach the necessary areas to stimulate research. In 1999, the United States Federal Government sponsored an interagency task force to create '*a Public Health Action Plan to Combat AMR.* The Action Plan includes action items organized into four focus areas: Surveillance, Prevention and Control, Research, and Product Development (Centers for Disease Control and Prevention, 1999b). Despite this effort, funding that was authorised for the activities of the task force was never appropriately allocated, and each member of the task force was left to its own devices to make good on the action items with which they were charged (Rice, 2008). Currently there are large gaps in our knowledge about how to combat antibiotic resistance. Examples of areas where antibiotic research is trailing behind include, but are not limited to:

- Definitions for minimal lengths of treatment (Rice, 2008)
- The benefit of antibiotic therapy over placebo (Peters *et al.*, 2008)
- The use of a combination of antibiotics without conclusive evidence of benefit (Rice, 2008)
- The utility of basic infection-control measures (Rice, 2008)
- Best mechanisms for disseminating this knowledge in a way that will change clinicians practices (Rice, 2008)
- Lack of rapid, sensitive and specific diagnostic tests for invasive bacterial infections (O'Neill, 2016b) which identify not only the pathogen's current resistance profile but also its future potential for evolution of resistance removing barriers for the clinical application of selection-inverting treatment strategies (Baym, Stone and Kishony, 2016)
- Lack of detailed information regarding the Pharmacokinetics (describes the drug concentration-time courses in body fluids resulting from administration of a certain drug dose (Meibohm and Derendorf, 1997) and Pharmacodynamics (the observed effect resulting from a certain drug concentration (Meibohm and Derendorf, 1997) of antibiotics (PK/PD) (Sarkar *et al*., 2007)
- Lack of effective vaccines against bacterial pathogens (O'Neill, 2016b)
- Lack of well-designed and executed clinical trials are crucial to the rational use of antibiotics. Clinical trials need to address standard-of-care antibiotic treatment versus shorter durations of therapy or no antibiotic therapy at all (Peters *et al.*, 2008; O'Neill, 2016b)
- Modelling evolution of drug-resistant phenotypes where common phenotypic and genotypic changes observed in parallel-evolving populations provide promising building blocks (Furusawa *et al.*, 2018)

Historically, antibiotic research has been underfunded when compared with research into chemotherapy options for treating the Human Immunodeficiency Virus (HIV) and cancer (Rice, 2008). This lack of funds results in a lack of research and as such, the science behind antibiotics and resistance has barely

progressed since the golden era of discovery. Instead, there has been a reliance on the Pharmaceutical industry to tackle antibiotics through a contribution of funds and the conducting of research (O'Neill, 2016b).

1.1.6 The Failure of Pharma: The Lean Drug Discovery Pipeline

As life expectancy increases due to medical advancements, so does the proportion of the population who are vulnerable to infection. This includes the elderly as well as those who are immunosuppressed due to cancer treatments or organ transplants (Davies, 2011). The risk of mortality and morbidity caused by infections increases as the incidence of antibiotic resistance increases and the limited number of new antibiotic agents currently in development remains stagnant (Magiorakos *et al.*, 2012). The Infectious Diseases Society of America provided a concerning update about the lean pipeline for novel therapeutics to treat drug-resistant infections, especially those caused by Gram-negative pathogens (Boucher *et al.*, 2009). A U.K report stated that global burden will be in a large part due to the lack of current antibiotics coming through the discovery pipeline, whilst estimating it could be ten years before new antibiotics may become available to treat the worst of the antibiotic resistant bacteria (O'Neill, 2016a).

The leanness of the discovery pipeline is exacerbated by the difficulty of a therapeutic agent to reach a stage where it is clinically relevant. The Pew Charitable Trusts track products in clinical development globally with the potential to treat or prevent serious bacterial infections (The PEW Charitable Trusts, 2020). As of December 2019, there were 41 new antibiotics in development. Of the 41 antibiotics in development, 15 were in Phase 1 clinical trials, 12 in Phase 2, 13 in Phase 3, one has had a new drug application submitted, four have been approved. Historically, about 60 percent of drugs that enter Phase 3 will be approved. They summarised these findings saying:

> *"These drugs would potentially address many, but not all, resistant bacteria. However, given the inevitability that some of these antibiotics will fail to win approval, and that resistance will eventually develop to those that are approved, it is clear that there are too few drugs in development to meet current and anticipated patient needs" (The PEW Charitable Trusts, 2020).*

To date, there has been an over-reliance on the pharmaceutical industry to solve this problem by bolstering the antibiotic discovery pipeline. It is argued that it is not in pharmaceutical companies' best interests to protect the effectiveness of antibiotics by minimising antibiotic usage. Their departure from R&D is merely an example of their profit-driven approach that is understandable from a business perspective (Rice, 2008). In 2018, Lord Jim O'Neill, author of the Review on AMR, condemned the pharma industry.

> *"I am shocked at the endless words that come from the pharma industry about their collective belief in the need to fight AMR, but the lack of concrete initiatives and more importantly, money, they are prepared to underwrite. In January 2016, a much larger number than we ever envisaged signed what is now known as the Davos*

declaration. There were more than 100 signatories in the end. In their important first benchmark on AMR, Access to Medicine could only find eight big Pharma companies that were actively working on new drugs. I personally think that was very generous… there is not really more than three." (O'Neill, 2018).

Over 95 percent of the products in development today are based on research taking place in small pharmaceutical companies rather than the large pharmaceutical firms that once dominated this field (The PEW Charitable Trusts, 2020). Davies (2011) stated very clearly in her annual report that she feels the leanness of the antibiotic discovery pipeline is in part down to a market failure. Antibiotics are used for short durations in small amounts. They will always become less efficient over time as resistance inevitably develops and some last resort antibiotics will be withheld indefinitely. This limits their profitability and decreases the incentive for new antibiotic production. This statement was echoed in a 2018 update (O'Neill, 2018). To summarise, the issue we face as a society is that the supply of new antibiotic agents has slowed whilst levels of antibiotic resistance are increasing, limiting our treatment options (Davies, 2011).

1.2 Tackling Antibiotic Resistance

1.2.1 The Ten Commandments

There are two specific issues associated with antibiotic resistance; the decreased supply of new antibiotics and the increased incidence of antibiotic resistance. These issues can be tackled and should be tackled using a variety of different approaches. In 2016, the comprehensive U.K Review on AMR put forward ten 'commandments' to revolutionise the fight against antibiotic resistance (O'Neill, 2016b). These commandments are:

1. Launch a massive global public awareness campaign.
2. Improve hygiene and prevent the spread of infection.
3. Reduce unnecessary use of antimicrobials in agriculture and their dissemination into the environment.
4. Improve global surveillance of drug resistance in humans and animals.
5. Promote new, rapid diagnostics to cut unnecessary use of antibiotics.
6. Promote the development and use of vaccines and alternatives.
7. Improve the numbers, pay and recognition of people working in infectious disease.
8. Establish a Global Innovation Fund for early-stage and non-commercial research.
9. Better incentives to promote investment for new drugs and improving existing ones.
10. Build a global coalition for real action – via the G20 and the United Nations (UN).

In 2018, a subsequent update to the original report was published detailing what had been achieved since publication of the review in 2016. This update

reported that only two of the ten areas were identified as having experienced encouraging progress (O'Neill, 2018). Human capital (Commandment 7) had increased as the numbers, pay and recognition of people working in infectious disease had increased as exemplified through growth in new university AMR centres in the U.K. In addition, the establishment of a Global Innovation Fund was partially achieved, however early stage R&D received special mention (Commandment 8):

> *…If I tally all the announced initiatives from the U.K, German and especially U.S.A governments, and the Welcome Trust, and that recently of the Danish pharmaceutical company, Novo, the amounts announced in the past two years, would equate if continued on a five year basis, to the $2 billion in new global funding, we specifically suggested was needed (O'Neill, 2018).*

This leaves eight categories where progress had been minimal. The O'Neill update (O'Neill, 2018) only indicated partial progress for improved public awareness, sanitation and hygiene, unnecessary use in agriculture, global surveillance of drug resistance and a global coalition via the G20 and UN. It reported no progress for rapid diagnostics, development and use of vaccines and alternatives and incentives to promote investment for new or improved drugs. If we are to tackle antibiotic resistance as a society, it is important that progress is made in all ten of these areas.

In 2019 an independent evaluation of the 2016 O' Neill report was published (Clift, 2019). This evaluation suggested further progress had been achieved; with significant advances in reducing antibiotic use in agriculture in high-income countries (commandment 3), greater investment in awareness campaigns (commandment 1) and investments made in improving surveillance of antibiotic use and resistance (commandment 4). It caveated this by noting that the central and most expensive recommendations had not progressed including investment in the antibiotic discovery pipeline (commandment 8 and 9). Further, of those areas that had seen progress - noticeably investment in awareness - questions still remained about its impact and effectiveness in eliciting behaviour change among the public (Clift, 2019).

This Doctor of Philosophy (PhD) focuses on the issues highlighted in section 1.1, the decreased rate of novel antibiotic discovery and the increased incidence of antibiotic resistance. To tackle the first problem, I go on to discuss the reinvigoration of the antibiotic discovery pipeline. To tackle the second problem, I discuss the need to re-examine how increased awareness is achieved (Commandment 1), noting the issues suffered by public health campaigns to date and advocating for the use of public engagement and project evaluation.

1.2.2 Reinvigorating the drug discovery pipeline

Bacterial resistance driven by human use of antibiotics has been observed since the discovery of penicillin in 1928 and the consequential introduction of antibiotics in the 1930s and 40s. As resistance to one type or class of antibiotic

emerged, new types or classes of antibiotic were developed (Davies, 2011). See Table 1-5 for the different classes of antibiotics.

This was until the drug discovery pipeline began to dry up. O'Neill (2016a) stated that despite a growing unmet clinical demand and affluent potential markets, the pipeline for new antibiotics has experienced a long-term decline. The global pipeline of antibiotics has too few drugs in development to meet current and anticipated patient needs (The PEW Charitable Trusts, 2020).

It could be viewed in a positive light that the antibiotic pipeline is still producing a handful of products, but the concern is that these will not be effective against the urgent and serious threats highlighted in Table 1-3. For example, history has shown that antibiotic resistance in clinical settings has often developed before antibiotics are available for clinical use (Bowater, 2017). There is rising resistance to carbapenems (Table 1-5), a class of antibiotics that constitute doctors' last good line of defence against a range of potentially life-threatening infections, however only three compounds under development have the potential to be active against the vast majority of bacteria resistant to carbapenems (O'Neill, 2016a).

One key reason the antibiotic pipeline has slowed dramatically is that until resistance against a previous generation of drugs has emerged, there is no certainty of profitable development of a new antibiotic. In addition, as we are trying to reduce the number of antibiotics we use, a drug may not be used until it is near/past the end of its patent, minimising corporate revenue to the point where development costs may not be recovered. The Chief Medical Officer Professor Dame Sally Davies in her annual report for the U.K Department of Health put forward 'Recommendation 7' which suggested that AMR is placed on the National Security Risk Assessment register. As part of this, collaborations to ensure the development of new antimicrobials and vaccines such as Private Public Partnerships would be developed (Davies, 2011).

The urgent need to supply funding in this area has been recognised by Governments globally. In the U.K, the Government has been forthcoming in setting up the Fleming Fund, contributing $375 million USD to improve disease surveillance in low and middle income countries, and has worked with China to contribute $72 million USD to a new Global Innovation Fund which aims to kick-start early research into new antimicrobials and diagnostics (O'Neill *et al.*, 2015). In the U.S.A, the Obama administration hoped to accelerate basic and applied R&D for new antibiotics. They aimed to have at least two new antibiotic drug candidates to prevent human disease and at least three drug candidates/probiotic treatments as candidates for promoting growth/preventing disease in animals by 2020. Finally, they wanted to promote innovation and increase the number of antimicrobials in the pipeline. Funding to do this was increased in President Obama's 2016 Budget, which nearly doubled the amount of Federal funding for combating and preventing antibiotic resistance to more than $1.2 billion (Obama and The Task Force for Combating Antibitic-Resistant Bacteria, 2015).

Table 1-5. Depicts the different classes of antibiotics with well-known examples. Classes of antibiotics in capital letters, with well-known examples underneath. Types of antibiotics belonging to each class in bold. Taken from Moore (2016).

Classes of Antibiotics							
PENICILLINS	CEPHALOSPORINS	FLUOROQUINOLONES	AMINOGLYCOSIDES	MONOBACTAMS	CARBAPENEMS	MACROLIDES	OTHERS
Natural Penicillin G Penicillin V-K	**First Generation** Cefazolin Cephapririn	Ciprofloxacin Moxifloxacin	Gentamycin Kanamycin	Aztreonam	Ertapenem Meropenem	Erythromycin	Vancomycin Tetracycline
Penicillinase Resistant Methicillin Oxacillin	**Second Generation** Cefacor						
Aminopenicillins Ampicillin	**Third Generation** Ceftriaxone						
	Fourth Generation Cefepime						

An independent report as part of the Review on AMR also focused on making antibiotics R&D commercially sustainable (O'Neill, 2016b) by jump-starting blue sky research. They proposed a system by which a global organisation has the authority and resources to commit lump-sum payments to successful drug developers. Payments would be set against selective criteria agreed in advance, removing the link between drug sales and profitability. Instead the financial incentive would come from the savings of treating resistance. For example, the cost of treating a patient with TB increases from $12,000 USD for a patient with a drug-susceptible strain to $180,000 USD for a patient with an MDR strain (Lieberman and Wootan, 1998). Furthermore, according to the CDC, AMR adds a 20 billion USD surplus in direct healthcare costs in the U.S.A, which is exclusive of about 35 billion dollars in loss of productivity annually (Centers for Disease Control and Prevention, 2013a). In comparison, the O'Neill report estimates that a comprehensive package of interventions could cost as little 16 billion USD and no more than 37 billion USD over the course of 10 years and would be sufficient to radically overhaul the antibiotics pipeline, which is less than the 40 billion USD per year we pay for antibiotics today (O'Neill, 2016b). Since 2016, the report has updated its recommendation to suggest that if pharma don't come forward soon with some action, governments should seriously consider implementing a so-called Play or Pay model. This charges a surcharge on sales from pharmaceutical companies that don't attempt to research new drugs and uses these funds to finance individual Market Entry Rewards (O'Neill, 2018).

Outside of government action plans, the need for antibiotic discovery has been widely understood by the U.K public. In 2014, the public voted that the most important challenge to humanity was point-of-care diagnostic tests which would aid in antimicrobial stewardship (Nesta, 2017) and in 2017, the British public voted antibiotics as Britain's Greatest Invention[5] (BBC TWO, 2017). Both victories resulted in funds being set up to encourage antibiotic research.

These government backed action plans and the U.K public take one key stance, that in order to reinvigorate the drug discovery pipeline funds must be committed to antibiotic research and drug discovery.

1.2.3 Preserving the effectiveness of current antibiotics

The golden era of antibiotic discovery solved the problem of emerging resistance by discovering new drugs faster than bacteria could develop resistance to them. However, it is clear now that this is not a successful long-term strategy as new drugs are no longer being found with such success.

[5] Antibiotics are not Britain's invention, moulds and plant extracts were used by some of the earliest civilisations. Paul Ehrlich (a German physician) discovered an antibacterial chemical in 1909. Selman Waksman (Ukrainian-American inventor) used the term 'antibiotic' 30 years later and discovered 20 antibiotics in his lifetime (Microbiology Society, 2017c). Penicillin was discovered by the British Alexander Fleming in 1928, but the process for mass producing the drug was patented in the U.S.A (Bowater, 2017).

Instead, we must be smarter with our antibiotic usage at the point of prescription but also with regards to proper use, consumption and adherence.

Proper prescribing practices are required to prevent further development of antibiotic resistance. When managed poorly, the result of antibiotic use in humans can be devastating. In the U.S.A in 1987, antibiotic-resistant *pneumococci* had not been encountered. By 1997, as many as 40 percent of *Pneumococcus.* spp were resistant to penicillin and other commonly used antibiotics. A large-scale study of U.S.A hospital records by the Health Care Financing Administration showed that 63% of orders for vancomycin, the last line of defence against severe hospital-acquired infections, were inconsistent with CDC guidelines (Lieberman and Wootan, 1998). In U.K hospitals, 15% of antimicrobials did not have the reason for their use recorded in the medical notes (Health Protection Agency, 2012). The Chief Medical Officer for England published an annual report in 2013 which highlighted the failure of treatment of *Neisseria gonorrhoea*, and our reliance on 'last line' antibiotics for which we have no replacements (Davies *et al.*, 2013).

However, case studies have examined the effects of regulating prescription practices with mixed results. An example of a successful intervention occurred in Finland between 1992 and 1998 when a single clone of *Streptococcus pyogenes* gained macrolide-resistance (erythromycin). Public Health authorities noted this increased resistance rate (15%) coincided with a tripling in use of macrolide over the previous 15 years. By reducing the prescription rates of macrolides for upper respiratory infections, the prevalence of erythromycin resistant *S. pyogenes* dropped from 16.5% to 8.6% between 1992 and 1996 (Seppälä *et al.*, 1997).

Interventions are not always this successful. Over the last 80 years *Neisseria gonorrhoea* has gained resistance to penicillin (Martin *et al.*, 1970), fluoroquinolones (Fenton *et al.*, 2003; Lewis, 2010), cefixime (Bignell, 2001; Ison *et al.*, 2013), ceftriaxone (Bignell, 2004; Ison *et al.*, 2013) and azithromycin (Bignell, 2004). This is despite several guidelines being issued and implemented by health care providers to try and reduce resistance to each antibiotic as resistance arose.

Interventions can also provide mixed results, as seen in *Clostridium difficile* infections between 1990 and 2000 in the U.K. Infections were seen to greatly increase in elderly patients who had recently received antibiotics, specifically cephalosporins (Wilcox *et al.*, 2004) and fluoroquinolones (Cooke *et al.*, 2015). These infections resulted in diarrhoea (Public Health England, 2014). Intervention achieved 60-80% reductions in the use of these two antibiotic classes. Furthermore, combining this with an emphasis on hospital hygiene saw a reduction in *C. difficile* associated infection by 77.9% from 2007/08 (Thelwall *et al.*, 2018). However, in replacing these two classes of antibiotic with penicillin-β-lactamase inhibitor combinations, selection pressure was then placed on the β-lactamase inhibitor combinations. This is of concern as *Enterobacteriaceae* with carbapenemase resistance (an urgent health threat, Table 1-3) are consistently resistant to this class of antibiotics (Livermore *et al.*, 2013). This emphasises the point that resistance to one class of antibiotic leads to change in prescription practices to a new prescribing regime. This in turn leads to increased resistance to the new antibiotic being prescribed.

Perhaps one of the more exciting developments is the idea of using combination therapy. This is where simultaneous addition of multiple drugs results in interactions which may bias evolutionary outcomes of bacteria, making it unfavourable to develop resistance (Furusawa, Horinouchi and Maeda, 2018). There are two ways in which combination therapy can work. If becoming resistant to one drug removes a bacterium's protection against a second drug, resistant mutants would be disadvantaged and as such resistance would be unfavourable. If mutations that confer resistance to a drug result in the combined effect of the drug and another compound to be greater than the summative effect, the mutations would be counteracted. Combination therapy relies on trade-offs between resistances to different compounds such that resistance to one antibiotic causes collateral sensitivity to another antibiotic or to a compound whose toxicity is mediated by the resistance mechanism (Baym, Stone and Kishony, 2016).

There are several trial studies which aim to control resistance evolution by adding multiple antibiotics. The author of a review which looked at these trials (Baym, Stone and Kishony, 2016) concluded:

> *"It is possible to invert the selective advantage of resistant bacteria and reverse the evolution of antibiotic resistance" (Baym, Stone and Kishony, 2016).*

Altering prescribing practices to correct the mistakes of misuse or overuse of antibiotics is a key tool in the fight against antibiotic resistance. However, misuse and overuse are not only influenced by the prescriber. The final report and recommendations of The Review on Antimicrobial Resistance states that the only sustainable, long-term solution to the global problems of antibiotic resistance lies in action to address the 'demand' side, thus reducing the burden on our current antibiotics (O'Neill, 2016b).

This demand for antibiotics is driven by industry and the public. In industry, a large quantity of antibiotics are used every year in veterinary practice, whilst the fishing and farming industries provide a further vehicle for the development of antibiotic resistance (Davies, 2011). For example in the U.S.A, the Minnesota State Department of Health showed that only two years after fluoroquinolones, a second-line drug, were approved for use in poultry, there was an increase in fluoroquinolone resistance in bacteria isolated from chickens, turkeys, and humans (Lieberman and Wootan, 1998). A solution, recommended by the U.K Chief Medical Officer (Recommendation 8), is to manage antibiotics jointly between the Department of Health and the Department for Environment, Food and Rural Affairs (Davies, 2011). In the U.S.A, as well as calls to stop the use of antibiotics in agriculture, there are also calls to ban their use as pesticides. To do this, similar collaboration is requested between the U.S.A Food and Drug Administration and the U.S.A Environmental Protection Agency (Lieberman and Wootan, 1998).

The public creates a demand for antibiotics even when antibiotic prescription is not the correct treatment. In 2014, 19.5% of a randomly sampled cross sectional survey of the Australian population (n=1509) reported that they would expect the doctor to prescribe antibiotics, effective only against bacterial infections, for viral infections such as cold or flu (Gaarslev et al., 2016). Of 190

patient, parents and caregivers, 53% incorrectly believed that antibiotics work well for treating viral infections. These patients were more than twice as likely to expect a provider to give them an antibiotic for a cough or common cold (Davis *et al.*, 2017). This is a particular concern considering patients who expect to receive antibiotics at an outpatient visit were prescribed antibiotics more frequently than those who were not expecting them (Coenen *et al*., 2006; Cals *et al*., 2007; Coenen *et al*., 2013; Cole, 2*014*; Sirota *et al.*, 2017). This links to the discovery that providers report feeling pressured to prescribe antibiotics even when they believe them to not be needed *(Brookes-Howell et al*., 2012; Coenen *et al.,* 2013; Little *et al.,* 2004; Macfarlane *et al.,* 1997; Watkins *et al.,* 2015).

It is important to tackle the public demand for antibiotics to help reduce the number of prescriptions and improve current prescribing practices. An obvious target is reducing prescriptions that happen unnecessarily, for example when the infection is not bacterial and is therefore not treatable with antibiotics. However, this approach must be done in parallel with increasing awareness among members of the general public and patients about the need for smarter, evidence-based prescribing in order to protect the usefulness of antibiotics. Critically, it also requires the public to play their part by taking the antibiotics as prescribed by health-care providers and improving compliance.

1.3 Public Perception of Antibiotic Resistance

1.3.1 Key Topics Emerging from the Literature

In order to raise awareness among members of the public about antibiotic resistance (Commandment 1, 1.2.1), it is first important to understand the public discourse surrounding this topic. By identifying areas where public awareness is lacking, especially if this lack of awareness leads to negative behavioural choices with regards to antibiotics, a targeted effort can be made to address this. In order to identify key areas or topics that are important with regards to behaviour, I undertook a literature review of research. The search terms included: public perception, public beliefs, patient expectations, patient interpretations, public attitudes towards antibiotics. This search identified research papers from which, other relevant literature was established. This provided 20 papers spanning a 21-year period that examined public perception of antibiotics and antibiotic resistance. As well as examining the key topics emerging from this body of literature, I have presented the key features of each paper: the number of participants, participant demographic and the data collection tools used (Table 1-6).

Table 1-6. Summary table of the key features of 20 papers which examine the public's perception of antibiotics and antibiotic resistance. The key features of each paper, with reference, include the number of participants, participant demographic and the data collection tools used.

Reference	Number of participants	Information about participants and country of origin	Data collection tool
(Macfarlane *et al.,* 1997)	1014	Previously well adults in Britain presenting to GP with illness defined as a Lower Respiratory Tract Infections	Quantitative intervention study, half received leaflets, half did not. Endpoint was reconsultation for the same symptoms within one month
(Butler *et al.,*1998)	38	GPs and their patients in South Wales who had recently consulted for a sore throat or Upper Respiratory Tract Infection	Qualitative study with semi-structured interviews
(Welschen *et al.,* 2004)	1014	Patients from GP practices in The Netherlands presenting with acute respiratory tract symptoms	Quantitative yes/no questionnaire
(Van Driel *et al.*, 2006)	298	Patients of family physicians in Belgium making visit for acute sore throat	Quantitative observational post visit questionnaire survey (4-point Likert scale)
(McNulty *et al.*, 2007)	7120	Household Survey of randomly selected adults in the U.K	Quantitative face-to-face questionnaires (4-point Likert scale)
(Hawkings, Wood and Butler, 2007)	46	Members of the Welsh public	Qualitative grounded theory interview study
(Brooks *et al.,* 2008)	23	Primary care patients in low prescribing affluent practice vs high prescribing, deprived practice in the U.K	Qualitative focus groups (5 groups, 23 patients) followed by 20 qualitative interviews
(Filipetto *et al.,* 2008)	98	Individuals in three family medicine practices (U.S.A)	Quantitative questionnaire. Mix of yes/no, numbered or 5-point Likert Scale responses
(Hawkings, Butler and Wood, 2008)	46	Members of the South Wales public	Qualitative semi-structured interviews.
(European Commission, 2010)	26762	Members of the public across 27 EU member states	Quantitative Eurobarometer Survey

(McNulty *et al*., 2012)	17 & 5,180	Participants with acute Respiratory Tract Infections at English pharmacies & members of the English public	Qualitative interviews & Quantitative face-to-face questionnaire survey
(Brookes-Howell et al., 2012)	121	Primary care patients with symptoms of Lower Respiratory Tract Infections based in 9 EU member states	Qualitative face-to face semi-structured interviews
(European Commission., 2013)	27680	Members of the public across 28 EU member states	Quantitative Eurobarometer Survey
(Norris *et al.*, 2013)	21 &101	Focus group participants & household members from the New Zealand public	Qualitative focus groups & interviews/diaries
(Gudnadottir *et al.*, 2013)	100	Patients with health care-associated infections placed in contact precautions in the U.S.A	Quantitative questionnaire
(Hawker *et al.*, 2014)	3800000	Active patients across U.K General Practices between 1995-2011	Descriptive study on diagnosis and prescription
(McNulty *et al.*, 2016)	1625	Members of randomly selected households across England	Quantitative, face-to-face computer-assisted survey
(European Commission, 2016)	27,969	Members of the public across 28 EU member states	Quantitative data from interview data gathered face-to-face at home in their own language
(Hawking *et al.*, 2017)	53 & 21	Adolescents (16-18) at educational establishments in the South of England	Qualitative semi-structured focus groups & interviews
(Dyar *et al*., 2018)	255	Students training to be healthcare professionals at U.K Universities	Quantitative cross-sectional multi-centre web-based survey

These 20 studies examine public perceptions regarding either antibiotics and/or antibiotic resistance (Table 1-6) from across the U.K, the EU as well as New Zealand and the U.S.A. Data was collected using a variety of quantitative and qualitative tools and sampled a varied range of individuals. An analysis of these 20 studies identified recurring themes. Whilst this list is not exhaustive, it provides a useful insight into some of the main perspectives individuals might hold towards antibiotics and antibiotic resistance. These six topics are:

1. Antibiotics and their side effects
2. The concept of the Resistant Human Body
3. The Irresponsible Other
4. Factual knowledge and sensible use of antibiotics
5. Public Awareness of antibiotics and antibiotic resistance
6. Understanding why users take antibiotics

Each of these themes are discussed in detail using information from the research studies highlighted in Table 1-6.

1.3.2 Antibiotics and their side effects

One of the broader themes emerging from the literature is the negative consequences of antibiotic use, including the negative side effects. Out of the 20 papers, eight demonstrated this theme.

As early as 1998, Butler *et al.,* showed that patients were aware antibiotics are not a trouble-free solution to coughs, colds and sore throats and provided association between antibiotics and thrush, rashes and oral contraceptive failure (Butler *et al* 1998). This may reflect the fact that these contraindications are often highlighted by health care providers when prescribing antibiotics. Research undertaken by McNulty *et al* (2007), on members of the British Public supported this finding; less than half of participants agreed that microbial flora is good for your health and that antibiotics kill this flora. In general however, participants believed that overuse of antibiotics increased the chance of resistance (McNulty *et al.*, 2007). This finding was also apparent in a separate study (Hawkings *et al.*, 2007). In this study a quarter of participants – members of the Welsh Public - described adaptation and mutation of bacteria in association with antibiotic use. However, some of these participants, notably those with a science background, also highlighted that not finishing the full course of antibiotics could account for treatment failure. In 2012, another study by Brookes-Howell *et al* (2012) found that almost all participants, patients attending a General Practitioner (GP) for a respiratory tract infection, linked unnecessary antibiotic use with resistance. Table 1-7 shows three subsequent Eurobarometer surveys on antibiotic resistance conducted by the European Commission (European Commission, 2010, 2013, 2016). With regards to side effects, these randomly sampled EU wide surveys showed that participants understood unnecessary use of antibiotics reduces their efficacy. These data show that in 2016, 84% of respondents agreed that taking antibiotics can increase antibiotic resistance. This number has not changed since 2010 (83%). Further, two-thirds of participants agreed that taking antibiotics often has side effects such as diarrhoea. The data showed 66% of respondents in

2016 agreed that antibiotics can cause significant side effects such as diarrhoea, a number which has not changed since 2010 (68%).

Table 1-7. Eurobarometer data mapping the change in % of population's understanding of antibiotics, their uses and the impacts of misuse. Data obtained from three subsequent versions of the Eurobarometer Survey spanning six years in 2010, 2013 and 2016 (European Commission, 2010, 2013, 2016).

Year	2010	2013	2016
Agreed Antibiotics kill viruses.	53%	49%	46%
Agreed Antibiotics effective against colds and flu.	47%	41%	36%
Agreed unnecessary use of antibiotics makes antibiotics ineffective	83%	84%	84%
Agreed antibiotic use cause side-effects	68%	66%	66%

This understanding, that frequent use of antibiotic can be detrimental to health, was also seen in the study by Norris *et al.* This study of the New Zealand public showed that the public acknowledged adverse effects of antibiotics as the main reason to avoid taking them (Norris *et al.*, 2013). In 2017, students studying a health-care provider course identified that overuse of antibiotics results in drugs that might not work in the future. Some participants avoided antibiotics and painkillers because they were concerned that they would become reliant on them or felt that they were harmful (Hawking *et al.*, 2017). A study by Dyar *et al* (2018) focused on university students who discussed the cause of antibiotic resistance. They agreed that excessive antibiotic prescriptions (100%), excessive use in livestock and food production (98%), too low dosing of antibiotic prescriptions (83%) and too long duration of antibiotic therapy (75%) were parts of the problem. They generally agreed that overuse of antibiotics makes them less effective (96%) and that antibiotics kill commensal bacteria as well as pathogenic bacteria (88%) (Dyar *et al*., 2018). However, you would expect that undergraduates studying on a health care degree programme would have a thorough understanding of the role of antibiotics and the growing problem of antibiotic resistance.

To summarise, evidence has shown that awareness that antibiotics are not a trouble-free solution and that there are consequences when using antibiotics is apparent within the general public. There is also a belief that sensible use, for example taking the full course, can minimise the effect of these consequences. Finally, the negative consequences of antibiotic use are a powerful motivator in discouraging the use of antibiotics. This is an important outcome for clinical practice.

1.3.3 The concept of the Resistant Human Body

A specific theme emerging from the literature is coined “The Resistant Human Body”, where participants understand that the reason antibiotics stop working is a result of the human body developing resistance or immunity to them. Five studies from the 20 I examined reported this finding.

In 2007, Hawkings *et al* found that 13% of participants, members of the general public residing in Wales, understood that resistant infections might cause treatment failure. Participants generally related this failure to the bodies response to repeated antibiotic use rather than changes in resistance characteristics of bacterial populations. The body becomes “used to” or “immune” to antibiotics (Hawkings *et a*l., 2007). In 2008, the human body becoming resistant to antibiotics and not the infectious agent was considered a common misconception in a study by Brooks *et al* who interviewed patients with both a high and low income background in GP practices (Brooks *et al*., 2008). In 2012, 43 of 121 participants with suspected respiratory tract infections from GP practices from nine member states of the EU suggested that resistance occurred in individuals’ bodies, whereas 28 of 121 identified that resistance is a property of an infectious body. This demonstrates that this misunderstanding is not restricted to the U.K. These authors coined the terms ‘Resistant Body’ and ‘Resistant Bacterium’ to explain these opposing perspectives (Brookes-Howell *et al*., 2012). The following year, it was also reported that resistance is used commonly in terms of the body developing resistance or becoming accustomed to medication. Further, immunity was considered a property of humans, rather than the bacteria in New Zealand (Norris *et al.*, 2013). In 2017, pupils that did not self-identify as science students suggested that the body or person becomes resistant to antibiotics (Hawking *et al.*, 2017), suggesting that this misconception is already established by the time a person reaches their teens.

There is a clear misconception among the public, other than perhaps those partaking in scientific study, that resistance is a property of the human body rather than an infectious agent. This perception was coined the resistant body theory. With regards to clinical practice however, the idea that overuse of antibiotics leads to their decreased efficacy, regardless of whether this is a result of the body or an infectious agent, likely results in the avoidance of antibiotic overuse.

1.3.4 The Irresponsible Other

Five papers attempted to understand whether members of the public feel they play a role in resolving the issue of antibiotic resistance. Overwhelmingly, the perception of an “irresponsible other” emerged from the literature: other people’s irresponsible behaviour is exacerbating the problem.

In a study by Hawkings, Wood and Butler (2007), few participants from the Welsh general public mentioned the individual’s potential contribution to controlling bacterial resistance. Instead, they focused on antibiotic resistance being caused by overprescribing by GPs, poor environmental hygiene in hospitals and receiving the incorrect dose for too short a time period. Notably, a few of the participants mentioned the role handwashing can play in

controlling resistant infections. Interestingly, the authors suggested that the attitudes towards infections influenced attitudes towards bacterial resistance. This considers germs as being something you caught from someone else or something else, not something that an individual can control. This led to the perspective that one has little individual responsibility for the control of bacterial resistance and instead that the responsibility for managing antibiotic resistance lies with the government or National Health Service (NHS) managers (Hawkings, Wood and Butler, 2007). I note that participants with a science background described the responsibility as belonging to the government and society as a whole (Hawkings *et al*., 2007). In 2008, the term "Irresponsible Other" was coined by Brooks *et al* (2008). Participants generally suggested that the individuals role was minimal and the solution to antibiotic resistance was out of their personal control, that they did not have the knowledge to contribute. Further, it was 'other' 'irresponsible' patients who misuse and overuse antibiotics. Participants did not just blame other patients, suggesting that GPs, agriculture and veterinary medicine are misusing antibiotics. When considering misuse by patients, participants identified needless use, failure to complete the course and sharing antibiotics as issues other people have, reflecting the idea that antibiotic use can have negative consequences (Brooks *et al*., 2008). There were members of the public who did not apportion blame. In these cases, they said antibiotic resistance is being 'dealt with' (Brooks *et al*., 2008). In 2017, adolescent participants suggested that their peers take a lot of antibiotics and treat them as a quick fix, a 'cure-all' like painkillers (Hawking *et al.*, 2017). Parents were also blamed as they have an advisory role; they could influence intentions to take antibiotics, despite some feeling that their parents were less knowledgeable about antibiotics than themselves. Participants also expressed the view that it is the GPs responsibility to prescribe appropriately and monitor antibiotic usage, and it is not their concern (Hawking *et al.*, 2017). In 2018, 31% of 255 university students training for health-care professions did not believe that the antibiotics they would prescribe, administer or dispense would contribute to the problem of antibiotic resistance. They did however believe that prescribing, dispensing or administering inappropriate or unnecessary antibiotics is professionally unethical, perhaps suggesting that they did not believe they would be found prescribing unnecessary antibiotics (Dyar et al., 2018). Whilst I am focusing on public perception, an interesting piece of research in 1998 showed that GPs may also blame 'irresponsible others'. GPs felt that patients expect antibiotics and attempting to educate them is time consuming and unrewarding. Furthermore, antibiotic resistance was seen as a community issue rather than the GPs issue, which instead was the wellbeing of the patient (Butler *et al*.,1998).

In general, members of the public, be it those who feel they lack knowledge to contribute to the solution of antibiotic resistance, student health-care providers or current GPs, have a tendency to blame 'Irresponsible Others' rather than considering their own contribution to the problem of antibiotic resistance. Whilst not conclusive, individuals with a background in science were the only reported participants that saw the responsibility of solving antibiotic resistance as a societal issue. It is important to raise awareness that individuals can make a difference in controlling resistant infections. This has been demonstrated in

the recent Covid-19 pandemic. The U.K government has moved the responsibility of containing the spread of the virus to individuals, expecting them to adhere to guidance such as 'Hands, Face, Space', in order to control the rate of infection (Department of Health and Social Care, 2020).

1.3.5 Factual knowledge and sensible use of antibiotics

Five studies reported the link between better factual knowledge and desirable human behaviour with regards to antibiotic resistance. In this section, I examine how individuals take responsibility for their own actions and whether better factual knowledge has any affect.

In 2007, McNulty *et al* reported that respondents with no formal qualifications were twice as likely to respond incorrectly to questions about antibiotics and resistance as those with a degree level of education. Further, young women or those with lower levels of education were most likely to be prescribed antibiotics. However, 87% of people who didn't finish their course of antibiotics knew they should have (McNulty *et al.*, 2007). In 2008, Brooks *et al* demonstrated that participants who believed the solution to antibiotic resistance was out of their personal control, and felt they did not have the knowledge to contribute, said that the individual's role was minimal (Brooks *et al*., 2008). In 2013, a survey of 27,680 people living in the EU showed that 36% of participants changed their views on antibiotics after receiving information on misuse. Those with low levels of education were more likely to be influenced, which resulted in 74% of those individuals saying they would consult a doctor about needing antibiotics in the future. This research also noted that this information was more effective when coming from a doctor (80%), rather than the media (69%). Finally, those with better factual knowledge were more likely to take antibiotics solely for illnesses that can be treated with antibiotics (European Commission, 2010, 2013, 2016). McNulty *et al (*2016) showed that participants from higher social grades and those educated to a higher level were more likely to report that they had been given information about antibiotics or caring for their infection. People would more readily trust their GP's advice on whether they need antibiotics (88%), compared to a nurse (69%) or a pharmacist (66%) (McNulty *et al.*, 2016). In 2017, student participants who demonstrated more knowledge of antibiotic resistance, mostly science students, felt that it was an important issue and awareness should be increased among the general public (Hawking *et al.*, 2017).

Individuals with lower levels of formal education seem to have less factual knowledge about antibiotic resistance. Participants with less factual knowledge about antibiotic resistance believe they cannot contribute effectively to the solution and are less likely to consider this an important issue. However, when information is provided on antibiotics and resistance, those with lower levels of education were more likely to be positively influenced to change their behaviours, especially if this information came from their GP. This must be a target for public health campaigns. Nevertheless, individuals with better objective knowledge are more likely to take antibiotics in the correct instance, yet even when these individuals know they should complete the full course, they often do not.

1.3.6 Public Awareness of antibiotics and antibiotic resistance

Eleven papers reported on the measures of public awareness of antibiotics and antibiotic resistance. I go on to specifically discuss public health campaigns and their effectiveness in more detail in 1.4.

In 1997, Macfarlane *et al* found that participants, patients attending their GP with a respiratory tract infection, who were provided with information on antibiotic resistance, whether or not they were prescribed antibiotics, were less likely to reconsult their GP with the same symptoms within a month because that had developed an awareness of antibiotic resistance (Macfarlane *et al*., 1997).

Ten years later in 2007, McNulty *et al* found that only 20% of English respondents, randomly chosen members of the U.K public, had heard of the national U.K Andy-Biotic Public Heath campaign focused in GP surgeries aimed at discouraging people from unnecessarily taking antibiotics for cold and flu. This same study found that 79% of participants had heard about antibiotic resistance. Data revealed less awareness about the effectiveness of antibiotics to treat viral infections, with only 38% of respondents realising that antibiotics cannot be used to treat viral infections. Participants had a lack of knowledge about a specific side effect of antibiotics: 43% of respondents were unaware that antibiotics can harm our normal microbiota (McNulty *et al*., 2007).

Hawkings, Butler and Wood (2008) reported similar findings. They conducted interviews with respondents and found that all had used antibiotics and were confident in the efficacy and safety of antibiotics but unfamiliar with potential disadvantages or side effects. They published another study from the same interview carried out with the same respondents that showed participants had little awareness of the causes and consequences of antibiotic resistance, and participants viewed this with a low sense of perceived importance or personal threat (Hawkings *et al*., 2007). In this study, participants reported that their main source of information on this topic was the media: Television, followed to a lesser extent by newspapers and radio reports (Hawkings *et al*., 2007).

In 2008, three-quarters of participants, patients at a GP surgery, who took part in a study by Brooks *et al* had heard of antibiotic resistance. However, from these positive responses, three-quarters had trouble explaining what this term meant, what caused antibiotic resistance and its implications. The authors showed that no participants reported hearing about antibiotic resistance through public information campaigns and only one individual had been informed by a health professional (Brooks et al., 2008).

This finding contrasts sharply with a separate research study conducted the same year that found doctors had discussed antibiotic resistance with 68% of participants, whilst 88% of participants thought doctors should have done so (Filipetto et al., 2008).

In 2010, a pan EU survey discovered that 37% of participants reported that they had seen anti-antibiotics information within the last year (European Commission, 2010). The same question asked three years later in 2013, indicated that this number had fallen to 33%. Percentages of participants differed greatly across European countries and the results were nuanced with

higher rates in some countries compared with others. Of these 33%, 11% received this information from health professionals and 9% specifically from a doctor. As discussed in the previous section (1.3.5), those who did encounter antibiotic awareness campaigns generally changed their views on antibiotics or enacted more desirable behaviours (European Commission., 2013).

Another 2013 study that took place in the U.S.A detailed the types of information patients received and how patients felt about this information. As would be hoped for, most patients with healthcare associated infections that took part in the survey had heard of multi-drug resistant organisms (MDROs). Most participants reported that this information came from their physician. They also indicated that internet-based information was agreed to be the most helpful source of information, followed by written and then verbal information. Further, 94% of patients thought that getting information about MDROs would definitely or probably help them make choices that would improve their care (Gudnadottir *et al.*, 2013).

Another study by McNulty *et al,* published in 2016, indicated that half of the participants lacked knowledge about antibiotic resistance. Half of those who consulted for an infection and 67% of those prescribed an antibiotic were given advice from a health professional. Only 8% of participants were specifically given information on antibiotic resistance after antibiotic prescription (McNulty *et al*., 2016).

In 2017, most adolescents interviewed by Hawking *et a*l perceived that they understood the term antibiotic resistance. Upon further investigation, the authors concluded that the participants had a poor understanding of the scientifically correct use of this term (Hawking *et al.*, 2017).

In 2018, 80% of university students training to be health-care professionals felt they did not have sufficient knowledge of antibiotic use for their future clinical practice. The students indicated that they wanted more information on resistance to antibiotics (63%), as well as the links between health of humans, animals and the environment (49%), prescription of antibiotics (45%) and how to use antibiotics effectively (25%) (Dyar *et al*., 2018).

Initial data suggests that information on antibiotics may reduce the numbers of re-consultations. Although data indicates that current awareness of antibiotic resistance is not pervasive among participants in the different studies, even those who self-report having heard of resistance cannot provide an explanation that has scientific validity. There is large room for improvement with the dissemination of information from GPs. Most people trust GPs to give the correct information and want more information on antibiotics and antibiotic resistance, but GPs are not providing this. Finally, it seems unlikely that antibiotic campaigns are reaching the public. If they are, they are not having the desired effect of improving public awareness and engendering behavioural change.

1.3.7 Understanding why users take antibiotics

Nine papers reported participant perspectives on what they would take antibiotics for. Participants were asked specifically about whether antibiotics

are useful to treat Cold and Flu in eight studies, with viruses and bacteria also being mentioned on several occasions. Cold and Flu is particularly important as this is a common target for public health campaigns which is discussed further in 1.4.

The number of participants correctly disagreeing with the statement 'antibiotics kill colds and flu' has been asked by several researchers in several studies. Answers were:

1. 68% (McNulty et al., 2007),
2. 55% (Filipetto et al., 2008),
3. 60% in 2008 and 69% in 2011 (McNulty et al., 2012).

Whilst there are fluctuations, between 2007 and 2011 the number of participants disagreeing with this statement remained steady. The EU has also sought to establish the knowledge of citizens from member states, concerning antibiotics using the following questions:

1. - Antibiotics kill viruses;
2. - Antibiotics are effective against colds and flu;
3. - Unnecessary use of antibiotics makes them become ineffective;
4. - Taking antibiotics often has side-effects, such as diarrhoea.

Respondents options were a binary choice of either true or false and percentage of respondents to each questions can be seen in Table 1-7. Table 1-7 clearly indicates that over time, members of the EU have increased their awareness that antibiotics are not effective treatments for colds and flu (European Commission, 2010, 2013, 2016). This contrasts with no real increase in understanding about the side effects of antibiotic use (1.3.2).

In addition, other more qualitative studies indicate that few participants would take antibiotics for colds and flu, expressing a feeling that these infections were simple and could be treated at home with "natural remedies" (Hawkings *et al*., 2007). Participants were quoted as having said: "*I would never take antibiotics for coughs and colds*" (Brooks *et al.*, 2008) and *"Antibiotics should be saved for dire emergencies, and not coughs and colds"* (Norris *et al.*, 2013).

Studies have also reported on actual treatment for coughs and colds, rather than self-reported actions of the public. Hawker *et al*., examined real prescription data retrieved from 2.97 million episodes of cough/cold diagnoses from 1995-2011, 46.9% were prescribed antibiotics in 1995, this reduced to 26.5% in 1999 but increased once more to 50.8% in 2011: similar levels to 1995 (Hawker *et al.*, 2014).

Eurobarometer data gathered between 2010-16 (Table 1-8) indicated a small decrease in the number of respondents taking antibiotics for cold or flu symptoms (European Commission, 2010, 2013, 2016).

Table 1-8. Eurobarometer data mapping the change in % of population who had taken antibiotics for viral infections. Data obtained from three subsequent versions of the Eurobarometer Survey spanning six years in 2010, 2013 and 2016 (European Commission, 2010, 2013, 2016).

Year	2010	2013	2016
Treated for Flu	20	18	16
Treated for Common Cold	14	13	11

Furthermore, several papers reported on specific infections that individuals might take antibiotics for. In 1997, 72% of participants said they would take antibiotics for a lower/acute respiratory tract infection (Macfarlane, Holmes and Macfarlane, 1997). A similar question was asked by Welschen *et al* in 2004, where only 44% of participants said they would take antibiotics for a lower/acute respiratory tract infection (Welschen *et al.*, 2004).

In a 2015 face-to-face computer-assisted survey of 1625 people in the U.K, aimed at identifying the public's reported use of antibiotics, 42% would take antibiotics for a runny nose or cold and a cough, 54% for flu symptoms, 60% for sinus infections, 62% for throat infections and 67% for ear infections (McNulty *et al.*, 2016). This opens a topic which needs further exploration, whether respondents realise that the pathogen most likely responsible for each for these infections is viral.

Further, Filipetto *et al* (2008) demonstrated that as questions include more technical language, answers become increasingly incorrect. The authors asked whether a discursive object required antibiotic treatment and summarised the percentage of participants that agreed they did. In the study, 45% agreed for colds, 70% agreed for viruses and 86% for yellow nasal mucous. I posit that this may be the result of a disconnect between the infection itself and the causative agent, be it bacterial or viral. This is something that might be considered when comparing different studies.

Outside of specific types of infection users may take antibiotics for, a 2006 study discovered that patients who hoped for an antibiotic felt more severely ill, had more faith in antibiotics to speed recovery, were less convinced that acute sore throat is a self-limiting disease and that frequent use of antibiotics is harmful for their own health or a threat to the public (Van Driel *et al.*, 2006).

Overall, data suggests that public awareness that coughs and colds cannot be treated with antibiotics has increased since 2007, as has the understanding that antibiotics cannot treat viral infections. This is perhaps one of the bigger success stories for public health campaigns. The different responses as technical language increased led me to ensure language remained identical between interviews, discussed in greater detail in 2.7.1

1.3.8 Summary

This section focused on the six key themes emerging from literature spanning 21 years from 1997 to 2018 which covered public perceptions of antibiotics and antibiotic resistance. These emerging themes, whilst not exhaustive, provide a foundation on which a coding schedule for my own interviews could

be developed (2.7.1). Further, these themes provide direction for clinical practice and can guide future public health campaign.

1.4 Public Understanding and Public Health campaigns

In 1.3, I examined public awareness of antibiotics and antibiotic resistance through the discussion of six key themes emerging from public discourse. In doing so, I highlighted key gaps in awareness which likely result in undesirable behaviour, such as not taking personal responsibility for the issue. This is important because the public are key stakeholders in implementing solutions to antibiotic resistance. In the following section I examine the method of public understanding to raise awareness about these topics through examination of public health campaigns. I discuss the foundation of public understanding, before discussing the extent to which campaigns based on this principle have been successful.

1.4.1 The Foundation of Public Understanding

The public are a diverse array of people and organisations who have a vested interest in finding a solution to antibiotic resistance (Phillips *et al*., 2014). Increasing the publics' scientific literacy is based on the principle that, as the public have a stake in shaping what happens in response to scientific challenges, they need to strengthen their understanding of how science works (Dunn *et al.*, 2016). Lack of scientific knowledge in the public domain isn't a new problem. Indeed, this was picked up in the seminal report by Snow more than 60 years ago (1959):

> *"I felt I was moving among two groups-comparable in intelligence, identical in race, not grossly different in social origin, earning about the same incomes, who has ceased to communicate at all. Two polar groups: at one pole we have the literary intellectuals, who incidentally took to referring to themselves as 'intellectuals' as though there were no others… – at the other, scientists. Between the two, a gulf of mutual incomprehension… but most of all a lack of understanding" (Snow, 1959).*

Almost 30 years later, a report by the Royal Society authored by Bodmer prompted the beginning of the Public Understanding of Science movement, which focused on educating the general public in matters of science:

> *"More than ever, people need some understanding of science… "Science and technology play a major role in most aspects of our daily lives both at home and at work. Our industry and thus our national prosperity depend on them. Almost all public policy issues have scientific or technological implications. Everybody, therefore, needs some understanding of science, its accomplishments and its limitations" (Bodmer, 1985).*

Public health campaigns arose from this movement; attempting to foster understanding of matters of science in members of the public, bridging the gap

between the sciences and the humanities. I now discuss what public health campaigns are, provide examples of public health campaigns and to what extent the public understanding of science movement has or has not been successful.

1.4.2 Designing a Public Health campaign

Public Health campaigns attempt to convince a defined public to refrain from behaviours that can be considered detrimental (Wakefield *et al*., 2010). They use a mixture of negative and positive messages to convey their point, often stressing the importance of the problem at hand, for example; how big an issue antibiotic resistance is (Huttner *et al*., 2010) or how important washing hands, covering mouth and nose and keeping two metres distance between individuals (Hands, Face, Space) is to prevent the spread of Covid-19 (Department of Health and Social Care, 2020).

Public Health campaigns focusing on antibiotic use or antibiotic resistance target specific demographics to convey specific messages to help solve a specific problem. In section 1.2.3, I discussed how both the prescriber and the recipient of antibiotics can contribute to overuse of antibiotics and as such, these are two groups that are targeted by campaigns. For example, parents are targets for campaigns as prescriptions written for young children diagnosed as having colds, upper respiratory tract infections and bronchitis represent a substantial proportion of total prescriptions to children *(*Nyquist *et al*., 1998). Campaigns also focus on the relationship between two groups of people. The doctor-patient relationship was targeted by Public Health England with the release of the “Keep Antibiotics Working” campaign in October 2017 (Public Health England., 2017).

I summarised some of the key behaviours that have been targeted by public health campaigns with regards to antibiotics and antibiotic resistance between 2000 and 2020 in the U.K (Table 1-9).

Table 1-9. Key themes surrounding antibiotics and antibiotic resistance promoted by public health campaigns. Summarised over a 20 year period between 2000 and 2020 in the U.K.

Key Themes:
Misuse of antibiotics promotes bacterial resistance
Most respiratory tract infections are caused by viruses and cannot be treated with antibiotics
Finish the course of antibiotic treatment as prescribed to avoid selection of resistant organisms (Take the lot, no matter what).
Follow the prescription and do not skip doses.
Hand washing prevents the spread of infectious diseases
Antibiotics need a prescription
Do not share antibiotics or keep leftover antibiotics.

As Public Health campaigns are disseminated to members of the public, research is undertaken to identify the effect they are having. This allows themes to be adapted if necessary. For example, the “take the lot, no matter what” (Table 1-9) message has been called into question because research suggested prolonged treatment might result in higher resistance rates (Rice,

2008) and identified that antibiotic use results in 140,000 emergency department visits every year in the U.S.A for antibiotic-associated adverse events (Shehab et al., 2008). Public Health campaigns must also consider in what way they their information or message will be disseminated.

1.4.3 The Success of Public Health campaigns.

Public Health campaigns have been successful. A review of 22 campaigns done at the national or regional level in high-income countries, aimed at reducing antibiotic use, indicated that campaigns are associated with a reduction in the use of antibiotics and resistance to antibiotics (Huttner *et al.*, 2010). This most likely occurred by influencing prescription provision and self-medication of outpatients, as well as a reduction in the number of consultations.

In the U.S.A, a reduction in antibiotic use was seen after exposure to mass-media campaigns compared within a controlled community (Gonzales *et al.*, 2008). The National Ambulatory Medical Care Survey showed that between 1995 and 2006 office visits decreased by 17%, a number that was accompanied by a 36% decrease in prescription of antibiotics for acute respiratory tract infections in children younger than 5 years old in the U.S.A (Grijalva *et al.*, 2009).

Public Health campaigns are most effective when they are multifaceted and repeated over several years (Huttner *et al.*, 2010), aim to change behaviour and target all relevant groups especially parents, children, day-care staff, and healthcare professionals (Finch *et al.*, 2004). The use of prime-time Television has been noted as elevating the impact Public Health campaigns have (Finch *et al.*, 2004; Huttner *et al.*, 2010), highlighting that in order to be successful, a campaign must most importantly reach its target audience.

1.4.4 Issues Identified with Public Health campaigns

Whilst Public Health campaigns have been successful, for example in the reduction in use of antibiotics, reviews of Public Health campaigns are highly critical.

One issue is correlation and causality. It is difficult to distinguish to what degree the observed effects of campaigns on the use of antibiotics are because of a change in the behaviour of physicians, patients, or both, and how important the effect is beyond trends happening in the absence of campaigns (Huttner *et al.*, 2010). In many cases, confounding effects like seasonal variation of viral respiratory tract infections on antibiotic prescriptions are not accounted for (Bauraind *et al.*, 2004). Campaigns often do not have a control population and pre-intervention trends are rarely assessed (Huttner *et al.*, 2010).

A separate issue is that the effect of a campaign might not necessarily be desired. A survey in the U.K showed that awareness of the “Andy-biotic” campaign was associated with increased knowledge about antibiotics, but interestingly this campaign also increased the likelihood of self-medication. Also in England a decrease in antibiotic use between 1993 and 2005 was linked to an increase in mortality associated with community-acquired

pneumonia (Price *et al.*, 2004). This was felt to be a warning that antibiotics must be prescribed when appropriate. This was further evidenced in a retrospective analysis of data from a large primary care database in the U.K in 2004, that found patients prescribed an antibiotic on the day of diagnosis of a lower respiratory tract infection had a lower likelihood of admission to hospital or death (Winchester *et al*., 2009).

Other issues include questionable messages not necessarily backed up by literature, incomplete evaluation of the effect of the campaign on the target, a missed opportunity to base campaigns on behavioural change theory and absence of cost effectiveness data.

1.4.5 Public Health campaigns moving forward.

Whilst Public Health campaigns do suffer from several issues, it is agreed that they are still a highly effective method to influence public behaviour. In knowing the issues suffered by Public Health campaigns, analysis of the literature has provided suggestions from researchers that might improve the success of Public Health Campaign outcomes (Table 1-10).

These suggestions include targeting the most relevant communities such as young women or those with lower levels of education, using patient-based interventions, conducting proper evaluation, promotion individual responsibility, using natural aversion to antibiotics because of side-effects and appealing to trusted authority.

Table 1-10. Suggestions to improve public health campaigns through general adaptations or specific messages. Messages pulled from public perception papers that mention Public Health campaigns.

Message	Author
Campaigns need to be better targeted (younger women or those with lower levels of education) and consultation behaviour/patients' expectations for antibiotics needs to be modified, perhaps through delaying antibiotic prescriptions or shortening the course prescribed.	(McNulty *et al*., 2007)
There is an urgent need for patient-based interventions, especially in the U.K. Better information regarding what antibiotic resistance is and what it does could go some way to reduce public uncertainty. A particularly focused message that any future intervention needs to explain why antibiotic resistance develops and include a dimension of causality/responsibility of individuals.	(Brooks *et al.*, 2008)
Evaluation is key. Examples of evaluations going forward could include but are not limited to; negative health effects and complication rates, unintended consequences of campaigns, confounding factors like seasonal variation, success rates of different campaign methods in changing public behaviour and how cost-effective each campaign method is when considering the cost of reducing spread of antibiotic resistance	(Huttner *et al.*, 2010)
The message that misuse of antibiotics promotes resistance may reinforce the idea that antibiotic resistance is an individual, rather than a community, problem. The authors speculate that connecting the individual to the social benefit, explaining the spread of resistance, may allow for a positive message that you can share to affect change.	(Brookes-Howell *et al*., 2012)
Focusing on people's concerns of and aversion to antibiotics by encouraging for example, natural remedies, may aid patient education and health promotion. They finish with the speculation that campaigns that stress and build on traditional ways of avoiding infection, building up resistance and treating minor infections may be more successful than those that attempt directly to limit antibiotic use.	(Norris *et al.*, 2013)
Media campaigns can work but need to be better targeted and then called upon the help of the 'trusted and influential authorities', like doctors and pharmacies as they have a key role to play in changing views a behaviour	(European Commission., 2013, 2016).

1.5 Public Engagement and Citizen Science

In the previous section, 1.4, I discussed how the movement of public understanding of science aims to increase the public's knowledge of science through the provision of facts, for example through the use of Public Health campaigns. I highlighted how these campaigns are not flawless in their approach. A different movement, one that is becoming increasingly popular, is that of public engagement with science and technology. Public engagement aims, instead of providing facts, to engage people with science through discovery led research. In the following section I define a type of public engagement, citizen science before discussing the extent to which citizen science projects have been successful

1.5.1 Defining Citizen Science

The term 'Citizen Science' was coined in the 1990s in both the U.S.A (Bonney., 1991) and the U.K (Irwin., 1995) separately.

Although the term was coined in the 1990s, citizen science projects and citizen scientists have been around for centuries. If you consider a citizen scientist to be a non-scientist making a major scientific discovery, you might argue the American Colonialists who recorded the weather in the 1600's were citizen scientists. If you consider citizen science projects to be projects when a non-scientist collaborates with a scientist, then you might suggest the first was The North American Bird Phenology Program that started in 1881 (Patuxent Wildlife Research Center., 1970).

Modern definitions of citizen science are constantly evolving, as is this field of research. At the start of this PhD project, the most up to date definitions considered citizen science to be:

- a project that includes the involvement of volunteers in science (Dickinson, Zuckerberg and Bonter, 2010),
- the engagement of volunteers and scientists in collaborative research to generate new science-based knowledge (Phillips *et al.*, 2014)
- science where the public participate in the scientific process that allows anyone to be involved in discoveries and simultaneously learn how science works (Dunn et al., 2016).

As this project progressed, so has the definition of citizen science. It progressed beyond being work where amateurs participate in research and moved towards projects that have defined methodologies. This was largely in response to criticisms that citizen science projects deliver poor science with great communication potential (Heigl & Dörler., 2017). The most recent and comprehensive attempt to define citizen science considers it to be:

> *Public participation and collaboration in scientific research with the aim to increase scientific knowledge (Robinson et al., 2018).*

As well as this flexible concept, Robinson *et al* (2018) also produced ten key principles that underpin good practice in citizen science:

1. Citizen science projects actively involve citizens in scientific endeavour that generates new knowledge or understanding.
2. Citizen science projects have a genuine science outcome.
3. Citizen science provides benefits to both science and society.
4. Citizen scientists may participate in various stages of the scientific process.
5. Citizen scientists receive feedback from the project.
6. Citizen science, as with all forms of scientific inquiry, has limitations and biases that should be considered and controlled for.
7. Where possible and suitable, project data and meta-data from citizen science projects are made publicly available and results are published in an open access format.
8. Citizen scientists are suitably acknowledged for their efforts in the projects.
9. Citizen science programs offer a range of benefits and outcomes which should be acknowledged and considered in project evaluation.
10. The leaders of citizen science projects take into consideration legal and ethical considerations of the project.

These most recent criteria are perceived as the current standard to which all citizen science projects can be evaluated (Heigl *et al.*, 2018). In order to understand how citizen science was being used at the conception of this project, I present a cross section of citizen science literature, first across all science disciplines, and then another specifically in the field of Microbiology.

1.5.2 Citizen Science and the focus on scientific output

Kullenberg & Kasperowski (2016) published a meta-analysis looking at which scientific fields used citizen science. They also examined to what extent citizen scientists were involved. Firstly, the analysis highlighted that citizen science has gained a substantial presence in the scientific literature since 2006. In quantitative terms, the largest scientific output was found in the fields of ornithology, astronomy, meteorology and microbiology. Out of 490 projects, only 78 had a scientific output in terms of publications that represent new scientific knowledge. I note in the 1.1.1, critics of citizen science perceived it as poor science with great communication potential (Heigl & Dörler., 2017). Further, of those studies which did produce scientific output, participant outcomes were rarely considered.

In 2016, as part of my initial literature review, I utilised the search string presented by Kullenberg & Kasperowski (2016) to identify citizen science studies with scientific output that had been published in the year since the meta-analysis was conducted. This search string highlighted 19 PubMed publications that contained the term “citizen science” in the title or abstract and had a scientific output. The aim was to determine the extent to which citizen science projects lacked scientific output.

From the 19 research papers I examined, two showed participant outcomes whilst not necessarily increasing scientific knowledge. Caruso *et al* (2016) showed that the introduction of a citizen-science project improved student performance of non-science majors at Florida Atlantic University. Kridelbaugh

(2016) based a course on projects on the SciStarter website (http://scistarter.com/), and the non-science major students attained a 93% average on the report. As shown in the Caruso *et al* (2016) study, the student participants benefitted from taking part in a citizen project, however there was limited scientific output (Kridelbaugh, 2016). Of the remaining 17 papers, 16 had strong scientific outputs but seemed to have little in the way of participant outcomes.

Three papers, notably all focused on marine science, provided and/or evaluated new tools by which citizen scientists could expand the amount of data collected. Raoult *et a*l (2016) suggested the use of GoPros™, video capture devices, as a citizen science mechanism to increase research output for motion surveying of desired marine locations. Bardaji *et al* (2016) suggested a $100 Do-It-Yourself machine that has been compared to commercial instruments in its ability to assess the environmental status of water bodies through the transparency of the water. Parkinson *et al* (2016) offered a simple and inexpensive method for allowing citizens to determine coral symbiont quality by observing the colour of the host.

Thirteen papers used databases or large numbers of participants to collect or classify data that would help assist scientific output. Schultz *et al* (2016) verified researcher detected patterns of echinoderm abundance using the Reef Environmental Education Foundation (REEF) citizen science database (REEF, 2011). Lintott *et a*l (2016) used data from the National Bat Monitoring Programme, a long-running citizen science scheme, to assess how two cryptic European bat species respond to the urban landscape. Deguines (2016) developed a Photographic Survey of Flower Visitors, now Spipoll, that gave a new dataset containing 7167 insects sampled on 1606 plants across France over 3 years. Other citizen science projects have used existing data sets but mined them or evaluated them to address new research questions to generate new scientific knowledge or understanding .Cleary *et a*l (2016) explored bird baths and their effect on south-east-Australian bird assemblages. They did this by analysing 992 citizen science datasets, collected online at the Atlas of Living Australia (ALA) website (ala.org.au). Vallejos et al (2016) used a citizen science programme to look for taxonomic homogenisation in terrestrial birds in the south Brazilian Atlantic Forest along a human-altered landscape (HAL) gradient. Mair & Ruete (2016) examined the effect of urbanisation, using data based on voluntary observations made in Sweden between 2000 and 2014 recorded on Swedish Lifewatch (www.svenskalifewatch.se). Johnson (2016) used data from Project BudBurst, a national citizen-science project that tracks bloom times and other phenological data for plants across the country, to measure the effects of climate change. Gosling *et al* (2016) used citizen scientists to quantify tar spot symptoms on sycamore across England. Roy *et al* (2016) used 13,000 school children (7-11 years old) to sample 26,868 bumblebees providing evidence that local proximity to flowers, being within five metres of a focal plant, had a significant effect on bumblebee abundance as part of the Big Bumblebee Discovery. Miyazaki *et a*l (2016) used web data mining of Twitter photographs to detail the first apparent illegal introduction of *Lepomismacrochirus macrochirus* in Japan since the Invasive Alien Species Act (IASA) was adopted. Daume & Galaz (2016) used data mining on social media to capture important environmental information through opportunistic

biodiversity observations. Kong *et al* (2016) developed a smartphone app, MyShake, to accurately record magnitude 5 earthquakes, to establish the location and magnitude of an earthquake, before sending real time information to issue an alert of forthcoming ground shaking. Anderson-Lee *et al* (2016), in the first paper based on dominant writing contribution and co-lead authorship by non-expert citizen scientists recruited through a video game, used tens of thousands of human participants and three automated algorithms in the Eterna massive open library to present several secondary structure elements and structural idiosyncrasies that lead to difficult RNA design problems.

Only in one instance did a citizen science project provide both scientific and participant outcomes. Cardamone *et al* (2016) used students to analyse data from the Dawn Mission as part of the project Asteroid Mappers, as well as examine interaction between human and natural systems by monitoring the health of a local river, as part of Charles River Watershed Association (CRWA). Student reflection showed engagement improved and students felt they were more capable of finding a job as a result of the course.

1.5.3 Citizen Science and Microbiology and the focus on scientific output

I examined citizen science in the field of microbiology separately as it was specifically relevant to this PhD. I undertook a systematic review based on the search terms presented by Kullenberg and Kasperowski (2016). The authors search terms were modified to identify only citizen science projects emerging from the field of Microbiology in 2016 (Appendix A-1). Five papers were identified as relevant.

Of the five papers that were identified, four used citizen scientists to collect or classify data. Fang *et al* (2016) had undergraduate biology majors plate bacteria from facial skin swabs in a Microbiology Laboratory, specifically to select for *Staphylococcus* and test for antibiotic resistance. The students checked these results against their own, and 9,000 other students' demographic and lifestyle variables. Agate *et al* (2016) had college students collaborate with secondary school students to investigate bacterial species in the local watershed, specifically the prevalence of bacteria that produce violacein, a potential treatment for the chytrid fungus that is causing rapid amphibian decline. Dunn *et al* (2016) had middle and high school students culture environmental microorganisms to highlight the differing microbial diversity of plant root soil compared to adjacent bulk soil to highlight the positive role of microorganisms. Schnetzer *et a*l (2016) used willing members of the public, citizen scientists, to enrich data collected by international scientists with regards to microbial diversity on a single Ocean Sampling Day. These four studies suggest that citizen science was used as a method of producing scientific output, with little regard for the participant outcomes. This aligns with the conclusion of Kullenberg and Kasperowski (2016), that the focal point for volunteer contributions consists of participation in observations, classification and collection of data, which in turn are used by scientists

The remaining study also produced strong scientific output, but went further to survey the participants to understand how taking part in the project had

affected them. Seifert *et al* (2016) had university students and high school students collaborate to promote Lyme disease prevention and to cultivate an interest in science through a citizen-science project. Over 2,000 students across 9 different educational facilities collected a total of 170 ticks from ~40 different locations, from which a novel strain of *Borrelia burgdorferi* has been cultured. A survey of the participants after the activity showed increased confidence in tick differentiation, and Lyme disease symptom recognition as well as a greater interest in pursuing a degree in science.

Having examined a cross-section of citizen science publications in 2016, both in science generally and in the field of microbiology, the criticism that those citizen science projects that do produce scientific output use citizen scientists for data collection and classification or observations, both in general fields of science and microbiology seems founded. However, whilst in the minority, two studies did produce novel scientific output and reveal participant outcomes. These criticisms were taken on board and provided a basis on which to develop a citizen science study which achieved scientific and participant outcomes.

1.5.4 Levels of Engagement within Citizen Science.

In the previous sections, I have explored citizen science projects through the lens of scientific output, that is by examining studies published in scientific journals. In the following section, I examine citizen science through the lens of participant outcomes. Bonney *et al* (2009) identified projects as either “top-down” or “bottom-up”. Most citizen science projects fit the top-down model. This is where participants primarily collect and submit data under the guidance of a scientific organization. The bottom-up model is where participants notice issues they wish to resolve and work together with scientists to do so. Within both top-down and bottom-up projects, Bonney *et a*l (2009) identified three models of citizen science that focus on the degree to which participants are included in various aspects of scientific investigation.

The top-down projects that focus on collection or classification of data were labelled “Contributory”. This is where a participant collects and analyses samples. Collaborative projects build on this, having participants develop explanations, design data collection methods and analyse data. Finally, Co-created projects do the same, whilst also having the citizen scientist define a question or issue, gather information about said issue before interpreting data and concluding, disseminating these conclusions and discussing their results or inquiring further.

To highlight the rarity of co-created citizen science projects, Follett & Strezov 2015) identified only four of 888 articles in which the projects were initiated and driven by the public. I note that the way that scientific research is funded makes co-created projects difficult to enact, as often funding has been granted for a specific area of research before the data collection method, in which one may decide to include citizen scientists, has been considered. In this final level of engagement, the citizens are effectively involved in the entire scientific method from synthesis of a project to measuring results and coming to an evidence-

based conclusion. This is the aim of all citizen science projects but is understandably difficult to achieve.

1.5.5 Citizen Science Outcomes

It is not only the scientist and the participant who benefit from citizen science. Shirk *et al* (2012) suggested that outside of research outcomes (scientific findings) and participant outcomes (acquiring new skills or knowledge), there are social-ecological system outcomes. Participation in collaborative and community-based monitoring has resulted in community-level outcomes, such as increased functioning of social groups through interpersonal relationships (Adger and Anger, 2003), increased ability for a given community to leverage resources to solve collective problems (Donoghue and Sturtevant, 2007), and trust between scientists, managers, and the public (Fernandez-Gimenez *et al.*, 2008). A successful citizen science project can inform ongoing exercises in setting goals and objectives for future research projects, policy shift and funding decisions (Phillips *et al.*, 2014). Perez *et al* (2016) suggested that involvement in citizen science may contribute to the natural aggregation of social norms that influence trends in political positions, opinions, cultural traits and scientific progress.

Many of the citizen science projects that achieve this outcome revolve around the monitoring of a specific data point, often as part of a national or global monitoring network. For example, Danielsen *et al* (2007) reported on experienced hunters and anglers living near protected areas in the Philippines who monitor and react to changes in resource use related to wildlife populations. Separately, the Jamaican Water Resource Authority required data on water levels from remote sites and so trained volunteers to read river gauges at assigned locations, gathering data needed to implement protective measures before floods (IFRC, 2000). The Famine Early Warnings Systems Network enlisted local monitors to report data such as rainfall and staple food prices around the world for use in ensuring food security (Barbara, 2011).The sea turtle monitoring network Grupo Tortuguero supports a body of hypothesis-driven scientific work. The consequent collaboration between biologists, agencies, and communities helped to establish marine protected areas and sustainable fisheries practices that are sensitive to the wellbeing of both turtle populations and local livelihoods (Delgado and Nichols, 2005).

Whilst difficult to achieve, a possible and perhaps desirable outcome for a citizen science project would be to provide scientific findings, influence an individual's skills and knowledge, as well as to effect change in a broader context, within a community or through policy changes. However, in order to recognise the outcomes of a project, a project must be carefully evaluated.

1.5.6 Evaluating Citizen Science Projects.

In the previous section, I discussed the potential of citizen science to contribute to change in science and society. The power of citizen science to achieve these outcomes has been recognised and as a result, new funding schemes have appeared (Kieslinger *et al.*, 2018). In order to continue convincing funding bodies of the power of citizen science, evaluation is important to fill the gaps

in understanding of the effectiveness of citizen science. As of 2014, Phillips *et a*l (2014) highlighted the less than rigorous evaluations or neglected evaluation altogether of the majority of citizen-science projects. The success and increased funding for citizen science calls for context-adaptable evaluation criteria that assess the impact of citizen science programmes on science, society and policy (Kieslinger *et al.*, 2018).

Shirk *et al* (2012) suggested that there are three dimensions by which citizen science projects can be evaluated. These are project scientific impact (research outcomes), learning and empowerment of participants (participant outcomes) and impacts for wider society. research outcomes (social-ecological system outcomes). Evaluation must include outcome-based evaluation and process-based evaluation, assessing the overall goals of activities or programmes and the benefits to participants and recipients of the results as well as the operational strengths and weakness of activities or programmes, respectively (Kieslinger *et al.*, 2018).

An open framework for citizen science evaluation, based on the Ten Principles of Citizen Science (1.5.1)(Robinson *et al.*, 2018), was put forward by Kieslinger *et al* (2018) and is shown in Table 1-11.

Table 1-11. Citizen Science Evaluation Framework. Framework evaluating three core dimensions: 1) Scientific dimension, 2) Participant dimension and 3) socio-ecological dimension. For each dimension, criteria are proposed at the "process and feasibility" level, and the "outcome and impact" level. Taken from (Kieslinger *et al.*, 2018).

Dimension	Process and Feasibility	Outcome and Impact
Scientific	• Scientific objectives • Data and systems • Evaluation and adaptation • Collaboration and synergies	• Scientific knowledge and publications • New research fields and structures • New knowledge resources
Participant	• Target group alignment • Degree of involvement • Facilitation and communication	• Knowledge and science literacy • Behaviour and ownership • Motivation and engagement
Socio-ecological and economic	• Target group alignment • Active involvement • Collaboration and synergies	• Societal impact • Ecological impact • Wider innovation potential

Kieslinger *et a*l (2018) noted that as the project lifecycle advances, the emphasis of evaluation would gradually shift from process and feasibility to outcome and impact. As this is the most comprehensive evaluation framework for citizen science projects to date, and it emerged towards the end of my project, I use this framework to assess the extent to which this PhD project was a successful citizen science project (6.1.4).

Whilst this framework is the most comprehensive to date, it had not been developed at the conception of this project when it was necessary to prepare the groundwork for upcoming activities by engaging with concepts, methodologies and adaptive planning. Instead, I developed a logic model that depicted the relationship between this studies inputs and activities and their intended effects (Appendix B-1). I also mapped my expected outcomes for the citizen scientist to the framework for evaluating citizen science learning outcomes (Phillips *et al.*, 2014). This framework focused only on the participant outcomes, breaking these into six categories:

1. Behaviour & Stewardship
2. Skills of Science Inquiry
3. Knowledge of the Nature of Science
4. Interest in Science & the Environment
5. Self-efficacy
6. Motivation

Whilst I do not use these outcomes to evaluate my project in Chapter 6, this framework was used for project development and was a useful tool for preparing the groundwork for the participant learning outcomes in this citizen science project.

In summary, citizen science can be defined as public participation and collaboration in scientific research with the aim to increase scientific knowledge. Citizen science often lacks scientific outcomes. Of those projects that do achieve scientific outcomes, they often neglect participant outcomes scientific power and are used in the most part as a methodology for collecting and classifying data produced by members of the public (Kullenberg and Kasperowski, 2016). If the focus is changed to improve knowledge and skills amongst citizen scientists, often the research outcomes suffer. Despite the success of projects in encouraging interested participants to take part, there is room for improvement. This improvement focuses on, among others, concerns of data quality and ensuring a link between scientific objectives and social outcomes (Bonney et al., 2014). In order to tackle the need for better evaluation, linking three dimensions of outcomes, an open framework has been created to help evaluate the success of citizen science projects (Kieslinger *et al.*, 2018). I use both this framework and the ten principles underlying good citizen science practice (Robinson *et al.*, 2018) to assess the success of this study as a citizen science project in Chapter 6.

1.6 Antibiotics Unearthed

Antibiotic discovery revolutionised modern medicine, however each discovery of a novel antibiotic was quickly followed by resultant antibiotic resistance in bacteria. Failures to develop long term strategies to tackle this has led to a global health crisis. Ten commandments were developed to highlight areas in which we could be doing better to tackle antibiotic resistance (O'Neill, 2016b). Two key methods of tackling antibiotic resistance are reinvigorating the drug discovery pipeline and preserving the effectiveness of current antibiotics. A key part of both, and the first commandment, is a massive global public awareness campaign. There are gaps in the public awareness of antibiotic

resistance. This awareness, or lack of, as reported in scientific literature is well explained through six key themes (1.3). In understanding these themes, recommendations can be made for Public Health campaigns and Citizen Science projects which aim to raise awareness. Public health campaigns are a useful tool for increasing public awareness, however, suffer issues in terms of engaging hard-to-reach individuals, often whom are most in need of being reached. Public engagement through citizen science projects is another way of attempting to engage members of the public in order to raise awareness. Citizen science projects also suffer issues, however strong frameworks for evaluation can go some way to reducing these by presenting a case for the power of citizen science for scientific research, participant learning and societal outcomes. In the next section, I present the Small World Initiative, and examine how this PhD project has adapted it to form a comprehensive citizen science project focused on reinvigorating the drug discovery pipeline and preserving the effectiveness of current antibiotics.

1.6.1 Small World Initiative, Yale.

The Small World Initiative^{TM} was a Yale University project developed by Dr. Jo Handelsman to address the global health threat of antibiotic resistance. The initiative identified two problems, a growing economic need for more Science, Technology, Engineering and Mathematics (STEM) graduates and the issue of AMR. The Small World Initiative programme is fundamentally a biology course that provides original research opportunities for its University and College students with the aim of collecting soil samples, isolating diverse bacteria and testing these for antibiotic or antifungal production against clinically relevant microorganisms. The Small World Initiative has currently trained instructors at more than 330 schools across 45 U.S.A States and 15 countries. Over 10,000 students have taken or are taking the Small World Initiative's introductory biology course (Small World Initiative., 2019).

1.6.2 Antibiotics Unearthed and the Microbiology Society.

In 2015 the Microbiology Society worked with the Small World Initiative to implement a spin off programme, Antibiotics Unearthed. The aim was to engage schools, undergraduates and the public in the U.K and Ireland in a search for novel antibiotics in collaboration with scientists. Like the Small World Initiative's biology course, schools and universities could apply for support from the Microbiology Society to run the Antibiotics Unearthed programme. Students would then learn how to look for new antimicrobial compounds in soil samples through a series of laboratory sessions. The aims of the Microbiology Society aligned strongly with those outlined at Yale (Microbiology Society, 2019a). To help the school or university partnership, the Microbiology Society provided full training for teachers and technicians, protocols for the research and all consumables that the institution would need for the experiments. Results were presented at the Microbiology Society's Annual Conference. In the case that an interesting and perhaps novel antimicrobial was discovered, staff and students would be encouraged to carry out more detailed analysis on the compounds. This initiative proved popular

(Microbiology Society, 2017a, 2017b) and was part of undergraduate courses at five universities and courses at seven schools.

1.6.3 Contextualising this PhD: Antibiotics Unearthed

This PhD was a match-funded project between the Microbiology Society and the University of East Anglia, using the Antibiotics Unearthed methodology of collecting soil samples to identify novel antimicrobial agents. A key feature of the project was the design of a citizen science protocol. This protocol included pop up stands, interviews, social media interaction and portfolios all with members of the public (2.6 and 2.7). These tools were designed to engage the public with the issues of antibiotic discovery and antibiotic resistance, whilst tracking how this engagement facilitated a participants exploration of these two topics, in the hope of ultimately leading to behaviours that help preserve the effectiveness of current antibiotics.

This chapter has presented the history of antibiotics, the emergence of resistance to these antibiotics, the problems antibiotic resistance presents and the potential solutions to this problem. It has also examined the current literature on public perception of antibiotics and antibiotic resistance, as well as the history of public understanding and public engagement. It defined citizen science and highlighted what it takes to produce a successful citizen science project. It finally discussed how this PhD project was conceived. The next chapters discuss the methods used to undertake this interdisciplinary project and the results that came from both the pursuit of novel antimicrobials and the pursuit of public perception data over short, medium and long terms. It draws conclusions from these results to determine the extent to which it was successful at reinvigorating the drug discovery pipeline and preserving the effectiveness of current antibiotics. Finally, it provides suggestions on how future citizen science projects could use this project as a toolkit to prepare and undertake a successful citizen science project (Table 1-12).

Table 1-12. Outline of the thesis chapter numbers, chapter titles and an insight into the chapter content.

Chapter No.	Chapter Title	Chapter Content
Chapter 1	Introduction	Literature Review of antibiotics and antibiotic resistance. Public perception of antibiotic resistance. Review of public health campaigns. The history and state of citizen science projects in general, and in the field of microbiology. The role of the Antibiotics Unearthed Project.
Chapter 2	Materials and Methods	Methods used for the microbiology research. Methodological underpinnings of the social science research and the methods used.
Chapter 3	The microbial composition and antimicrobial activity of citizen science soil samples	Data collection and analysis, followed by results of isolating antibiotic producing bacteria from soil sample, screening them against medically relevant pathogens, whole genome sequencing a select few colonies and identifying the nearest relatives and biosynthetic loci coding for antibiotic and secondary metabolite production.
Chapter 4	Public understandings of and attitudes to antibiotics and antibiotic resistance: Findings from a citizen science project	Design of data collection and analysis including pop up stands and interviews. Descriptive and thematic analysis of interview data using pivotal codes based on key themes extracted from public perception literature.
Chapter 5	Medium- and long-term study of public understandings of and attitudes to antibiotics and antibiotic resistance: Findings from a citizen science project	Design of data collection and analysis of social media interaction and portfolio entries. Descriptive analysis of social media data. Thematic analysis of portfolio data providing evidence of participant learning.
Chapter 6	Conclusion	Discussed the research questions in the light of methodological reflection. Reflected on strengths and limitations and provided concluding remarks.
Chapter 7	References	References to literature discussed throughout the PhD.
Chapter 8	Appendices	Supplementary material.

Chapter 2 Materials and Methods

Part One: Natural Sciences Materials and Methods

2.1 Materials

Chemicals and reagents used are laboratory standard grade or above, purchased from Fisher Scientific (U.K) or Sigma Aldrich (U.K) unless stated otherwise. Distilled water (dH_2O) was used in all media and solutions.

2.2 Bacterial strains

All strains used in this study are listed in Table 2-1.

2.3 Strain isolation and culture conditions

2.3.1 Media

Media compositions are shown in Appendix C-1. When required, media were supplemented with appropriate antifungals (Appendix D-1) at the following concentrations: cycloheximide (100 µg/mL) and nystatin (20 µg/mL) unless otherwise stated. All media were autoclaved at 125°C for 30 minutes at 15 p.s.i unless otherwise stated.

2.3.2 Soil sample collection

Soil samples were collected from topsoil by members of the public from five locations around the U.K: Garwnant Forest, Glasgow Botanical Gardens, Kielder Forest, Thetford Forest and the University of East Anglia, Norwich. They used faecal sample collection tubes which contained a spoon to collect between 2-10 g of soil.

Table 2-1. Strains used throughout this study.

Strain	Description	Reference
Indicator Strains		
168	*Bacillus subtilis*	(Anagnostopoulos and Spizizen, 1961)
CA6	*Candida albicans*	(Marconi *et al.*, 1976)
Kp2	*Klebsiella pneumoniae*	(Clarke, Yousafzai and Eady, 1999)
	Methicillin-resistant *Staphylococcus aureus*	(Y. Qin *et al.*, 2017)
DT104	*Salmonella enterica* serovar Typhimurium	(Poppe *et al.*, 1998)
SL1344	*Salmonella enterica* serovar Typhimurium 4/74, *hisG*, *rpsL*	(Hoiseth and Stocker, 1981; McClelland *et al.*, 2001)
ATCC 14990	*Staphylococcus epidermidis*	(Dale *et al.*, 1995)
B16.06226	Vancomycin-resistant *Enterococcus faecium*	(Y. Qin *et al.*, 2017)
Environmental isolates		
ELD01	*Bacillus subtilis*	This study
ELD02	*Bacillus altitudinis*	This study
ELD03	*Bacillus velezensis*	This study
ELD04	*Bacillus altitudinis*	This study
ELD05	*Paenibacillus peoriae*	This study
ELD06	*Sporosarcina aquamarina*	This study
ELD07	*Bacillus subtilis*	This study
ELD08	*Paenibacillus peoriae*	This study
ELD09	*Bacillus subtilis*	This study
ELD10	*Bacillus simplex*	This study
ELD11	*Bacillus subtilis*	This study
ELD12	*Pseudomonas vranovensis*	This study
ELD13	*Bacillus psychrodurans*	This study
ELD14	*Bacillus altitudinis*	This study
ELD15	*Pseudomonas vranovensis*	This study

2.3.3 Overnight culture of environmental strains

2.3.3.1 In-situ protocol

One gram of soil was mixed with 10 mL sterilised tap water *in situ* and shaken by hand. Using a Pasteur pipette, five drops (~50 µL) of soil solution were transferred on to an LB Agar plate containing antifungals for spread plating. The plate was then packaged and transported to the University of East Anglia and incubated at 30°C for up to 48 hours and stored at 4°C. Sterilised tap water was plated to rule out potential sources of contamination. After incubation, digital photographs of the isolates were taken using a Panasonic DCM-FZ18 LUMIX camera inside a home-made black box.

2.3.3.2 Laboratory protocol

One gram of soil was resuspended in 10 mL dH_2O, homogenised on a vortex at 13000 rpm for 60 seconds and diluted 1:10; 100 µL soil solution and 900 µL dH_2O. 100 µL of this 10^{-1} solution was spread plated on to Brain Heart Infusion (BHI) Agar and All Culture (AC) Agar containing antifungals. Plates were incubated at 30°C for up to 48 hours and stored at 4°C. Photos were taken as stated in 2.3.3.1.

2.3.4 Isolating potential antibiotic-producing colonies

Single colonies that appeared to be inhibiting the growth of neighbouring colonies were picked from the LB, BHI or AC agar plates and subcultured to obtain pure cultures (isolates). Isolates were maintained on the media they were originally picked from, but no antifungals were added to the subcultured plates. Isolates were incubated at 30°C and stored at 4°C. Photos were taken as stated in 2.3.3.1.

2.3.5 Long-term strain stocks

Glycerol stocks produced by adding 1 mL of fresh overnight culture grown in their respective media to 900 µL of 40% (v/v) glycerol in a 2 mL cryogen tube, inverted to mix and stored at -80°C. Microbank™ bead stocks were produced by following manufacturer's instructions (Prolab Diagnostics), stored at -80°C

2.4 Inhibition of medically relevant laboratory isolates

2.4.1.1 Overnight cultures

Indicator strains were aseptically streaked on to LB agar plates from glycerol stocks or Microbank™ bead stocks and incubated overnight at 30°C and stored at 4°C. 10 mL LB cultures inoculated with a single colony were cultured overnight (~15 hrs) at 30°C, 200 rpm. 200 µL cultures of environmental strains, inoculated with a single colony, were grown overnight (~15 hrs) in a 96 well plate using the media they were isolated from. 96-well plates were left stationary overnight at 30°C. All work was completed in a category 2 microbial safety hood.

2.4.2 Drop plate assay

After overnight growth in a respective broth, indicator strains were spread as a lawn using sterile cotton swabs and left to dry on 4% agar plates. Isolates of interest were added on top of this dry lawn using an 8-tip multi-pipette with 6 pipette tips attached. 5 µL samples were taken from the 96-well plates and dispensed onto the indicator strains in a 6 x 4 grid. Plates were left to dry and incubated at 30ºC overnight (Figure 2-1). Photos were taken as stated in 2.3.3.1. All work was completed in a category 2 microbial safety hood.

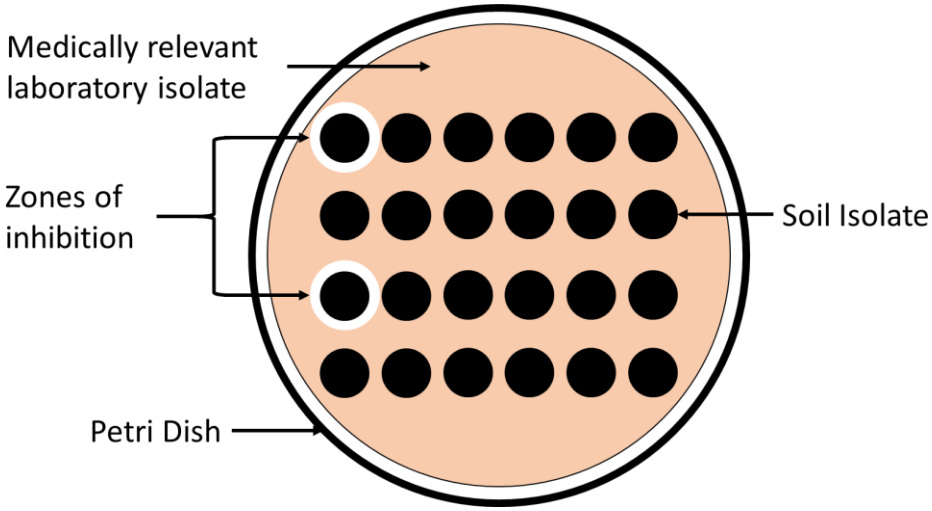


Figure 2-1. Drop plate assay to test inhibition of medically relevant laboratory isolates. Indicator strains grown overnight in liquid broth were spread as a lawn using sterile cotton swabs and left to dry on 4% agar plates. Isolates of interest were added on top of this dry lawn using an 8-tip multi-pipette with 6 pipette tips attached. 5 µL samples were taken from 96-well plates and dispensed onto the indicator strains in a 6 x 4 grid. Plates were left to dry and incubated at 30^0C overnight. Zones of inhibition left a clear circle around the environmental isolate where no indicator strain could grow.

2.5 Genome sequencing of inhibitory isolates

2.5.1 Samples and DNA extraction

To rationalise which inhibitory isolates were selected for genome sequencing, soil samples or forest locations with multiple inhibitory isolates had to be morphologically distinct from each other and have a unique inhibitory profile to be selected. From this, 15 distinct isolates were grown overnight in 5 mL broth and harvested using centrifugation. Pellets were prepared and snap frozen using liquid nitrogen. Genomic DNA (gDNA) was extracted using QIagen ATL lysis buffer plus DTT. After isolation, liquid nitrogen was used to flash freeze the gDNA which was then transported on dry ice.

2.5.2 MiSeq Genome Sequencing

The DNA Libraries for all samples (*n* = 15) were prepared using the Nextera® XT (Illumina, Inc.) sample preparation, and the 600-cycle v3 reagent kit by myself and Dr. Hurst. After DNA libraries were prepared, samples were sequenced using paired 300 bp reads by the group of Prof. Cooper, School of Medicine, University of East Anglia.

2.5.3 Genome Sequencing data analysis

The Cooper Lab uploaded all sequence data to BaceSpace™, Illumina's integrated sequencing hub, and produced coverage plots and average quality scores. They annotated and assembled genomes with the Prokka annotation pipeline V1.12 (Seemann, 2014) and provided FastQ files, in a text-based format with quality scores. Reads of inserts had been filtered by quality and read length by the Cooper Lab. I conducted 16S rRNA analysis using applications within the BaseSpace™ suite, namely MetaPhlAn (Segata *et al.*,

2012), 16S Metagenomics (Wang *et al.*, 2007) and Kraken Metagenomics (Wood and Salzberg, 2014).

2.5.4 Phylogenetic analysis

The 16S rRNA sequences of isolates were retrieved from their genomes and compared with sequences of 16S rRNA gene sequences using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST), specifically BLASTN (Altschul *et al.*, 1990). Type strains which had a similarity of higher than 97% with each isolate were used for sequence analysis. *Stenotrophomonas maltophilia* (DSM 20030^T) was used as the Gram-positive outgroup, while *Acinetobacter baumannii* (DSM 30007^T) was used as the Gram-negative outgroup.

The trees of the 16S rRNA gene were constructed. Phylogenetic analysis was conducted using the MEGA software package version 7.0 (Kumar, Stecher and Tamura, 2016) after alignment of the data via Muscle (Edgar, 2004). Phylogenetic trees were constructed by neighbour-joining (NJ) (Saitou and Nei, 1987) algorithms and the topology of the NJ tree was evaluated using bootstrap analysis on the basis of 500 replications (Felsenstein, 1985).

2.5.4.1 Detecting secondary metabolite biosynthetic gene clusters

FASTA files were uploaded to the antiSMASH 3.0 (Weber *et al.*, 2015) web server. Gene prediction was performed by Glimmer3 (Delcher *et al.*, 2007). A search was conducted using 'KnownClusterBlast', 'SmCOG analysis', 'ActiveSite finder' and 'SubClusterBlast' to identify the secondary metabolite biosynthetic gene clusters (BGCs). Probabilistic detection was turned off.

Part Two: Ontological and epistemological assumptions of this study

Ontological assumptions give rise to epistemological assumptions; these, in turn, give rise to methodological considerations; and these, in turn, give rise to issues of instrumentation and data collection. These assumptions, both implicitly and explicitly, underpin the way one looks at social reality (Cohen, Manion and Morrison, 2007). This section explores briefly each of these sets of assumptions underpinning social science at large, and this study specifically.

Ontological assumptions concern the very nature or essence of the social phenomena being investigated. A key question asked at this stage is whether the author sees social reality external to individuals, or as the product of individual consciousness. Reality could be of an objective nature or the result of individual cognition. The phenomena may be a given in the world, or it may be created by an individual's mind. Ontologically, one is either a realist or a nominalist. A realist contends that objects have an independent existence and are not dependent for it on the knower, whilst a nominalist suggests that objects of thought are merely words and that there is no independently accessible thing constituting the meaning of a word (Cohen, Manion and Morrison, 2007).

Epistemological assumptions concern the very bases of knowledge; its nature and forms, how it can be acquired and communicated to other human beings. One's epistemological assumptions affect how they go about producing knowledge. Epistemologically, one can be a positivist, or non-positivist. A positivist holds the view that knowledge is hard, objective and tangible. This mindset demands of researchers an observer role, together with an allegiance to the methods of natural science. A non-positivist sees knowledge as personal and subjective. This imposes on researchers an involvement with their subjects and a distancing from the ways of the natural scientist (Cohen, Manion and Morrison, 2007).

A final set of assumptions pertains specifically to the nature of social science as the study of humans. These assumptions concern human nature, particularly the relationship between human beings and their environment. In social science, human beings are both the subject and object of study. A social scientist can endorse determinist or voluntarist assumptions. Determinism portrays humans as responding mechanically deterministically to their environment; as products of the environment, controlled like puppets. Voluntarism portrays humans as initiators of their own actions with free will and creativity, and co-producers of their own environments (Cohen, Manion and Morrison, 2007).

Methodological decisions are made in the light of ontological and epistemological assumptions. An investigator who takes a positivist (or objectivist) approach to the social world assumes the world of natural phenomena is real and external to the individual, and most often will opt for surveys and experiments. An investigator who takes a non-positivist (or subjectivist) approach to the social world assumes the world to be personal

and humanly created, and most often will opt for personal accounts accessed, for example, via interview and participant observation (Cohen, Manion and Morrison, 2007).

The positivist would employ scientific investigation directed at analysing the relationships and regularities between selected factors in that world. Their investigation will be predominantly quantitative and will be concerned with identifying and defining elements and discovering ways in which their relationships can be expressed. The non-positivist's principal concern is with an understanding of the way in which the individual creates, modifies and interprets the world in which they find themselves. This approach may takes on a qualitative as well as quantitative aspect (Cohen, Manion and Morrison, 2007).

As the investigator of this PhD, I am coming from a scientific paradigm whereby I consider myself a realist and a positivist carrying out research which is quantitative in nature. I am transitioning into a citizen science paradigm, which navigates across the nominalist and the realist paradigms as well as across the non-positivist and positivist paradigms. I note that as human behaviour is one focus of the study, my study also navigates between voluntarism and determinism. This naturally leads to a mixed methods approach, in which one can utilise both quantitative and qualitative methodologies in order to explore the natural phenomena that are the focus of the study (for example, the lab-based analysis of the data collected by the participants) and the social phenomena (for example, the evolution of the participants' discourses on science over the course of their engagement with the study). Citizen science design itself, where a researcher is engaging human participants with scientific research, calls for a mixed methods approach (1.5). Part Three considers the issues of instrumentation and data collection associated with the study of humans interacting with the natural sciences as part of the social science arm of the project.

Part Three: Social Sciences Methods

2.6 Access to participants

I focused in depth on a convenience sample of members of the public selected at pop-up stands in five locations across the U.K (Table 2-2). The five events took place over a 14-month period, beginning 9th September 2016 and ending 19th October 2017, in Wales, England and Scotland. The forest locations were chosen by the Microbiology Society communications team. Convenience sampling was used to identify a relevant population for study. Convenience sampling is a type of nonprobability sampling in which people are sampled simply because they are available and accessible sources of data for researchers (Battaglia, 2008). Convenience sampling is cost effective, simple and can lead to hypothesis generation in a short duration of time. However, like all non-probability sampling, it is prone to volunteer bias and therefore could have a high level of sampling error. Due to the nature of convenience sampling, whereby we are reliant on volunteers, sample size was limited by the factors which influenced the number of volunteers that could be obtained.

Table 2-2. Antibiotic Unearthed Pop-up events. Event locations, visit dates and who the location was managed by (noted as approval was given for us to collect samples and hold the intellectual property of any discoveries).

Event location	Date	Managed by
Brecon Beacons National Park, Coed Taf Fawr forest, Garwnant visitor centre, Merthyr Tydfil. OS grid reference SO003132.	9th September 2016	Forestry Commission Wales.
Kielder Forest, Northumberland. OS grid reference NY665905.	16th September 2016	Forestry Commission England
Thetford Forest, Norfolk. OS TL87168306.	4th May 2017	Forestry Commission England.
Glasgow Botanic Gardens, Glasgow, Kibble Palace.	7th September 2017	The Friends of Glasgow Botanic Gardens.
Norwich Science Festival, The Forum, Norwich. Soil collected from University of East Anglia, Norwich.	19th October 2017	Event sponsored, funded and partnered with multiple organisations. Soil and stand by the University of East Anglia.

Pop-up stands were used and provided a covered area in which Microbiology Society and University of East Anglia logos with the project's name Antibiotics Unearthed were made visible to the passing public. I engaged members of the public using a factual and narrative account of the potential for volunteers to help find novel antibiotics in soil bacteria (storytelling). Storytelling can be used to move audiences to action (Woodside, 2010). Explanations about the project were enticing, designed to pique interest but were kept deliberately vague so as not to provide answers to the questions that were to follow if a participant chose to take part in an interview (2.7.1). Once returning from soil collection, participants were asked by myself whether they would be comfortable

undertaking an interview. Upon agreeing to be interviewed, the participant was given reassurance on confidentiality and their right to withdraw at any time. It was clearly stated that this was an investigation into the participants' perception of the issue of antibiotics and antibiotic resistance. After completing or declining to complete the interview, participants were shown how to process their sample ready to be taken back to the University of East Anglia laboratory for further research (2.3.2).They were then advised that they could follow the progress of their own numbered soil samples on our Facebook page and they were provided with documents providing links to the relevant social media. Finally, participants were asked whether they would be willing to take a portfolio from us and contribute to long-term data collection. If participants agreed to take part in the project, they were given an Antibiotics Unearthed starter pack (Figure 2-2). Consent forms were given at each part of this process.

Figure 2-2. Packs were given to members of the public upon agreement to collect a soil sample. From top left participants were given a booklet explaining Antibiotics Unearthed, a booklet explaining antibiotic resistance, a marvellous microbes comic book, soil sample collection pots, a trowel, antibiotics unearthed wrist bands, antibiotics Unearthed factsheet, a Microbiology Society advertisement and an Antibiotics Unearthed toy.

At the pop-up stands, participants were asked to collect soil samples, undertake interviews, follow our social media presence and compose a portfolio. Participants were not required to undertake all these tasks. A total of 455 soil samples were collected, 32 interviews were recorded, 337 email addresses were taken, and 14 portfolios were handed out (Table 2-3).

Table 2-3. Sampling details for each pop-up event. The number of soil samples obtained, interviews conducted, email address collected for future social media engagement and portfolios handed out at each of the five pop-up event locations.

Event Location	Soil samples obtained	Interviews conducted	Email address collected	Portfolios handed out
Brecon Beacons	155	13	121	2
Kielder Forest,	144	10	96	3
Thetford Forest,	47	6	32	4
Glasgow Botanic Gardens	51	2	46	2
Norwich Science Festival,	57	1	42	3

2.7 Data Collection and Analysis

Phillips *et al* (2014) highlighted common data collection methods with description, strengths and weaknesses. From this, I selected short-, medium- and long-term tools for data collection. Data collection began on the 9th September 2016 and was completed in May 2018.

2.7.1 Interviews – Short Term

I conducted face-to-face, semi-structured interviews with open- and closed-ended questions with members of the public (6 - 37 minutes) at pop-up stands around the U.K. Participants were informed that the purpose of the interview was to understand public perceptions surrounding the topics of antibiotics and antibiotic resistance. Questions were based on exploring key themes emerging from a literature review (1.3). In total, four distinct topics were explored through fifteen questions (Table 2-4). The four topics were soil microbiota's role in medicine, antibiotic resistance, use of antibiotics in a medical setting and antibiotic discovery. Each topic was captured by one closed-ended question, whilst the remaining questions were open-ended. Questions were developed and refined in collaboration with the supervisory team. Questions were submitted to the Research in Mathematics Education (RME) group within the University of East Anglia School of Education for feedback. Feedback was incorporated into the final design of the interview questionnaire. The interview questionnaire was trialled at the University of East Anglia on a volunteer by the researcher with supervision from the supervisory team. Feedback from this process was also incorporated into the design and delivery of the interview. In total 32 interviews were conducted on 40 interviewees. In instances where there was a couple, rather than isolating an individual, questions were put to both. Whilst this deviated from the normal one-to-one nature of an interview, it still provided implicit and explicit data on public perceptions in the topics of interest.

Audiotaped interviews were transcribed using Microsoft Word. These word files were uploaded to NVivo 11, read twice, then discussed with an experienced qualitative researcher (Prof. Nardi). To ensure the anonymity of participants whilst allowing for participant identification, consecutive numbers were assigned to interviews (1-32). Participants are referred to by their interview number. In some interviews, two participants were present, and were so designated A and B. For convention, the first person to have spoken in the interview is 'A' and the second person to have spoken is 'B'. The interviewer, or researcher, is denoted as 'R'.

Coding schedules were agreed and piloted (Table 2-5). These codes represented key topics emerging from the public perception literature (1.3), conceptions of research and researchers (Brew, 2001) and a commognitive framework (Sfard, 2008). Interviews were coded in NVivo 11. One interview was selected at random to be double coded (Prof. Nardi); ambiguities were resolved in discussion. Coded interviews were distilled into factual accounts (Appendix E-1). Codes were reduced to major themes through ongoing discussion between researchers and the re-reading of factual summaries and transcripts.

Table 2-4. Antibiotic Unearthed Interview Questions. Fifteen questions were based on key topics of antibiotics and antibiotic resistance and were refined with the help of the University of East Anglia Research in Mathematics Education group.

Questions asked of respondents
1. Why are we looking for new antibiotics in the soil?
2. Antibiotics are made by bacteria, fungi, humans, plants, viruses?
3. Can you name infections we take antibiotics for?
4. Antibiotics are taken for bacterial, fungal or viral infections?
5. When was the last time we discovered new antibiotics?
6. Have you heard of teixobactin?
7. How many antibiotics have been discovered since 1962? ^
8. What do you know about antibiotic resistance?
9. Which of the following can become resistance to antibiotics, bacteria, fungi, humans, plants, viruses?
10. What's causing antibiotic resistance? *
11. How does antibiotic resistance spread?
12. Biggest problem for humans if antibiotic resistance spreads?
13. Can you make a difference to the spread of antibiotic resistance?
14. How are antibiotics used?
15. Are antibiotics misused?

*Question 10 was added to the original questionnaire for the start of the Thetford Forest event on the 4th May 2017 after looking back at transcripts from the first two events and identifying room for further questioning. Interviewee 24 to interviewee 29 were asked this question, and upon analysis of results the question was discarded for the remaining two events.

^Question 7 was removed midway through the Kielder Forest event on the 16th September for interviewee 17 to interviewee 21. Answers to this question were guesses, and there was a feel that it added confusion to the conversation. It was added back in after it was realised that it could still be used to quantitatively identify optimistic or pessimistic nature among interviewees.

Interviewee 23 was not asked question 6 or question 7 due to the breadth and nature of discussion following Q5. This meant these topics were discussed, but question 6 and 7 were not directly asked as phrased in the table.

Table 2-5. Coding schedule. Code abbreviation and description mapped against number of times code was applied in 32 interviews (references) and number of interviews it was applied in (sources). Codes were based on key topics emerging from public perception of antibiotics and antibiotic resistance literature and a framework discussing conceptions of research (Brew, 2001).

Code	Code description	References	Sources
SKO	Evidence of scientific knowledge	310	32
CON	Level of confidence a participant has in their response	212	30
SWU	Evidence of scientific word use	105	26
TER	Evidence of lay word use	79	24
IRO	Irresponsible others are to blame for spread of AMR	70	26
ABI	Understanding of which illnesses can be treated with antibiotics	61	25
PHN	Evidence of public health narrative rhetoric	55	28
ROD	Beliefs about the role of doctors in the problem of AMR	48	23
ReB	The belief that the human body becomes resistant to antibiotics rather than the infectious body	44	19
UTA	What users take antibiotics for	43	20
ABP	Knowledge surrounding antibiotics being used as prophylactics	35	11
MRB	Knowledge of medically relevant bacteria	29	16
UTO	Utopian attitude towards science	29	16
DRI	Confusion using the terms surrounding AMR and antibiotics	22	16
DYS	Dystopian attitude towards science	20	15
UURE	Knowledge that unnecessary use of antibiotics results in a reduced effectiveness	19	15
BAT	Evidence of avoidance of antibiotics due to belief that our body is a temple and antibiotics disrupt that	19	12
Trading	Scientific research as a means of product generation	19	12
Domino	Research as a physical process of daily tasks, events, things, activities, problems, techniques, experiments, issues, ideas and questions	18	14
CTD	Adherence to the narrative of completing the dose of antibiotics	15	11
Layer	Scientific research as bringing to light ideas, explanations and truths	15	8
SAS	Use of stories to explain science, almost like a mythology	14	10
UVT	Narrative of us versus them, can take several forms	12	8
KID	Narrative of elite actors deliberately keeping the public in the dark about emerging technologies	11	8
PON	The potential of nature to provide humans with medicines	10	7
PB	Science as releasing potential and unforeseen evil, likened to pandora's box	10	6
Ant	Knowledge of antibiotic campaigns	9	7

WMP	A belief that science is gaining wisdom by misusing power	9	5
MN	Ill-considered action will mess with preconceived morals and boundaries set by nature	7	4
BKM	Better objective knowledge about AMR leads to reduced misuse of antibiotics	6	5
RGR	Narrative that emerging technology helps the rich get richer and magnifies injustice and inequality	6	5
Journey	Research as engagement with activities which enable researcher growth or transformation	6	2
DID	Evidence of distinction between the two disciplines involved in this project, science and education	4	3
NAE	Narrative that the participant is not an expert, a qualifier on the potential of being wrong	4	3
SSP	Narrative that science is for smart people	3	3
PMI	Paternal or maternal instincts to protect a child would override the need to protect society	3	3
FUSE	Knowledge that frequent use of antibiotics leads to side effects	3	2
SAE	Narrative that science is evil or presents fake truths	1	1

Answers to the closed-ended questions were extracted and tabulated in Microsoft Excel. The data from the closed-ended questions were quantitatively scored. A yes response was given a score of 1, a no response a score of 2 and a don't know response a score of 3. Responses to closed-ended questions were analysed using GraphPad Prism 7. Because of the strict responses provided to the participants, there was no need to clean the data, or account for any missing data points.

2.7.2 Facebook – Medium Term

I provided links to the Antibiotics Unearthed Facebook page upon which various types of relevant content were published. Participants were informed that the purpose of the Facebook page was to follow along the progress of the natural science part of the project. Interested participants submitted email addresses on which to be alerted once images of their laboratory samples had been uploaded. 337 email address were collected, of which 309 were successfully sent email updates. The Facebook Page was public and so anybody could like the Page. The Page started with 476 likes on the 17th May 2016 and ended on 1798 likes on the 22nd May 2018 as data collection concluded. Content released included web links to relevant news articles, event links advertising upcoming pop-up stands, photos from the pop-up stand events, videos created by the Microbiology Society, images of relevant tools for data analysis and images of laboratory samples (Table 2-6). Details of web links to these posts, where available, can be found in Appendix F-1. Posts were designed or discussed and edited in collaboration with the Microbiology Society's Communications Team. Participant response guided the release of subsequent content. In total, 47 posts were released between the 5th July 2016 and the 21st February 2018.

Facebook metrics were downloaded to Microsoft Excel by the Microbiology Society's Communications Team at the end of the data collection period on the 22nd May 2018. When users commented, I wrote a response which was edited in collaboration with the Microbiology Society. These comments and replies were filed in Microsoft Word. These word files were uploaded to NVivo 11, then discussed with an experienced qualitative researcher (Prof. Nardi).

Coding schedules were agreed and piloted (Table 2-5). These codes represented key topics emerging from the public perception literature (1.3), conceptions of research and researchers (Brew, 2001) and a commognitive framework (Sfard, 2008). Facebook comments were coded in NVivo 11. A series of comments with one user was selected at random to be double coded (Prof. Nardi); ambiguities were resolved in discussion. .

Table 2-6. Chronological account of types of content released to Antibiotics Unearthed Facebook Page. Table shows the number of each of the 47 posts released as part of Antibiotics Unearthed social media engagement. The date of release of each post is shown, as well as the type of media that the post represented. Web links would take users to a page, such as a news article. Event links specifically advertised upcoming Antibiotics Unearthed pop-up stand events. Images provided visual aids to discussion. Videos linked to relevant media from YouTube. Photo refers to a change of cover photo. Sample Images were images of data emerging from the laboratory, labelled so users could identify their own soil samples.

Post Number	Date	Description of Facebook Post
1	05/07/16	Web Link to the Antibiotics Unearthed Webpages
2	18/07/16	Event Link to news event about the pop up stands to take place at Garwnant Visitor Centre in the Brecon Beacons, and Kielder Castle
3	25/07/16	Event Link to the Pop-Up Event taking place at Garwnant Forest
4	10/08/16	Photograph of a participants attending the Pop-Up Event at Garwnant Forest
5	16/08/16	Link to a BBC News article that discus finding a Colicin Resistant Bacteria found in Scotland
6	18/08/16	Video about Phage created by the Microbiology Society Attached to a Link to the Microbiology Today society magazine
7/8	23/08/16	New Image to represent Antibiotics Unearthed project and link to website
9/10	24/08/16	Photos of agar plates streaked from soil samples brought in by participants who attended the Pop-up event at Garwnant Forest and Kielder Forest during summer 2016 Graphic of Different Colony Morphology Images
11	22/09/16	Link to a news article about UN signing a landmark declaration to fight the global challenge of antibiotic resistance
12	23/09/16	Photos of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Pop-up event at Garwnant Forest and Kielder Forest during summer 2016
13	04/10/16	Link to News Story about The Microbiology Society staff trip to the Ministerial Side-Event on AMR held in New York to hear about the global commitment in tackling the issue
14	03/11/16	Link to an interactive microscope tool created by the Microbiology Society
15	14/11/16	Microbiology Society Fact sheet about Antibiotic Resistance released as part of Antibiotic Awareness Week
16	16/11/16	Link to You Tube animation about Antibiotic Resistance
17	23/11/16	Photo images of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop-up event at Garwnant Forest and Kielder Forest during summer 2016
18 & 19	21/12/16	Photos of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop-up event held at Garwnant Forest and Kielder Forest during summer 2016

		Advert for lecture at Nottingham University focused on O'Neil Review on AMR
20	09/01/17	Two photographs of agar plates from Samples taken the 2016 pop-up events at Garwnant Forest and Kielder Forest
21	12/01/17	News article about spider silk being used to augment antibiotic-releasing bandages
22	23/01/17	News article about a moth gut bacterium the defends its host by making antibiotics
23	26/01/17	Link to BBSRC highlighting a research call that address AMR in agriculture.
24	09/02/17	News article about scientists showing that currently available antibiotics can still stop resistant bacteria – by exerting enough force
25	22/02/17	News article from U.KRI about uncovering the molecular mechanisms that make MDR *Klebsiella pneumoniae* so resistant to antibiotics
26	27/02/17	NICE article:- Children and young people should be taught simple hygiene measures to curb the spread of infections
27	03/03/17	Announcement about the BBC Radio 4 drama called Resistance a three-part drama series about an outbreak of antibiotic resistance
28	24/03/17	School Pupil Winners of Antibiotic Unearthed Schools programme award
29	05/04/17	Agar plate demonstrating the microbial handprint of an eight-and-a-half-year-old boy after he'd been playing outside
30&31	16/05/17	Photo images of agar plates streaked from soil samples brought in by participants who attended the Glasgow Botanic Garden Pop up event that took place in May
32	30/05/17	Photos of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Thetford Forest pop up event in May
33	27/06/17	Photos of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop-up event at Thetford Forest in May
34	31/07/17	Lespar comment on the BMJ article that discusses how long people should take a course of antibiotics for
35	10/08/17	Photos of species identified by Ethan from soil samples brought to the pop-up event at Thetford Forest that took place in July. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected
36	19/09/17	Article about the up and coming launch of the Microbiology Society new microbiome-themed colouring book, that became available to pre-order
37	02/10/17	Photos of agar plates streaked from soil samples brought in by participants who attended the Glasgow Botanic Gardens Pop up event that took place in September

38&39	10/10/17	Photos of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Glasgow Botanic Garden pop up event Article about the launch of the Microbiology Society new microbiome-themed colouring book, that became available to pre-order
40	06/11/17	Photos of agar plates streaked from soil samples brought in by participants for the Norwich Science Festival event that took place in October 2017
41	09/11/17	Photos of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop-up event at Glasgow Botanic Gardens
42	15/11/17	Infographic from the WHO that explains the antibiotic resistance cycle. Published in Antibiotic Awareness week
43	20/11/17	Photos of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples brought in by participants to the Norwich Science Festival pop-up event in October 2017
44	05/12/17	Photos of species identified by Ethan from soil samples brought to the pop-up event at Glasgow Botanic Garden that took place in September. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected
45	06/12/17	Photos of zones of inhibition gathered from bacterial species purified from soil samples brought to the Norwich Science Festival
46	15/12/17	Photos of species identified by Ethan from soil samples brought to the Norwich Science Festival. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected
47	21/02/18	BBC news article: New Family of Antibiotics found in Dirt

2.7.3 Portfolios – Long Term

I handed out portfolios (Figure 2-3) at pop-up stands in England, Scotland and Wales. Participants were provided with a portfolio handbook which explained what the portfolio was designed for (Appendix G-1), provided detail on how to record each entry and then offered suggestions for topics to explore or types of entries to make. It concluded with a signed thank you note from myself, with University of East Anglia and Microbiology Society branding. The handbook was developed and refined in collaboration with the supervisory team. Entries were self-guided and could explore any topic the participant wished. Participants were given contact information including my email address and my social media information. One participant asked to receive a phone call to discuss what they should be including. In total, 14 portfolios were given out, one person withdrew from the study and eight did not respond to contact on request for return of the portfolios. In total, five portfolios were returned with varying levels of content.

Returned portfolios were transcribed using Microsoft Word. These word files were uploaded to NVivo 11, read twice, then discussed with an experienced qualitative researcher (Prof. Nardi). To ensure the anonymity of participants whilst allowing for participant identification, consecutive numbers were assigned to portfolios (1-5). A thank you note and a gift of The Microbes Fight Back: Antibiotic Resistance (Bowater, 2017) were sent to the participant,. If requested the portfolio was also returned.

Key themes that had emerged from coding of the interviews were agreed and piloted (Table 2-7). These themes represented key topics emerging from discourse analysis. Two characteristics: consistency and specificity were agreed; adapted from analysis to diagnose learning in teaching situations (Biza et al., 2018). Two learning levels, object and meta level, were agreed; taken from analysis which evidenced discursive transformations in learners (Sfard, 2008). Portfolios were distilled into factual accounts (Appendix H-1). Excerpts representing key themes which evidenced changes in specificity, consistency, object or meta level shifts were selected through re-reading of factual summaries and transcripts. Excerpts from one portfolio were selected to be checked (Prof. Nardi); ambiguities were resolved in discussion.

Table 2-7. Key themes emerging from discourse analysis of participant interviews. A list of the eight key themes emerging from coding of verbatim interview transcripts and more general reading of factual summaries.

Key Themes
1. The correct use of scientific terminology
2. Using personal experiences in ritualised ways to participant in a new discourse
3. Public Health Narratives
4. Human Health Narrative
5. Who is to blame?
6. What are scientists after?
7. Do people need a hero?
8. Do people make bad decisions in the face of better knowledge?

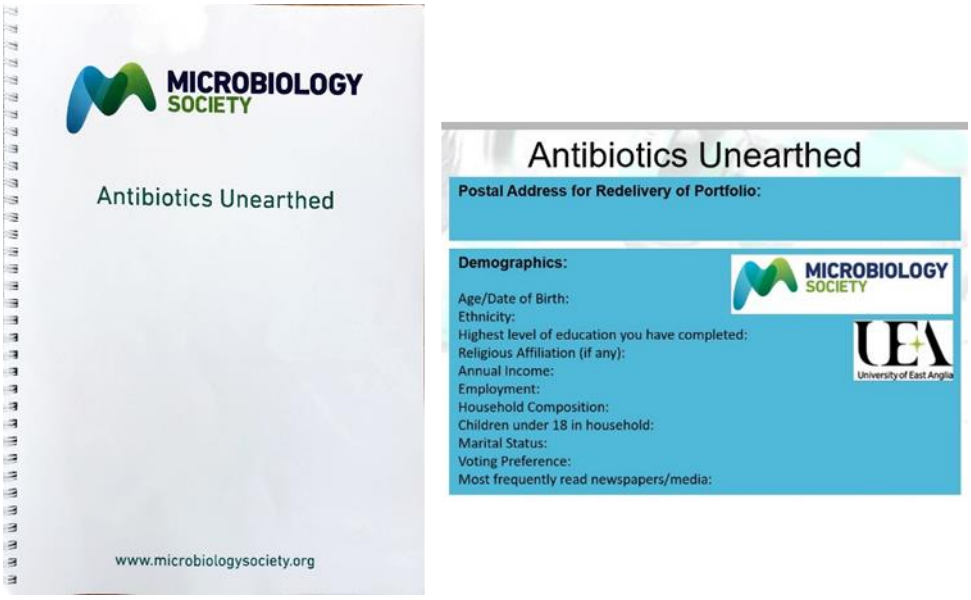


Figure 2-3. Front cover of the A4, ring bound Portfolio, displaying the project name and the Microbiology Society logo, and a demographics survey for the participant to complete should they wish.

2.8 Ethics

The University of East Anglia's Faculty of Education's Research Ethics Committee approved the study (Appendix I-1). As well as an application form, a participant information sheet (Appendix J-1) and participant consent forms for the interviews and social media (Appendix K-1) and the portfolios (Appendix K-2) were submitted. Ethics approval for the project was received on the 11th July 2016 from the EDU Chair of the Research Ethics Committee.

I made myself familiar with the data protection act and the freedom of information act. I promised to explain the purpose, procedures of the research and potential benefits and costs of participating (including time) to each participant before they took part in the project. I promised to treat participant data with respect regarding anonymity and confidentiality. I promised to obtain written consent from each participant and to not interview any persons under the age of 18 without consent from a parent. I promised not to put undue pressure on any individual to participate, and to not prejudice those who refused. Participants were informed that data generated by the research would only be used purely for the purposes of the research project.

I provided my full identity to potential participants as well as my University of East Anglia contact details and those of my supervisor in order that they could contact in relation to any aspect of the research. Participants were told of their right to withdrawal and that their data would not be held by a third party. Research was carried out at times designed to minimise the burden on participants. I conducted myself in an appropriate and professional manner, including respecting the views of all participants in the research. I took steps to ensure no harm resulted from participating in this project.

Chapter 3 The microbial composition and antimicrobial activity of citizen science soil samples

3.1 Introduction

Building on the antibiotic discoveries of the 1920s and 30s by Selman Waksman's laboratory (Bowater, 2017), there was a widespread introduction of antibiotics in the 1940s. This began with penicillin (Fleming, 1929) and Streptomycin (Schatz, Bugle and Waksman, 1944) and transformed modern medicine as we know it; curing the most deadly infectious diseases of the time. Novel classes of antibiotics known as the aminoglycosides, tetracyclines, macrolides, glycopeptides, amphenicols and carbapenems were discovered in the golden-era of antibiotics (Bowater, 2017).

However in the 1960s, overmining of the limited resource that is cultivable soil microorganisms brought the golden-era of natural antibiotic discovery to an end (Lewis, 2012). Researchers began increasingly rediscovering compounds produced by the overmined actinomycetes (Lewis, 2020). Actinomycetes are popular as a source of broad-spectrum antibiotics and their large genomes typically harbour a substantial 20 BGCs per isolate (Rutledge and Challis, 2015). Because of the ease at which these broad-spectrum antibiotics can be discovered, Baltz (2007) detailed the low probability of discovering a new broad-spectrum antibiotic, suggesting one would need to sieve through a library of at least 10^7 isolates.

Success of synthetic compounds, at the same time discovery of natural products was drying up, meant they took precedence (Lewis, 2020). These synthetic compounds however, ran into regulatory, commercial and scientific barriers (Payne *et al.*, 2007; Tommasi *et al.*, 2015). In the last 50 years, pathogens have continued to accumulate resistance to antibiotics despite a faltering anti-infective discovery platform (Lewis, 2020). Currently, antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice (Ling *et al.*, 2015). History suggests the development of resistance limits the lifespan of antibiotics, providing the need for a constant introduction of new compounds into the antibiotic pipeline (Bush *et al.*, 2011; Spellberg and Shlaes, 2014). I discussed the predictable emergence of resistance and the pathogenic bacteria which pose urgent or serious threats in chapter 1.1.3 and 1.1.4, respectively.

Broad-spectrum antibiotics of actinomycetes, effective against Gram-negative bacteria, have been overmined, however other bacterial groups have had to develop their own compounds to act against their Gram-negative competitors (Lewis, 2020). These underexploited bacteria have been shown to devote sizeable parts of their genome to BGCs (Crits-Christoph *et al.*, 2018). However, these bacteria are challenging to culture. In fact, it has been reported that only 1% of cells from natural environments like soil can, and have, been cultured (Winterberg, 1898; Lloyd *et al.*, 2018). The difficulty of culturing these organisms has been attributed to the requirement of some species for growth factors produced by their neighbours (D'Onofrio *et al.*, 2010), but this only accounts for around 10% of bacteria that do not readily grow *in vitro* (Lewis, 2020).

Approaches have been developed to grow these underexploited organisms in their natural environment, such as the iChip (Nichols *et al.*, 2010), from which came a variety of compounds including Teixobactin, a novel class of antibiotic

isolated from the soil bacterium *Eleftheria tereae* in 2015 (Ling *et al.*, 2015). *Eleftheria tereae* is a Gram-negative β-proteobacterium and was previously never known to produce antibiotics. In this study, growth media was tested which extracted nutrients and growth factors from the soil environment (soil extract media) to encourage a more diverse range of culturable organisms.

Facing the barrier of rediscovery, novel strategies and innovative techniques have been developed (Trautman, Eric and Crawford, 2015; Quinn *et al.*, 2017) including the integration of genome mining, silent pathway induction and Mass Spectrometry-based molecular networking (Trivella and de Felicio, 2018) and genetics-based bioinformatic tools such as AntiSMASH on behalf of genome mining to detect BGCs (Medema *et al.*, 2011). In 2018, a team of researchers developed a culture-independent discovery platform that involved sequencing, bioinformatic analysis and heterologous expression of BGCs captured on extracted DNA from environmental samples. In doing so, they discovered a new class of calcium-dependent antibiotics named Malacidins (Hover *et al.*, 2018).

3.2 Aim

The aim of the Antibiotics Unearthed Laboratory work (and this chapter) was to use citizen science to enhance sample collection. Further, a culture-based approach combined with WGS was used to identify genomes and predicted BGCs of cultured bacteria with antimicrobial potential against a range of medically relevant pathogens. To facilitate this an *in situ* and lab-based protocol was first established through manipulation of a variety of parameters including diluent, dilution factor, incubation temperature, contamination control and growth media. This allowed a balance between large sample processing and accuracy.

3.3 Results

3.3.1 Isolating Antibiotic Producing Bacteria from the Soil: Pilot Study

There are 10,000 bacterial species per gram of soil (Torsvik et al, 1990) and 830,000 species per 1 billion bacterial cells (Gans et al, 2005). Despite this considerable abundance and diversity, only 1% of cells can be cultured (Pham & Kim, 2012). An absence of information on natural habitat and growth requirements have contributed to this dilemma (Stewart, 2012; Chaudhary et al, 2019). However, these underexploited bacteria provide a potential reservoir for novel antibiotics. A pilot study was conducted to optimise growth conditions allowing the culture of a more diverse range of underexploited soil bacteria. (Figure 3-1).

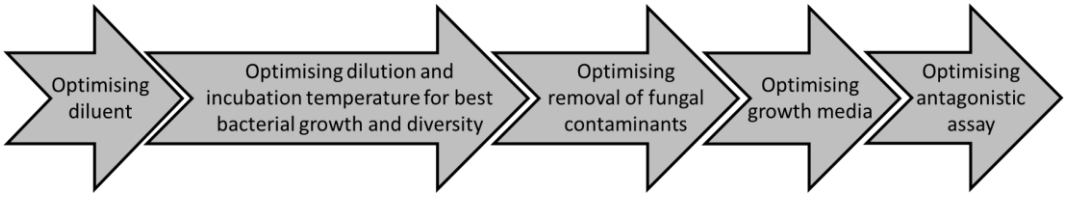


Figure 3-1. Developing a plan to pilot test citizen scientist's soil samples. Flow diagram which details step by step the processes needed to progress a soil sample through the testing pipeline. Samples were plated to determine CFU/sample at each stage to ensure the methods were optimised.

3.3.1.1 Optimising Diluent

Liquid diluent serves to transfer bacteria from their soil environment to the growth media and control their abundance. Two common diluents were tested: Phosphate-Buffered Saline (PBS) and dH_2O. Bacteria have been shown to survive in both for more than 30 weeks, however some bacterial species survived better than others (Liao and Shollenberger, 2003). One gram of soil, taken from participant soil samples (2.3.2), was added to 10 mL of diluent and homogenised. The samples were diluted to 10^{-2} to allow counting of Colony Forming Units (CFU) as CFU/g. 50 µl of soil water was added to each plate. Diluents were tested in triplicate across five growth media: Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich). The CFU/g for all media was averaged to give average CFU/g for dH_2O and PBS (Figure 3-2). Plates were incubated at 30°C which was determined to be the optimum temperature for growth (Table 3-1). Data showed that CFU/g of soil was similar using both dH_2O and PBS.

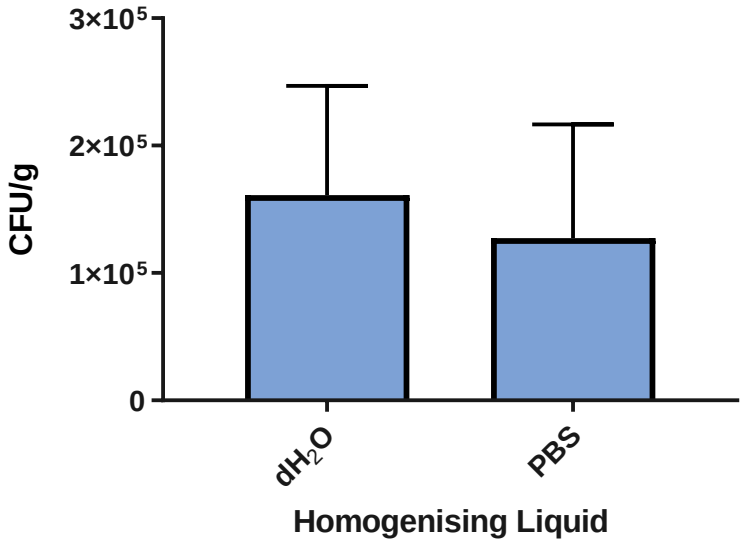


Figure 3-2. Homogenising diluent did not affect CFU per gram of soil bacteria. Average taken from 15 samples across 5 different media: Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich). CFU/g calculated from samples diluted at a concentration of 10^{-2}. SD bars show the amount of heterogeneity in the CFU per gram ranges.

The total number of colonies showing antagonistic bacteria was also determined for each diluent across the five media (Figure 3-3). Results indicated that dH_2O produced more inhibitory colonies (Figure 3.3a), however there is little or no difference between the two diluents with regards to the percentage of these inhibitory colonies which inhibit either bacteria or fungi (Figure 3.3b).

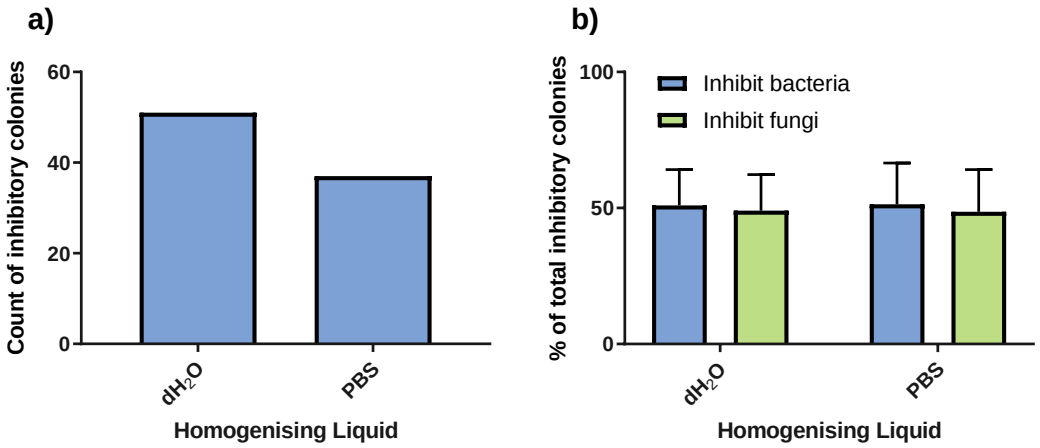


Figure 3-3. Total number of inhibitory colonies and antagonistic activity profiles compared between dH_2O and PBS. a) Total count of inhibitory colonies taken from triplicate samples across 5 different media: Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich) (n=15). Diluted at a concentration of 10^{-2}. B) Percentage of inhibitory colonies which inhibit either bacteria of fungi calculated from triplicate samples across 5 different media: Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich) (n=15). Diluted at a concentration of 10^{-2}. Media was LB, LB, NA, TSA, SFM and TA + $CaCl_2$.

Both datasets suggest a lack of significant difference either in the abundance of bacteria or the proportion of colonies showing antagonistic activity between the two diluents. dH_2O was selected as the trend showed a slightly higher CFU/g and inhibitory colony count.

3.3.1.2 Optimising Dilution Factor and Incubation Temperature for Bacterial Growth and Diversity

Different dilutions of dH_2O were tested at different temperatures to assess the effect on ability to culture individual colonies whilst maintaining neighbouring colony interaction. Serial dilutions of 10^0, 10^{-1} and 10^{-2} were tested in duplicate on LB agar media at three different temperatures, 25°C, 30°C and 37°C. 50 µl of soil water was added to each plate (Table 3-1).

Table 3-1. Soil samples diluted to 10^{-1} and grown at 30°C represent balance between single colonies and colony interaction. Colony counts of 10^{0}, 10^{-1} and 10^{-2} serial dilutions of 1 g of soil (n=2) mixed with 10 ml dH_2O, plated on LB agar and grown at 25°C, 30°C and 37°C.

Temperature (°C)	Sample 1 (CFU)			Sample 2 (CFU)		
	10^{0}	10^{-1}	10^{-2}	10^{0}	10^{-1}	10^{-2}
25	265	59	6	166	23	3
30	234	34	2	182	26	1
37	211	30	2	62	5	1

Results informed the decision to use a 10^{-1} dilution of dH_2O as this resulted in single colonies large enough to be isolated whilst presenting neighbouring colonies on which antagonistic activity could be noted (Figure 3-4). Each temperature provided a similar CFU/g count, however less fungal growth was observed at 30°C and so was selected.

Figure 3-4. Example of a plate showing single colonies that are interacting allowing zones of inhibition to be detected, plated at 10^{-1}. 50 µl soil water diluted 10^{-1} was added to an LB plate, spread and incubated at 30°C.

3.3.1.3 Optimising Removal of Fungal Contaminants

The rapid spread of fungi on the growth media (Figure 3-4) was a source of contamination when picking single colonies. Fungal growth was inhibited with the addition of antifungals. Cycloheximide and nystatin were tested. Cycloheximide had a working concentration of 50 µg/mL (Lawana, Korrapati and Mehendale, 2014), whilst nystatin was shown to have an MIC_{50} of 8 µg/mL on isolates of *Aspergillus* spp., *Candida* spp., and *C. neoformans*. For ease, a working concentration of 10 µg/mL was used. At these concentrations fungal contamination still occurred. Concentrations were doubled, preventing fungal contamination. Final working concentrations were 100 µg/mL cycloheximide and 20 µg/mL nystatin.

3.3.1.4 Optimising Growth Media

To create a baseline for abundance and diversity of soil bacteria on common lab media, readily available media from The Small World Initiative Research Protocols (Hernandez, Tsang and Handelsman, 2015) was selected. Five media were tested for abundance (Figure 3-5) and antagonistic profiles (Figure 3-6). These were LB Agar (BERTANI, 1951), NA (Wright, 1934), TSA (TSB; Sigma Aldrich), SFM Agar (Kieser et al. 2000) and TA + $CaCl_2$ (TSB; Sigma Aldrich)..

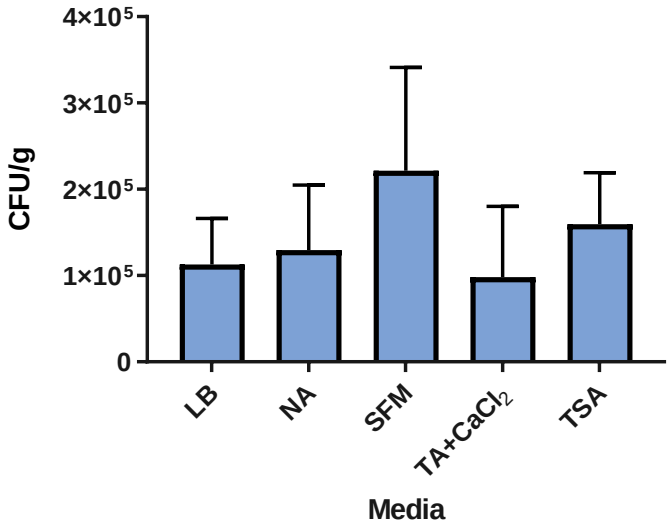


Figure 3-5. CFU per gram of soil does not different significantly across five media. Average taken from 6 samples across 2 dilution liquids (PBS and dH_2O). CFU per gram calculated from samples diluted at a concentration of 10^{-2}. SD bars show the amount of heterogeneity in the CFU per gram ranges.

Figure 3-5 suggests that whilst SFM and TSA seemed to have slightly higher abundance, the difference was not significant. Figure 3-6 suggests that a) the nutrient poor media (TA + $CaCl_2$) produced the fewest inhibitory colonies whilst TSA seemed to produce the most and b) that most of the TSA antagonism was seen against fungal colonies, whilst the nutrient poor TA media showed antagonism against bacteria only. LB and NA were similar whilst SFM had slightly more bacteria than fungi.

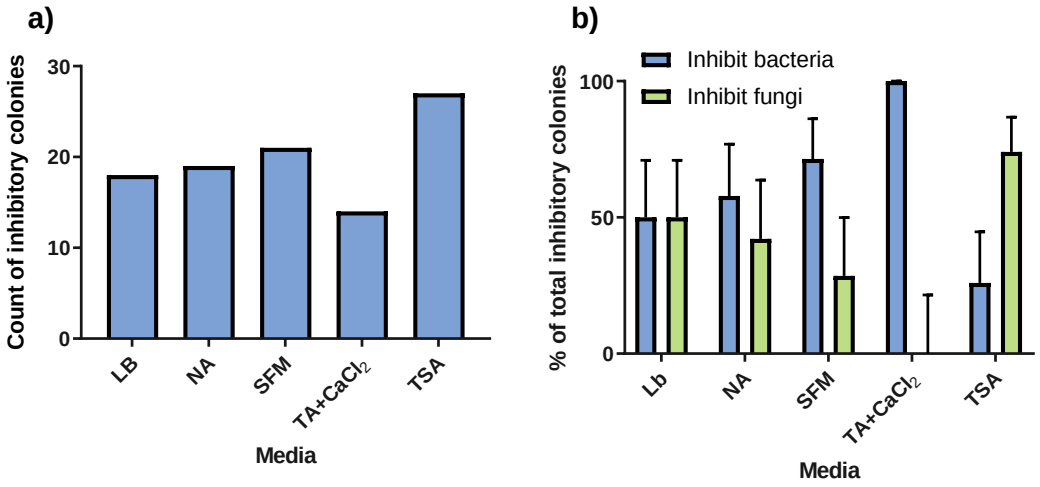


Figure 3-6. Total number of inhibitory colonies and antagonistic activity profiles compared between 5 different common lab growth media. a) Total count of inhibitory colonies on Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich) taken from 6 samples across 2 diluents (PBS and dH_2O) diluted at a concentration of 10^{-2}. B) Percentage of inhibitory colonies on Luria-Bertani (LB) Agar (BERTANI, 1951), Nutrient Agar (NA) (Wright, 1934), Tryptic Soy Agar (TSA) (TSB; Sigma Aldrich), Soy Flour Mannitol (SFM) Agar (Kieser et al. 2000) and Technical Agar with Calcium Chloride (TA + $CaCl_2$) (TSB; Sigma Aldrich)which inhibit either bacteria of fungi taken from 6 samples across 2 diluents (PBS and dH_2O) diluted at a concentration of 10^{-2}

In selecting potential lab media, CFU/g did not significantly differ. LB, NA and SFM had similar abundance of inhibitory colonies and did not significantly produce more colonies which inhibited bacteria of fungi. LB was selected as the baseline against which to compare the effects of utilising media which utilises nutrients and growth factors from the soil.

Research has shown that culture media made from a soil-extract increases success in isolating previously uncultured or novel soil bacteria (Hamaki et al., 2005; Liebeke et al., 2009). Media tested included Soil Extract Media (SEM) (adapted from Liebeke et al., 2009), dH_2O SEM, Autoclaved dH_2O SEM, Concentrated dH_2O SEM and LB (BERTANI, 1951) (Appendix C-1). Media was made as broth and mixed with 1.5% w/v agar for pouring with addition of antifungals.

Table 3-2. Concentrated SEM results in highest abundance, diversity and number of colonies showing antagonistic activity. On five media: Soil Extract Media (SEM) (adapted from Liebeke et al., 2009), three modified versions of SEM: dH_2O SEM, Autoclaved dH_2O SEM and Concentrated dH_2O SEM.LB (BERTANI, 1951), averages were taken from duplicate samples. Total colony count (abundance), count of morphologically distinct colonies (diversity) and count of bacteria showing antagonistic activity against neighbouring colonies (antagonistic activity) were taken from samples diluted at a concentration of 10^{-3}.

Sample	Abundance	Diversity	Antagonistic Activity
SEM	64	4	1.5
LB	66	4.5	0.5
dH2O SEM	58	3	0
Autoclaved dH_2O SEM	81.5	3	1.5
Concn dH_2O SEM	115.5	8	3.5

Table 3-2 shows Concentrated dH_2O SEM resulted in the highest average abundance (115.5 colonies), diversity (8 distinct colony types) and antagonistic activity (3.5 colonies showing antagonistic activity against neighbouring organisms). A physical comparison between LB and Concentrated dH_2O SEM can be seen in Figure 3-7. SEM and LB produced similar results, contrary to what was expected. dH_2O SEM produced the fewest total colonies (58) and the joint lowest diversity with the autoclaved dH_2O SEM (3). Based on these results, concentrated SEM was selected as appropriate for use in the testing pipeline.

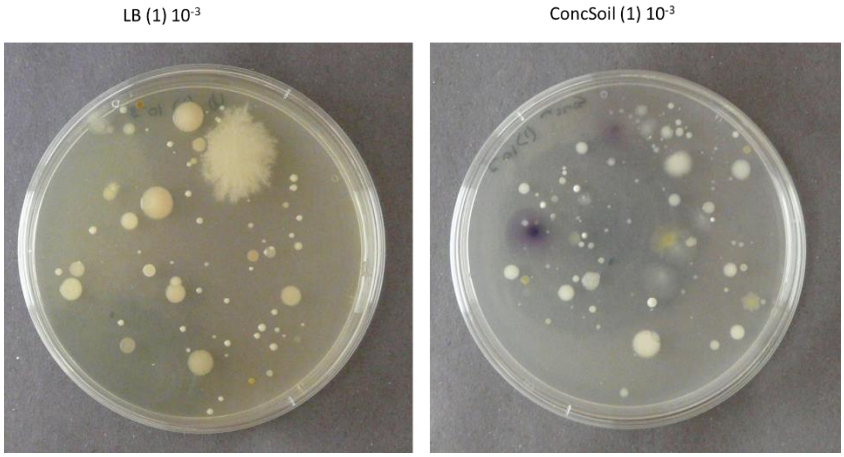


Figure 3-7. Soil extract media produces a more abundant, diverse and more antagonistic soil community than LB. Images of one University of East Anglia soil sample diluted to 10^{-3} on LB (left) and diluted to 10^{-3} on Concentrated Soil Extract Media (right). These plates were investigated for abundance, diversity and antagonistic activity.

Both testing and the Small World Initiative Research Protocols (Hernandez, Tsang and Handelsman, 2015) provided several options for growth media. These were LB, Brain Heart Infusion (BHI) Agar, All Culture (AC) Agar and concentrated dH_2O SEM. Which media were eventually selected is discussed in 3.3.2.

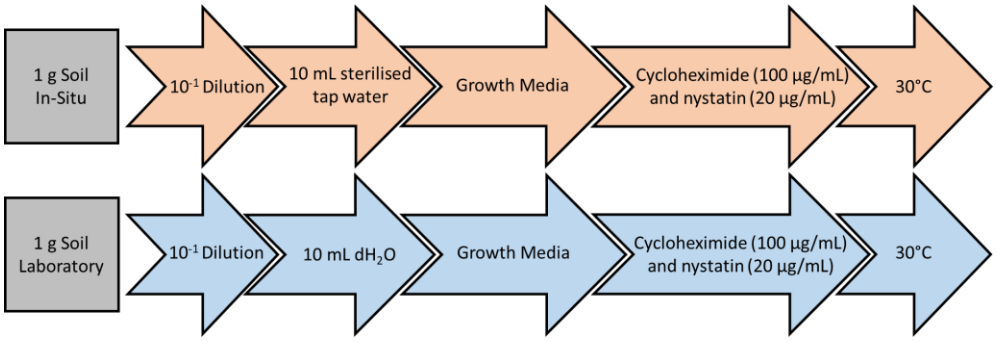


Figure 3-8. Optimised protocol for testing our citizen scientists soil samples. Flow diagram which details step by step the processes needed to progress a soil sample through our testing pipeline. The top diagram shows the protocol for in-situ testing at each pop-up event whilst the bottom diagram shows the protocol for laboratory testing. Square identifies amount of soil and where protocol took place. Soil in both protocols was taken from participant soil samples. Arrows show dilution, diluent, growth media, antifungals and growth temperature (left to right).

This pilot study provided a protocol through which citizen scientist's soil samples could be analysed. In practice, collection and analysis in the field

differed slightly from collection and analysis in the lab (Figure 3-8). In the field 1 gram of soil was mixed with 10 mL sterilised tap water and shaken by hand. Five drops of soil solution were moved using a Pasteur pipette (approx. 50 µL) on to growth media. In the lab, 1 gram of soil was resuspended in 10 mL dH_2O, homogenised and diluted 1:10; 100 µL soil solution and 900 µL dH_2O. 100 µL of this 10^{-1} solution was spread plated on to the selected media. All were incubated at 30°C, however the field plates had to be transported back to University of East Anglia first. To reduce fungal contamination, cycloheximide was added at 100 µg/mL and nystatin was added at 20 µg/mL. The method of plating bacteria differed between field and laboratory settings due to the need for a pragmatic approach which would allow better engagement with the public and a large throughput of participants in comparison to reproducible lab conditions.

3.3.1.5 Optimising Antagonistic Assay

The *in vitro* antimicrobial activity of environmental isolates was tested using an adaptation of the well diffusion method and the cross-streak method (Lertcanawanichakul & Sawangnop., 2008). The well-diffusion method takes an agar plate with a lawn of indicator strain, cuts wells in the agar and pipettes isolates in liquid media into these wells. The secondary metabolites produced by the bacterium of interest diffuse into the agar. One can then measure whether the secondary metabolites kill or prevent growth of the indicator strain. The cross-streak method takes an agar plate on which a line of indicator strain is swabbed. Across this line, isolates are swabbed. Once both grow, it is possible to see areas of inhibition where secondary metabolites kill the indicator strain. Upon testing both methods, I found that the well-diffusion method was too time consuming, and the cross-streak method lacked accuracy, especially if inhibition zones were large enough that it was difficult to tell which isolate was causing toxicity. Instead, I merged the two protocols to produce a simple, fast and repeatable antagonistic assay, the drop-plate assay (Figure 2-1).

Using this assay, colonies showing antagonistic activity against neighbouring colonies were selected and streaked to pure cultures. Medically relevant pathogens covering Fungi, Gram-positive and Gram-negative bacteria were selected as indicator strains (Table 2.1).

3.3.2 Citizen Science Field Studies

A protocol was devised where participants could safely collect suitable soil samples and return them to the pop-up stands to begin their initial experiments (2.3.2). Participants followed the *in-situ* protocol to transfer soil bacteria on to growth media and plates were taken back to the University of East Anglia to be incubated and imaged (2.3.3.1). Photos were edited on Microsoft PowerPoint and uploaded to Facebook with sample numbers, media type and forest location (Figure 3-9).

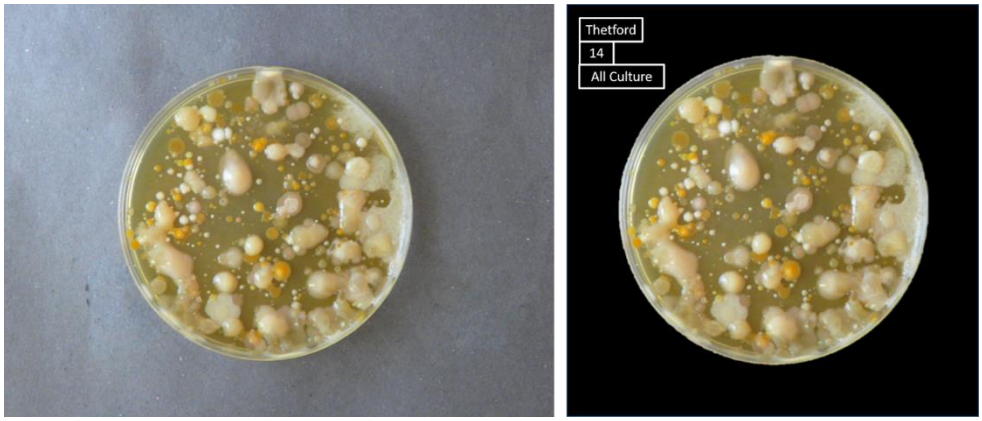


Figure 3-9. Example of soil sample grown in the lab and the result of editing on PowerPoint for upload to the public on Antibiotics Unearthed Facebook Page. Example of the raw image (left) and the edited image (right) of sample number 14, plated in the lab from a soil sample collected by a participant in Thetford Forest on an All Culture agar plate. The plate was incubated overnight at 30°C. The edited image was uploaded to Facebook as part of the Thetford AC album.

Once back at the University of East Anglia, soil samples were run through the laboratory protocol (2.3.3.2). In Kielder and Brecon, rather than AC and BHI as laid out in 2.3.3.2, Concentrated dH_2O SEM was used. Topsoil used to make the media was taken from the same forests samples were collected in so that nutrients and growth factors would most match the organism's natural environment. However, the resultant plates lacked diversity and showed no antagonistic activity (Figure 3-10).

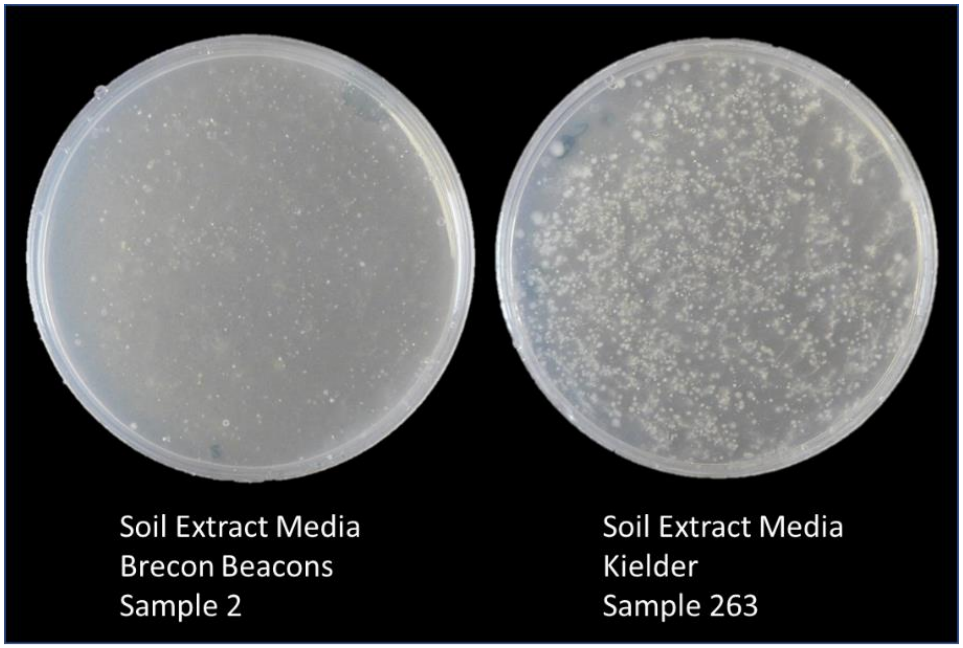


Figure 3-10. Soil extract media did not perform in practice. Images of soil samples grown on soil extract media taken from Brecon Beacons (left) and Kielder Forest (right). Soil used to make the extract was soil taken from the forest in which the samples were collected.

These results, together with the time-consuming nature of preparing SEM justified the decision to stop the use of Soil Extract Media. Instead, All Culture and Brain Heart Infusion Media were used for the laboratory protocol for the remaining three events. After the *in-situ* and laboratory images had been taken, single colonies that inhibited neighbouring colonies were streaked to pure (Figure 3-11) and stored (2.3.4 and 2.3.5).

Figure 3-11. Environmental isolates showing antagonistic activity against neighbouring colonies streaked to pure. This colony was taken from a plate made in the lab on All Culture media, from sample number 12 collected in Glasgow. This was the 14th colony to show antagonistic activity from Glasgow on AC media. The colony had 'nodules' forming on its surface which contained a clear liquid.

A total of 454 soil samples were collected and cultured over the course of five events (Table 2-3). 96 well plates were used to allow the simultaneous growth of a larger number of isolates. Upon shaking, cross-contamination between wells was noted and so shaking was removed. Drop plates were made with 4% w/v agar rather than 1.5% to limit colony motility. A total of 929 colonies were isolated that showed some level of antagonistic activity against neighbouring soil organisms. A total of 165 isolates showed antagonistic activity against one or more medically relevant pathogens (Table 3-3). Of the isolates which showed antagonistic activity against neighbouring soil organisms, 17.76% went on to show antagonistic activity against medically relevant indicator strains.

Table 3-3. Story of citizen scientist's soil samples. Sample location, date of collection, number of samples collected, number of colonies screened for inhibitory activity against neighbouring colonies and number of isolates inhibiting medically relevant organisms.

Event location	Number of soil samples collected	Number of colonies inhibiting neighbouring soil bacteria	Number of isolates inhibiting medically relevant organisms
Brecon Beacons	155	142	29
Kielder Forest,	144	62	11
Thetford Forest,	47	325	38
Glasgow Botanic Gardens	51	234	40
Norwich Science Festival	57	166	47

To aid with public engagement, photos were taken and uploaded to Facebook with media type, forest location and medically relevant pathogen attached to the respective images (Figure 3-12).

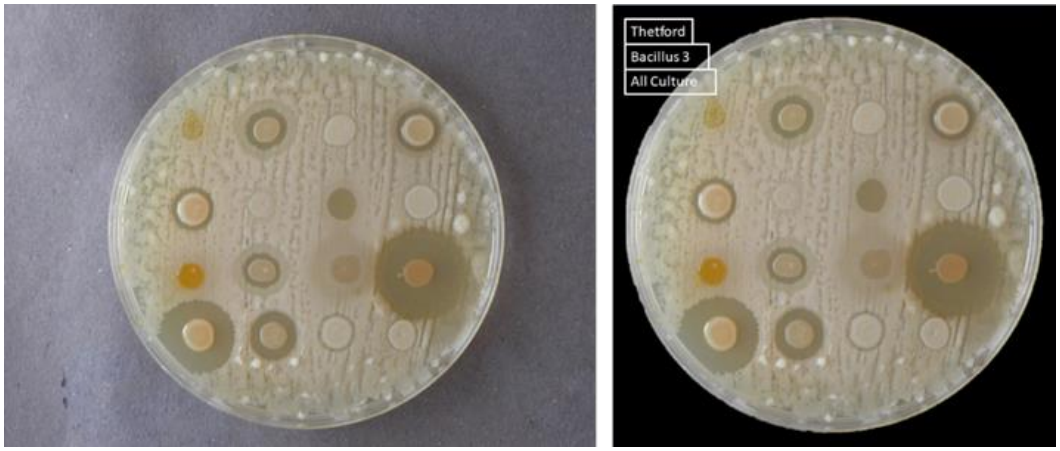


Figure 3-12. Example of Drop Plate assay uploaded to Facebook, where the antagonistic activity of soil isolates was tested against medically relevant pathogens. These plates show the raw (left) and edited (right) images of *Bacillus subtilis* indicator strain spread as a lawn using a sterile cotton swab and left to dry on a 4% AC agar plate. Isolates of interest were added on top of this dry lawn using an 8-tip multi-pipette with 6 pipette tips attached. 5 µL samples of the potential antagonistic isolates were taken from 96-well plates and dispensed onto the MRSA lawn in a grid. Plates were left to dry and incubated at 30^0C overnight. Transparent zones around some of the antagonistic isolate were taken to be zones of inhibition, for example in the 4^{th} isolate in the 3^{rd} row.

The antagonistic assay of the 165 isolates was conducted within a week of sample collection at the forest events. Isolates were separated into one of seven distinct activity profiles. In total, 45 isolates inhibited Fungi, Gram-positive and Gram-negative bacteria, 24 isolates inhibited Gram-positive and Gram-negative bacteria, eight isolates inhibited Fungi and Gram-positive bacteria, 23 isolates inhibited Fungi and Gram-negative bacteria, 31 isolates inhibited Gram-positive bacteria, 21 isolates inhibited Gram-negative bacteria and 13 isolates inhibited Fungi.

3.3.3 Identifying bacteria for genome sequencing

Isolates had to be selected for genome sequencing. To increase the chances of selecting underexploited bacteria which were more likely to produce novel antibiotics, a filtering process was designed. This was a two-step selection process and aimed to select 15 morphologically distinct isolates (Figure 3-13) with unique antagonistic profiles (Table 3-4). Antagonistic activity and morphology were determined by eye.

The 165 isolates were grouped into categories based on inhibitory activity against Fungi, Gram-positive and Gram-negative bacteria. Antagonistic activity was determined using the drop plate assay (Figure 3-12). Due to the nature of the interaction, sometimes the isolates grew in a transparent fashion and, whilst looking like an inhibition zone, were determined not to be one. This can be difficult to determine on a photo. Instead, the decision to consider this an inhibition zone or not was discussed with an expert (Dr. Rowley). After restreaking, isolates didn't always continue to show potential antimicrobial activity against some or all the medically relevant indicator strains. Considering this, in preparation for selection of isolates to be sent for Genomic Sequencing, a second assay was conducted. When examining the assay results for the 15 isolates, the bacteria were less antagonistic in the second assay (Table 3-5). After discussion with Dr. Rowley, results of the pre-genome sequence assay were used to distinguish the antagonistic activity profiles. Isolates with each

type of antagonistic activity profile were separated by colony morphology. This was a qualitative process after discussions with Dr. Rowley.

Of the 15 isolates, six originated from Glasgow Botanical Gardens, five from the Norwich Science Festival and four from Thetford Forest. These were grown on different growth media, with seven being isolated on All Culture Media, five on Brain Heart Infusion media and three on Luria Bertani media (Table 3-4). Some isolates inhibited penta-resistant *Salmonella (*DT104*)* and not the wild type (SL1344). Whilst this was unexpected, it could be a remnant of the drop-plate assay, where overnight cell density was not calibrated. It is possible that the wild-type *Salmonella* grew faster overnight, which in turn resulted in a higher cell density on the spread plate. The antagonistic activity was then not strong enough to create an obvious zone of inhibition. It is also possible that the zone of inhibition was so weak that it was difficult to determine by eye.

Whilst the rest of this chapter focuses on analysis of these 15 isolates, the remainder were stored as long-term glycerol stocks for future analysis. Details such as the participant plate number, colony number, growth media and antagonistic assay results have been stored and can be seen in (Appendix L-1, L-2 and L-3). Some isolates, notably those from Brecon Beacons and Kielder Forest, were not frozen because of contamination or poor performance in the 2nd round of testing.

Table 3-4. Antagonistic profile of 15 isolates selected for whole genome sequencing, as of the first and second wave of testing. ELD represents the names attributed to each isolate. Event represents the location from which the soil sample which led to the culture of each isolate was submitted from. G represents Glasgow, NSF the Norwich Science Festival and T represents Thetford. In the first wave of antagonistic testing, one Gram-positive *Bacillus subtilis*, two gram – *Salmonella enterica* serovar Typhimurium strains were used (WT SL1344 and penta-resistant DT104) and one *Candida albicans* fungi were used. In the second wave of antagonistic testing, three Gram-positive bacteria were used; *Bacillus subtilis*, Methicillin-resistant *Staphylococcus epidermis* and Vancomycin-resistant *Enterococcus faecium*. Two gram – *Salmonella enterica* serovar Typhimurium strains were used (WT SL1344 and penta-resistant DT104) and one *Candida albicans* fungi were used. A 'Y' is indicative of antagonistic activity against the medically relevant pathogen.

Collection Info			**Antagonistic Profile Post-Event**				**Antagonistic Profile Pre-WGS**					
Isolate	Event	Media	*B. subtilis*	*Salmonella* WT	*Salmonella* DT104	*C. albicans*	*B. subtilis*	MRSA	VRE	*Salmonella* WT	*Salmonella* DT104	*C. albicans*
ELD01	G	AC		Y	Y		Y		Y		Y	Y
ELD02	G	AC				Y					Y	
ELD03	G	BHI	Y	Y	Y					Y	Y	
ELD04	G	BHI			Y			Y				Y
ELD05	G	BHI				Y			Y	Y	Y	Y
ELD06	G	LB				Y			Y			Y
ELD07	NSF	AC				Y		Y			Y	Y
ELD08	NSF	AC		Y	Y			Y				
ELD09	NSF	AC				Y				Y	Y	Y
ELD10	NSF	AC		Y	Y	Y			Y		Y	
ELD11	NSF	BHI			Y	Y		Y		Y	Y	
ELD12	T	AC	Y	Y		Y		Y				Y
ELD13	T	BHI		Y						Y	Y	
ELD14	T	LB	Y	Y		Y	Y	Y				Y
ELD15	T	LB	Y	Y		Y						Y

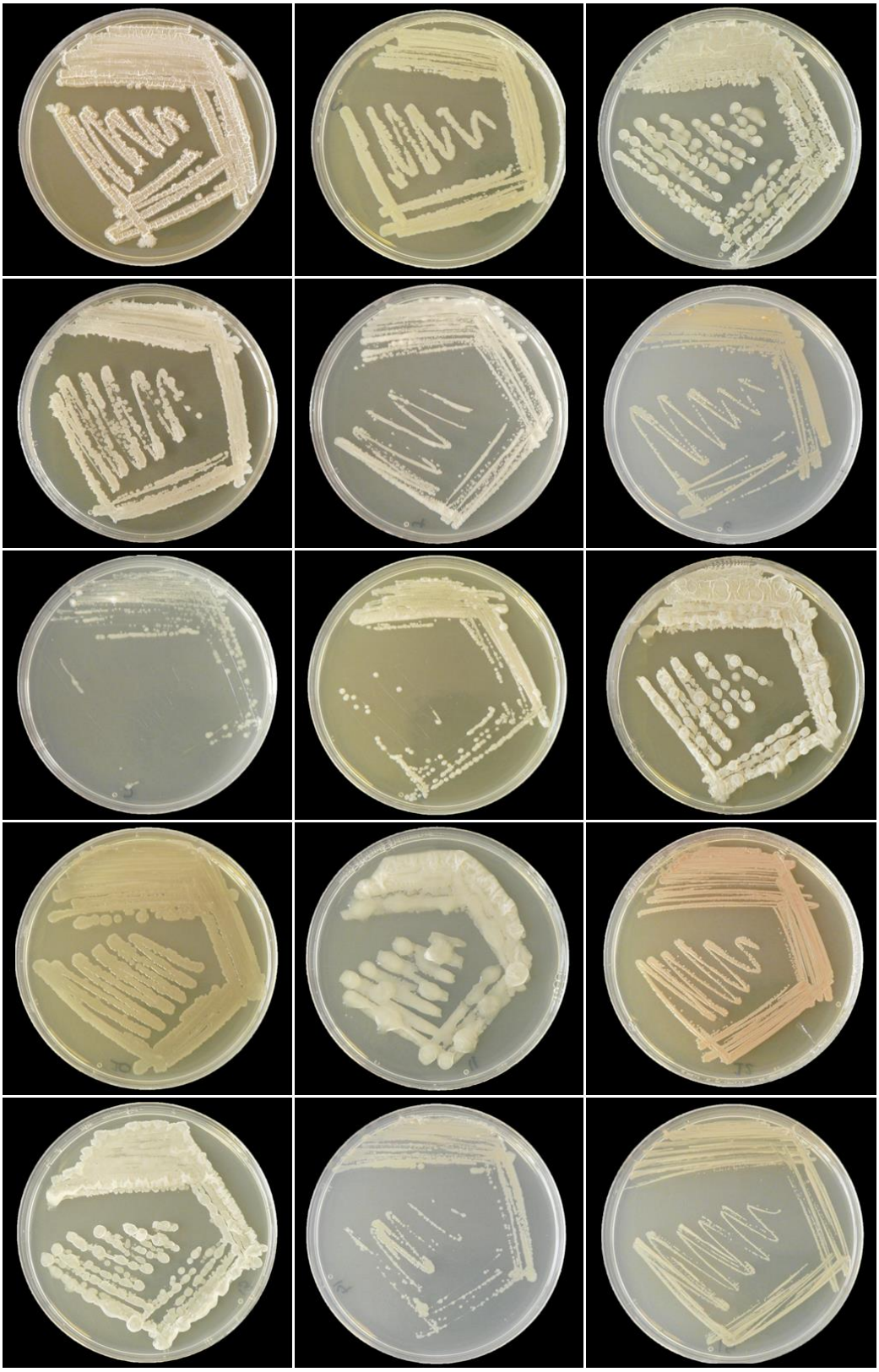


Figure 3-13. Morphologies of ELD01 – 15. From top left, ELD01, collected in Glasgow and grown on AC media. ELD02, collected in Glasgow and grown on AC media. ELD03, collected in Glasgow and grown on BHI media. ELD04, collected in Glasgow and grown on BHI media. ELD05, collected in Glasgow and grown on BHI media. ELD06, collected in Glasgow and grown on LB media. ELD07, collected at NSF and grown on AC media. ELD08, collected at NSF and grown on AC media. ELD09, collected at NSF and grown on AC media. ELD10, collected at NSF and grown on AC media. ELD11, collected at NSF and grown on BHI media. ELD12, collected in Thetford and grown on AC media. ELD13, collected in Thetford and grown on BHI media. ELD14, collected in Thetford and grown on LB media. ELD15, collected in Thetford and grown on LB media. Plates were incubated at 30°C for two days prior to imaging.

Table 3-5. Descriptive results of isolates showing antagonistic activity against medically relevant pathogens throughout the course of the project. The category of antagonistic activity is shown in column 1. Columns 2 and 3 and 4 show the number of isolates inhibiting medically relevant pathogens at different stages or at different scopes of the project. Column 2 shows the antagonistic profiles of each of the 15 isolates as they were tested in the first assay. Column 3 shows the antagonistic activity profiles of each of the 15 isolates as they were tested in the second assay. Between the first two events (Brecon and Kielder) and the final three events (Thetford, Glasgow and NSF) a different selection of ESKAPE and Medically relevant pathogens were chosen.

Activity Profile	Post-collection assay	Pre-genome sequencing assay
Gram-positive, Gram-negative and Fungi	9	3
Gram-positive and Gram-negative	2	2
Gram-positive and Fungi	1	4
Gram-negative and Fungi	2	1
Gram-positive	0	1
Gram-negative	1	3
Fungi	0	1

3.3.4 Assessing Quality of Whole Genome Sequencing of Citizen Scientists Soil Samples

Whole Genome Sequencing was carried out in collaboration with the Cooper Lab (2.5). Genomic DNA (gDNA) was extracted and DNA libraries were prepared using the Nextera® XT (Illumina, Inc.) sample preparation. Indexes, number of reads and original concentration of each sample were noted and compared to a blank to ensure adequate genomic DNA was collected (Table 3-6). Samples were sequenced using paired 300 bp reads by the group of Prof. Cooper, School of Medicine, University of East Anglia

Upon completion, sequencing data was assembled by our collaborators in the Cooper lab using the Prokka annotation pipeline V1.12 (Seemann, 2014). Assembly metrics such as number of contigs and longest read were reported to describe the sequence depth. Sequencing produced a high number of contigs (169-1538) and short read lengths, suggesting that the gDNA was fragmented (Table 3-7). This was considered in future analysis.

Table 3-6. The index, number of reads and original DNA concentration of each of the 15 isolates, as well as a blank for comparison.

Isolate Number	Index 1	Index 2	Number of reads	Original concentration (ng/ul)
ELD01	N701	S513	440,756	14.20
ELD02	N701	S515	471,386	10.40
ELD03	N702	S516	347,610	5.49
ELD04	N702	S517	520,044	10.10
ELD05	N703	S518	476,096	12.20
ELD06	N703	S520	599,602	13.00
ELD07	N704	S521	295,744	15.20
ELD08	N704	S522	448,418	7.49
ELD09	N705	S513	439,402	10.80
ELD10	N705	S515	474,272	8.48
ELD11	N706	S516	385,776	5.78
ELD12	N706	S517	456,390	2.09
ELD13	N707	S518	419,884	11.80
ELD14	N707	S520	254,366	19.50
ELD15	N710	S521	211,458	10.70
blank	N710	S522	224	0.071

Table 3-7. Assembly metrics for ELD01-15 sent for whole genome sequencing. Notably number of contigs, genome length, %GC and the longest read.

Isolate Number	no.Contigs	Genome Length	%GC	Longest
ELD01	411	4143494	43.73	80793
ELD02	457	3663406	41.57	50409
ELD03	169	4028619	46.26	128662
ELD04	232	3732733	41.35	114327
ELD05	1184	5853633	46.25	54853
ELD06	303	3430612	42.16	150587
ELD07	505	4014487	43.90	65232
ELD08	809	5894691	45.84	65647
ELD09	442	4141661	43.67	95893
ELD10	1069	5264469	40.59	56724
ELD11	305	4150537	43.54	133312
ELD12	700	5818588	62.62	156697
ELD13	736	4110725	37.43	56510
ELD14	400	3620520	41.45	69761
ELD15	1538	5827844	62.33	52042

It was decided that, in collaboration with the Cooper Lab, there was capacity to rerun three of the isolates to reduce the number of contigs and improve the read depth. ELD05, ELD08 and ELD15 were selected based on data presented in sections 3.3.5 and 3.3.6. Assembly metrics for these sequences showed better read quality. The number of contigs in ELD05 reduced from 1184 to 88, ELD08 from 809 to 44 and ELD15 from 1538 to 45. The longest read in ELD05 increased from 54853 to 752335, ELD08 from 65647 to 1385684 and ELD15 from 52042 to 539668 (Table 3-8).

Table 3-8. Assembly metrics for ELD05, ELD08 and ELD15 sent for whole genome sequencing. Notably number of contigs, genome length, %GC and the longest read.

Isolate Number	no.Contigs	Genome Length	%GC	Longest
ELD05	88	6108531	45.70	752335
ELD08	44	6020479	45.55	1385684
ELD15	45	6015083	62.36	539668

3.3.5 Discovering underexploited bacterial species using the nucleotide sequence of the 16S rRNA gene

WGS data from the first run of 15 isolates was provided by the Cooper Lab. The data was available on the Basespace (Illumina) Sequence Hub. 16S rRNA analysis was conducted using readily available analysis tools on the Basespace hub. These tools were MetaPhlAn (Segata *et al.*, 2012), 16S rRNA Metagenomics (Wang *et al.*, 2007) and Kraken Metagenomics (Wood and Salzberg, 2014). Running the 16S rRNA data through these tools provided a preliminary genus and species for each isolate (Table 3-9).

The 16S rRNA data was also extracted and analysed using the NCBI BLASTn database (Altschul *et al.*, 1990). This basic tool looks for regions of local similarity between sequences and offers several possible candidates for genus and species. Species-level classification for 16S rRNA gene sequences remains a serious challenge because existing taxonomic classification tools either do not provide species-level classification, or their classification results are unreliable (Gao *et al.*, 2017). Instead, only calls for bacterial genera were considered (Table 3-10).

Examining both tables, the 15 isolates represent four genera of bacteria. These were *Bacillus* (10/15), *Paenibacillus* (2/15), *Pseudomonas* (2/15) and *Sporosarcina* (1/15). The next step was to attempt to improve the resolution of this analysis and identify the bacterial species.

Table 3-9. 'First look' at 16*S* rRNA profile of 15 whole genome sequence isolates. Data compiled using integrated programmes within the Basespace (Illumina) software suite.

Isolate Number	MetaPhlAn analysis	16S rRNA Metagenomics	Kraken Metagenomics
ELD01	*Bacillus subtilis*	*Bacillus sp.*	*Bacillus subtilis*
ELD02		*Bacillus sp.*	
ELD03	*Bacillus amyloliquefaciens*	*Bacillus sp.*	*Bacillus amyloliquefaciens*
ELD04		*Bacillus aerophilus*	*Bacillus pumilus*
ELD05	*Paenibacillus polymyxa*	*Paenibacillus polymyxa*	*Paenibacillus polymyxa*
ELD06		*Sporosarcina sp.*	
ELD07		*Bacillus sp.*	*Bacillus subtilis*
ELD08	*Paenibacillus polymyxa*	*Paenibacillus polymyxa*	*Paenibacillus polymyxa*
ELD09	*Bacillus subtilis*	*Bacillus sp.*	*Bacillus subtilis*
ELD10		*Bacillus simplex*	
ELD11	*Bacillus subtilis*	*Bacillus sp.*	*Bacillus subtilis*
ELD12		*Pseudomonas sp.*	
ELD13		*Sporosarcina sp.*	
ELD14	*Bacillus pumilus*	*Bacillus aerophilus*	
ELD15	*Pseudomonas sp.*	*Pseudomonas sp.*	

Hits that came back as unsure are left out in this table.

Table 3-10. 16*S* rRNA comparison using NCBI BLASTn software for the 15 isolates selected for whole genome sequencing. Genera with >97% identity were reported.

Isolate Number	16S rRNA Call at Genus level using NCBI BLASTn Software >97% match		
ELD01	*Bacillus*		
ELD02	*Bacillus*		
ELD03	*Bacillus*		
ELD04	*Bacillus*		
ELD05	*Paenibacillus*	*Paenibacillus*	
ELD06	*Sporosarcina*		
ELD07	*Bacillus*		
ELD08	*Paenibacillus*		
ELD09	*Bacillus*		
ELD10	*Bacillus*	*Brevibacterium*	
ELD11	*Bacillus*		
ELD12	*Pseudomonas*		
ELD13	*Bacillus*	*Psychrobacillus*	*Renibacterium*
ELD14	*Bacillus*	*Geobacillus*	
ELD15	*Pseudomonas*		

16S rRNA data for each isolate was run through BLASTn software (Altschul *et al.*, 1990). Hits with a 97% or higher match were downloaded and imported into the MEGA software package version 7.0 (Kumar, Stecher and Tamura, 2016), along with the 16S rRNA data for each of the 15 isolates. GenBank (Benson *et al.*, 2013) was queried to identify the type strain for each species.

The nucleotide sequences of the 16S rRNA gene from ELD01 to ELD15 and the type strain of each of the species identified in the BLASTn search were determined and a phylogenetic trees based on these data were constructed by the NJ method (Figure 3-14 and Figure 3-15) using the genetic distances. Trees were routed on an outgroup, an organism not closely related to the isolates of interest which provides perspective for the level of relationship between closely matched genus and species. As 16S rRNA analysis had identified both Gram-positive and Gram – bacteria, two trees were made. The first compared the Gram-negative bacteria (Figure 3-14) and the second Gram-positive bacteria (Figure 3-15).

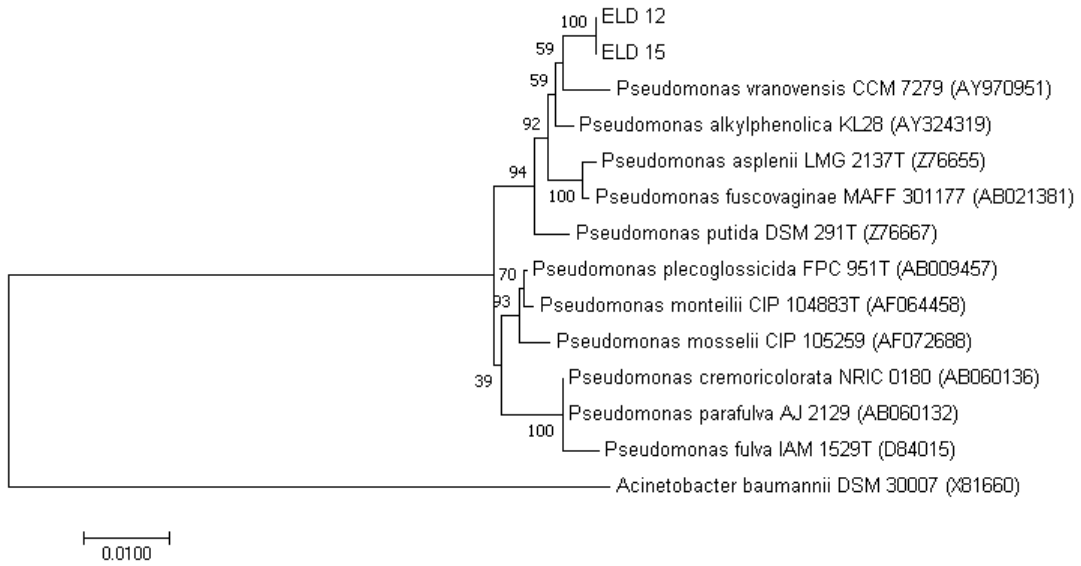


Figure 3-14. Gram-negative phylogenetic tree with ELD 12 and 15. Outgroup-rooted maximum-likelihood (ML) phylogenetic tree of 16rRNA sequences from *Pseudomonas* spp. and related species from environmental isolates (ELD12 and ELD15. Trees were constructed by using the neighbour-joining method and genetic distances were computed. Numerical values represent the percentage of bootstrap replications that support the respective node.

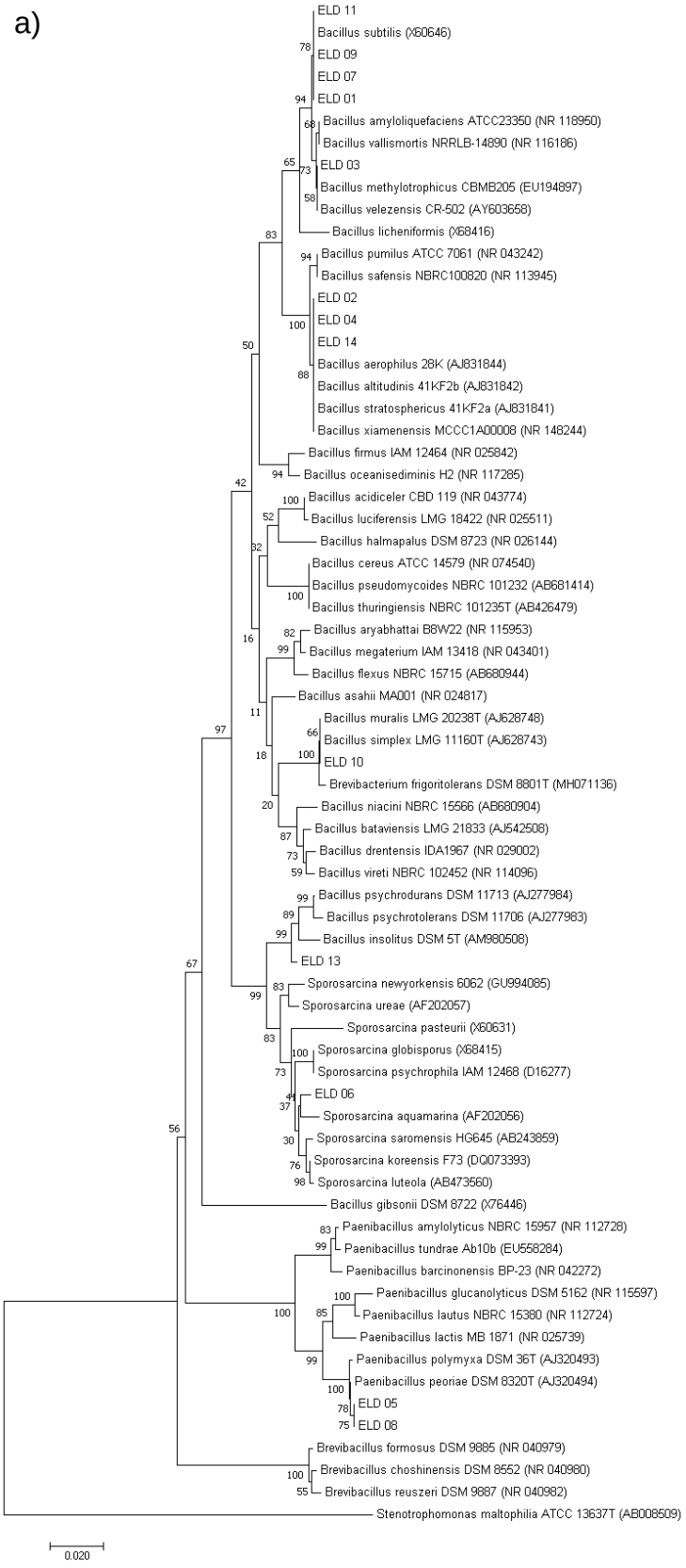
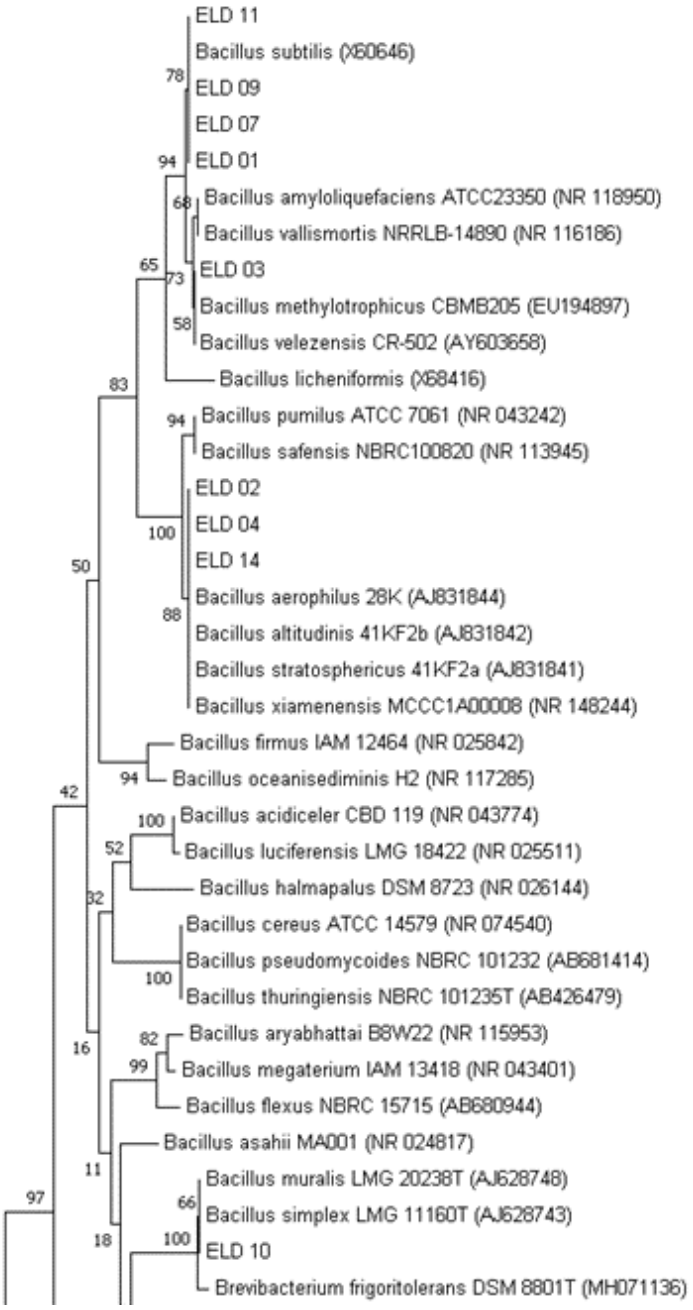
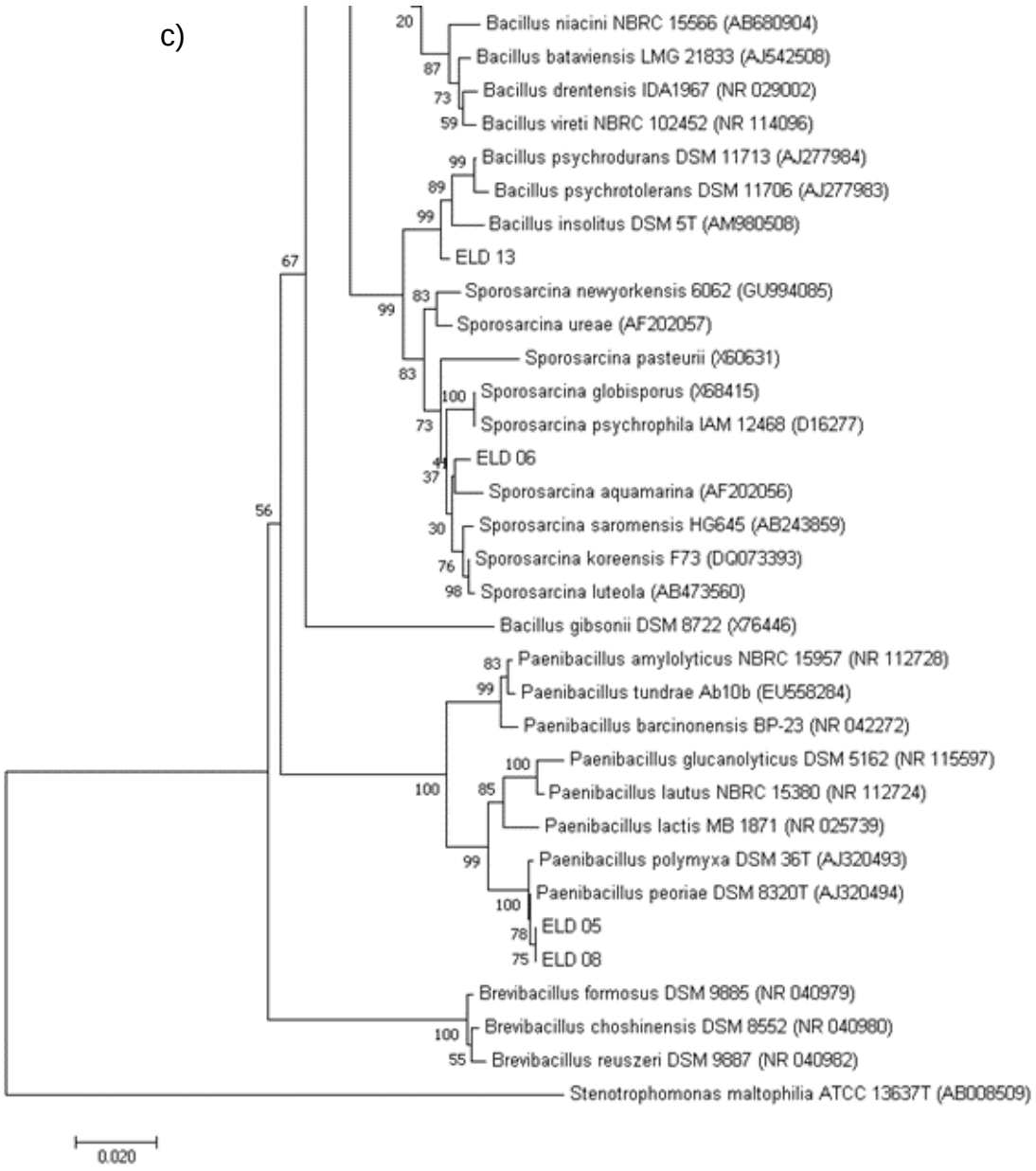


Figure 3-15. Gram-positive phylogenetic tree with ELD01-11, 13 and 14. Outgroup-rooted maximum-likelihood (ML) phylogenetic tree of 16rRNA sequences from *Bacillus* spp, *Sporosarcina* spp, *Paenibacillus* spp and *Brevibacillus* spp., as well as related species from environmental isolates (ELD01-ELD11, ELD13 and ELD14). Trees were constructed by using the neighbour-joining method and genetic distances were computed. Numerical values represent the percentage of bootstrap replications that support the respective node. Inset of overall tree (a) which has been split into 2 (b – top and c – bottom) for display purposes.

b)


ELD 11
Bacillus subtilis (X60646)
78
ELD 09
ELD 07
94
ELD 01
68
Bacillus amyloliquefaciens ATCC23350 (NR 118950)
Bacillus vallismortis NRRLB-14890 (NR 116186)
65
73
ELD 03
Bacillus methylotrophicus CBMB205 (EU194897)
58
Bacillus velezensis CR-502 (AY603658)
Bacillus licheniformis (X68416)
83
94
Bacillus pumilus ATCC 7061 (NR 043242)
Bacillus safensis NBRC100820 (NR 113945)
ELD 02
100
ELD 04
50
ELD 14
88
Bacillus aerophilus 28K (AJ831844)
Bacillus altitudinis 41KF2b (AJ831842)
Bacillus stratosphericus 41KF2a (AJ831841)
Bacillus xiamenensis MCCC1A00008 (NR 148244)
Bacillus firmus IAM 12464 (NR 025842)
94
Bacillus oceanisediminis H2 (NR 117285)
42
100
Bacillus acidiceler CBD 119 (NR 043774)
52
Bacillus luciferensis LMG 18422 (NR 025511)
Bacillus halmapalus DSM 8723 (NR 026144)
32
Bacillus cereus ATCC 14579 (NR 074540)
Bacillus pseudomycoides NBRC 101232 (AB681414)
100
Bacillus thuringiensis NBRC 101235T (AB426479)
16
82
Bacillus aryabhattai B8W22 (NR 115953)
99
Bacillus megaterium IAM 13418 (NR 043401)
Bacillus flexus NBRC 15715 (AB680944)
Bacillus asahii MA001 (NR 024817)
11
Bacillus muralis LMG 20238T (AJ628748)
97
66
Bacillus simplex LMG 11160T (AJ628743)
18
100
ELD 10
Brevibacterium frigoritolerans DSM 8801T (MH071136)

c)


Bacillus niacini NBRC 15566 (AB680904)
Bacillus bataviensis LMG 21833 (AJ542508)
Bacillus drentensis IDA1967 (NR 029002)
Bacillus vireti NBRC 102452 (NR 114096)
Bacillus psychrodurans DSM 11713 (AJ277984)
Bacillus psychrotolerans DSM 11706 (AJ277983)
Bacillus insolitus DSM 5T (AM980508)
ELD 13
Sporosarcina newyorkensis 6062 (GU994085)
Sporosarcina ureae (AF202057)
Sporosarcina pasteurii (X60631)
Sporosarcina globisporus (X68415)
Sporosarcina psychrophila IAM 12468 (D16277)
ELD 06
Sporosarcina aquamarina (AF202056)
Sporosarcina saromensis HG645 (AB243859)
Sporosarcina koreensis F73 (DQ073393)
Sporosarcina luteola (AB473560)
Bacillus gibsonii DSM 8722 (X76446)
Paenibacillus amylolyticus NBRC 15957 (NR 112728)
Paenibacillus tundrae Ab10b (EU558284)
Paenibacillus barcinonensis BP-23 (NR 042272)
Paenibacillus glucanolyticus DSM 5162 (NR 115597)
Paenibacillus lautus NBRC 15380 (NR 112724)
Paenibacillus lactis MB 1871 (NR 025739)
Paenibacillus polymyxa DSM 36T (AJ320493)
Paenibacillus peoriae DSM 8320T (AJ320494)
ELD 05
ELD 08
Brevibacillus formosus DSM 9885 (NR 040979)
Brevibacillus choshinensis DSM 8552 (NR 040980)
Brevibacillus reuszeri DSM 9887 (NR 040982)
Stenotrophomonas maltophilia ATCC 13637T (AB008509)
0.020

ELD12 and ELD15 had originally been called as part of the *Pseudomonas* genus belonging to the Phylum Proteobacteria using the Basespace hub. Figure 3-14 shows that these Gram-negative isolates were most closely related to *Pseudomonas vranovensis*, however they were more closely related to each other than they were their nearest relative. For this reason, ELD15 was selected for the second genome sequencing run to improve the read depth (3.3.4). *Pseudomonas vranovensis* is a Gram-negative soil bacterium (Tvrzová *et al.*, 2006).

All Gram-positive bacteria had been called as part of the Phylum firmicutes. ELD01, 02, 03, 04, 07, 09, 10, 11, 13 and 14 had been called as part of the *Bacillus* genus. Figure 3-15 showed that these Gram-positive isolates represented four distinct *Bacillus* spp. ELD01, 07, 09 and 11 were most closely related to *Bacillus subtilis*. ELD 03 was most closely related to *Bacillus velezensis*. ELD02, 04 and 14 were most closely related to *Bacillus altitudinis*. ELD10 was most closely related to *Bacillus simplex*. ELD13 was most closely related to *Bacillus psychrodurans*. ELD05 and ELD08 were originally called as *Paenibacillus* spp, now known to be most closely related to *Paenibacillus peoriae*. Finally, ELD06, called as *Sporosarcina* spp was shown to be most closely related to *Sporosarcina aquamarina*.

Bacillus subtilis (ELD01, 07, 09, 11) is a Gram-positive bacterium which inhabits the upper layers of the soil (Van Dijl & Hecker., 2013). *Bacillus simplex* (ELD10) is a Gram-positive bacterium which is ubiquitous in nature. A 2013 article showed isolation of one of these bacterium from the rhizosphere of a plant (Schwartz *et al.*, 2013). *Bacillus velezensis* (ELD03) is an aerobic, Gram-positive bacterium which is an important member of the nutrient-rich zone of soil around plant roots, known as the rhizosphere (Rabbee *et al.*, 2019). *Bacillus altitudinis* (ELD02, 04, 14) is a species of bacteria first isolated from cryogenic tubes for collecting air samples from high altitudes (Shivaji *et al.*, 2006). Like the other *Bacillus* spp, it is ubiquitous. It has been discovered in marine soil (Jin *et al.*, 2012) and recently, the rhizosphere of rice (Budiharjo et al., 2017). *Bacillus psychrodurans* (ELD13) is a psychrotolerant *Bacillus* species categorised in 2002 (Abd El-Rahman et al., 2002). Research has reported this bacterium in only limited locations.

Paenibacillus peoriae (ELD05, 08) is a genus of Gram-positive bacteria originally included within the genus *Bacillus* before being reclassified as separate in 1993 (Ash et al., 1993). *Paenibacillus* bacteria have been detected in a variety of environments, not least soil (Padda et al., 2017). *Sporosarcina aquamarina* (ELD06) represents a genus of Gram-positive, endospore forming bacteria. Species of this genus are known to inhabit soil (Eitinger et al., 2014).

The 15 isolates selected for this study represent two Phyla, four Genera and eight species and represent both Gram-positive and Gram-negative organisms.

3.3.6 Discovering Interesting Antibiotics

WGS data from the first run of 15 isolates (ELD01 to ELD15) was analysed using antiSMASH 3.0 (Weber *et al.*, 2015). This uses the antibiotics and Secondary Metabolite Analysis Shell algorithm to predict biosynthetic gene loci

for secondary metabolites. Gene prediction was performed by Glimmer3 (Delcher *et al.*, 2007). A search was conducted using 'KnownClusterBlast', 'SmCOG analysis', 'ActiveSite finder' and 'SubClusterBlast' to identify the secondary metabolite BGCs. Probabilistic detection was turned off. A representation of the number of BGCs appearing in each isolate can be seen in Figure 3-16. The number of distinct types of BGC are represented by the different colours.

Figure 3-16. Gene cluster predictions provided by the antiSMASH algorithm. The number refers to the isolate (ELD01-15). FASTA files were uploaded to the antiSMASH 3.0 (Weber *et al.*, 2015) web server. Gene prediction was performed by Glimmer3 (Delcher *et al.*, 2007). A search was conducted using 'KnownClusterBlast', 'SmCOG analysis', 'ActiveSite finder' and 'SubClusterBlast' to identify the secondary metabolite BGCs. Probabilistic detection was turned off. Coloured circles do not represent specific types of cluster, but instead identify individual BGCs. Where groups of same coloured clusters are together, these are the same type of cluster (e.g. ELD01, green clusters together are all NRPs). Provided as an overview of abundance and diversity of BGCs in each isolate.

There were 21 different types of secondary metabolite called across the 15 isolates (Table 3-11). Within these 21, nonribosomal peptide synthetase clusters (NRPS) were the dominant type. They represented five of the 21 types of secondary metabolite, yet 110 of the total 210 total BGCs. 96 of these were NRPS only, whilst the remaining 14 were NRPS linked with another type of secondary metabolite, such as Polyketide Synthase (PKS), shown as transAT-PKS-NRPS.

On average, isolates contained 14 gene clusters. This ranged from two gene clusters in ELD13, to 39 gene clusters in ELD08. The two Gram-negative isolates averaged 10.5 gene clusters, slightly lower than the 14.5 average in Gram-positives. On average, isolates were predicted to have six different types of antibiotic or secondary metabolite BGCs.

Isolates belonging to the genus *Paenibacillus* had the two highest predictions for numbers of gene clusters, 30 (ELD05) and 39 (ELD08). ELD08 had the most diverse range of antibiotics and secondary metabolite clusters, with

eleven predicted. For this reason, ELD05 and ELD08 was selected for the second genome sequencing run to improve the read depth (3.3.4). ELD06 and ELD13 had the least diverse range, containing only two types of cluster. ELD 06 and ELD13 were shown to represent different genera and species of bacteria but had similar secondary metabolite profiles. Both had only two distinct types of cluster, Type III PKS (T3PKS) and Terpene. ELD13 had one of each cluster, whilst ELD06 had one T3PKS and two Terpenes. ELD04, like ELD13, had one secondary metabolite cluster in each of its called types of cluster, however it had six types of cluster in total. These were Bacteriocin (or other unspecified ribosomally synthesised and post-translationally modified peptide product clusters (RiPP)), NRPS, other (cluster containing a secondary metabolite-related protein that does not fit into any other category), T3PKS, Terpene and Terpene-Siderophore. Further details of the types of clusters called for each of the isolates can be found in Table 3-11.

Table 3-11. Summary of BGCs in each of the 15 isolates. FASTA files were uploaded to the antiSMASH 3.0 (Weber *et al.,* 2015) web server. Gene prediction was performed by Glimmer3 (Delcher *et al.,* 2007). A search was conducted using 'KnownClusterBlast', 'SmCOG analysis', 'ActiveSite finder' and 'SubClusterBlast' to identify the secondary metabolite BGCs. Probabilistic detection was turned off. The number of BGCs and the type of BGCs are represented against the isolate number and the genus and species, as shown using a 16*S* rRNA phylogenetic trees and the neighbour-joining method.

Isolate	Genus and species	No. Antismash Clusters	Identified BGCs
ELD01	*Bacillus subtilis*	16	Ladderane, NRPS, NRPS-transAT-PKS-otherpks, other, sactipeptide-head to tail, T3PKS, terpenes
ELD02	*Bacillus altitudinis*	8	NRPS, Other, siderophore, T3PKS, terpenes
ELD03	*Bacillus velezensis*	20	Bacteriocin, NRPS, other, otherKS, phosphonate, T3PKS, terpenes, transAT-PKS, transAT-PKS-NRPS
ELD04	*Bacillus altitudinis*	6	Bacteriocin, NRPS, other, T3PKS, terpenes, terpene-siderophore
ELD05	*Paenibacillus peoriae*	30	Bacteriocin, lassopeptide, NRPS, NRPS-transAT-PKS-otherKS, other, phosphonate, siderophore, transAT-PKS, transAT-PKS-NRPS
ELD06	*Sporosarcina aquamarina*	3	T3PKS, terpenes
ELD07	*Bacillus subtilis*	17	NRPS, NRPS-transAT-PKS-other's, other, sactipeptide-head to tail, T3PKS, terpenes, transAT-PKS, transAT-PKS-NRPS
ELD08	*Paenibacillus peoriae*	39	Bacteriocin, lanthipeptide, lassopeptide, NRPS, NRPS-transAT-PKS-otherKS, other, phosphonate, siderophore, T1PKS-NRPS, transAT-PKS, transAT-PKS-NRPS
ELD09	*Bacillus subtilis*	15	Lanthipeptide, NRPS, other, sactipeptide, T3PKS, terpenes, transAT-PKS-otherKS-NRPS
ELD10	*Bacillus simplex*	9	Bacteriocin, NRPS, other, siderophore, T3PKS, terpene
ELD11	*Bacillus subtilis*	15	Lanthipeptide, NRPS, other, sactipeptide-head to tail, T3PKS, terpene, transAT-PKS-otherKS-NRPS
ELD12	*Pseudomonas vranovensis*	8	Arylpolyene, NRPS, other,
ELD13	*Bacillus psychrodurans*	2	T3PKS, terpenes
ELD14	*Bacillus altitudinis*	10	Bacteriocin, NRPS, other, siderophore, T3PKS, terpene
ELD15	*Pseudomonas vranovensis*	13	Arylpolyene, NRPS, other, ppysks,

3.4 Discussion and future work

3.4.1 Development of field and lab methods to isolate antibiotic producing bacteria from soil

The Small World initiative has instructed over 10,000 students on how to collect bacteria from soil samples across 45 U.S Sates and 15 countries (Small World Initiative., 2019). This protocol (Hernandez, Tsang and Handelsman, 2015) has been extensively field-tested. However, Antibiotics Unearthed aimed to move student learning out in to the public and as such, the protocol needed to be optimised and modified to reflect that. Modifications considered the nature of asking citizen scientists to experiment *in-situ*. This meant the strict and aseptic techniques had to be flexible, whilst repeatable. Further, the aim was to discover potentially novel bacteria producing potentially novel antimicrobials and as such, the protocol was optimised towards identifying underexploited organisms and improving abundance and diversity of antagonistic isolates producing antibiotics or secondary metabolites. Results of these modifications and optimisations are presented in Figure 3-8.

Antimicrobial drug discovery is particularly difficult (Payne et al., 2007). Some of the barriers to antimicrobial drug discovery are beyond the scope of this project, such as the need for antibiotics to pursue molecular targets not prone to rapid resistance development and the need for chemical diversity which can overcome barriers to bacterial entry and efflux mechanisms especially in the Gram-negative organisms (Silver., 2011). One barrier that this project aimed to target was that of the uncultured organisms. Approximately 99% of all species in external environments do not grow under laboratory conditions (Lewis, 2013). This is a promising source of novel antibiotics (Lewis et al., 2010), as was highlighted by the discovery of Teixobactin (Ling *et al.*, 2015). Whilst diluents, dilutions, growth temperature and fungal contamination were all optimised, growth media received the greatest focus. SEM has a long history of giving higher plate counts and encouraging growth of otherwise unculturable bacterial isolates compared to liquid media without soil-extract (Taylor, 1951; Nguyen *et al.*, 2018).Therefore, part of the method development of the Antibiotics Unearthed project attempted to incorporate the use of SEM into the protocol. However, whilst original results were encouraging, soils taken from event locations did not act as a successful growth media and this protocol was removed. Instead, AC and BHI media were selected from the Small World Initiative's list of media for successful growth of a range of soil organisms (Hernandez, Tsang and Handelsman, 2015).

The most significant problem posed by the antagonistic drop-plate assay was bacterial motility. Whilst a lawn was made of the medically relevant indicator strain, the droplets of antagonistic isolate were required to grow only in the location they were placed. Bacteria can swarm over the agar surface (Kearns, 2010). In lab strains, swarming motility has been selected against, however natural isolates are often motile. Whilst some bacteria can swarm over nearly any agar surface, most swarming bacteria require soft agar in a narrow range of agar concentrations (Kearns, 2010). Agar concentrations between 0.5% and 2% have been shown as ideal for bacterial swarming over the agar surface

(Harshey, 1994). To prevent this, 4% agar was selected for the antagonistic assays which successfully prevented this bacterial swarming motility. To improve the drop-plate assay further, overnight cell density of the clinically relevant indicator strains could be controlled to ensure that antagonistic activity, no matter how weak, could be identified clearly. Future work may consider the length of growth time before maximum antimicrobial activity is observed (Von Der Weid *et al.*, 2003).

Over five events, 454 soil samples were collected. Table 3-3 shows the story of these 454 soil samples. Of these 454 samples, 299 were collected at the first two events. Despite this large number of samples, less colonies which showed antagonistic activity against neighbouring soil colonies (204) and medically relevant pathogens (40) were isolated than in the remaining three events. The number of soil samples collected was reduced after the first two events to increase accuracy in the lab, allowing more time to be spent on each sample. This, along with further optimised protocol (using AC and BHI rather than SEM) resulted in more antagonistic colonies per plate. The modified and optimised protocol resulted in the analysis of a total of 929 bacterial isolates that showed antagonistic activity against neighbouring soil organisms and the selection of a total of 165 isolates which showed antagonistic activity against one or more medically relevant pathogens (Table 3-3). 15 isolates were selected for further analysis.

3.4.2 Whole Genome Sequencing

High throughput WGS has transformed the study of microbial genomics, not least through bacterial identification notably in infection control (Hasman et al., 2014; Walker et al., 2013), antibiotic resistance (Pallen et al., 2010) and antibiotic discovery (Ziemert et al., 2016). In collaboration with the Cooper Lab, WGS was used to characterise 15 antagonistic isolates, ELD01-15.

Double-barrelled shotgun sequencing utilises pairs of reads obtained from both ends of inserts of various sizes (Sundquist et al., 2007) which assists with assembling complex genomes. Illumina's MiSeq provided paired 300 bp read lengths. One of the limitations of reading comparatively short lengths of DNA is that more reads must be done to cover the same sequence. Further, stitching the results together into longer genomic sequences is a lot more complicated (Rogers and Venter, 2005). Producing high-quality assemblies with short reads is a challenge for bacterial genomes (Chaisson et al., 2004). This limitation was noticeable in the first round of sequencing which produced highly variable densities of reads across the length of the 15 genomes (Table 3-7). This must be considered when examining the results of analysis in the remaining sections.

A successful attempt was made to reanalyse the DNA from initial DNA extraction of three of the isolates. These three isolates were chosen based on phylogenetic analysis (3.3.5) and identification of BGCs (3.3.6). The Cooper Lab calculated recommended coverage and reran the samples, successfully reducing the number of contigs and increasing the depth of coverage (Table 3-8). Analysis of this data is part of future work for publication.

3.4.3 Discovery of interesting bacteria producing interesting antimicrobials

Actinomycetes have been overmined due to their substantial number of BGCs (Rutledge and Challis, 2015) and the ease at which broad spectrum antibiotics, particularly effective on troublesome Gram-negative pathogens, can be discovered (Baltz., 2007). However other underexploited bacteria have had to develop their own compounds to act against their competitors (Lewis, 2020). In this study, ELD01-15 were identified as eight species of bacteria (Figure 3-14, Figure 3-15): *Bacillus subtilis* (ELD01, 07, 09 and 11), *Bacillus altitudinis* ELD02, 04 and 14), *Bacillus velezensis* (ELD03), *Bacillus simplex* (ELD10), *Bacillus psychrodurans* (ELD13), *Paenibacillus peoriae* (ELD05 and 08), *Sporosarcina aquimarina* (ELD06) and *Pseudomonas vranovensis* (ELD12 and 15). Each of these bacteria are known soil organisms. Importantly, these organisms are not part of the overmined actinomycetes.

Underexploited bacteria have been shown to devote sizeable parts of their genome to BGCs (Crits-Christoph *et al.*, 2018). In this study, the use of genome mining software allowed the identification of a substantial number of BGCs in ELD01-ELD15.

3.4.3.1 Bacillus spp.

Of the eight species of bacteria, five are of the genus *Bacillus*. The genus *Bacillus* comprises 403 species (last update in November 2020) of Gram-positive, rod-shaped bacteria (Gordon et al.,1973). Like streptomycetes and fungi, members of the genus *Bacillus* are characterized by the presence of many genes coding for biosynthesis of secondary metabolites. The type species is *Bacillus subtilis* (Ehrenberg CG, 1835).

Whilst not a member of the actinomycetes, *Bacillus* spp*.,* are common lab strains and as such, their antibiotic production has been well documented. As predicted, the *Bacillus* isolates produced a wide range of antimicrobial compounds (AMCs). For any given strain of the *Bacillus* group, it is now estimated that at least 4–5% of its genome is devoted to AMCs production (Stein, 2005). The main clades of AMCs within the *Bacillus* group are the ribosomal peptides (RPs) (bacteriocins and enzymes), the PKS, the NRPs and the volatiles (Caulier *et al.*, 2019). This aligns with the laboratory results which indicated a broad spectrum of activity against Fungi, Gram-positive and Gram-negative bacteria.

To focus this discussion, I concentrate in more detail on the lesser explored species isolated in this project, namely *Paenibacillus* spp (3.4.3.2), *Sporosarcina* spp (3.4.3.3)., and *Pseudomonas* spp (3.4.3.4).

3.4.3.2 Paenibacillus spp.

The genus *Paenibacillus* comprises 262 species (last update in November 2020) of Gram-positive, rod-shaped, facultatively anaerobic bacteria (Ash, Priest and Collins, 1993). ELD05 and 08, identified as *Paenibacillus peoriae*, inhibited different indicator strains. ELD 05 inhibited *Candia albicans*, *Salmonella* WT, *Salmonella* DT104 and VRE. ELD08 however only inhibited MRSA. I note in the first round of testing, ELD08 also inhibited *Salmonella* WT

and *Salmonella* DT104. ELD05 was collected from Glasgow, where ELD08 was collected from the NSF.

Paenibacillus peoriae was first isolated from soil (Montefusco et al., 1993). The antibiotic and secondary metabolite production of this species is not well published, however *P. peoriae* generally shows a broad inhibition spectrum with activity against several taxonomic groups of bacteria and fungi (Von Der Weid *et al.*, 2003). For a more comprehensive understanding of the known secondary metabolite and antibiotic production of *Paenibacillus,* I look to the type species *Paenibacillus polymyxa* (Ash, Priest and Collins, 1993).

Several members of the genus *Paenibacillus* have been reported for the production of diverse antimicrobial peptides (Baindara et al., 2020). Genome sequencing of *P. polymyxa* strains revealed numerous antibiotic biosynthetic genes in the genome encoding NRPS, PKS and bacteriocins (Jeong et al., 2019).

Of the NRPS*, P. polymyxa* strains produce polymyxins (bactericidal activity against Gram-negative bacteria), Fusaricidins (Gram-positive and fungi) and Tridecaptins (Gram-negative). The NRPS-Trans-AT-PKS Paenilipoheptin is an antibiotic whose activity spectrum is yet to be elucidated . The lantibiotic Paenilan is effective against Gram-positive bacteria (Jeong et al., 2019), and has also been called antimicrobial (Daud *et al.*, 2019).

Genome analysis revealed the presence of additional, yet uncharacterized, antibiotic BGCs including Trans-AT-PKS-OtherKS-NRPS and lassopeptide gene clusters (Jeong et al., 2019).

Paenibacillus polymyxa produces antimicrobial volatile organic compounds (Benzothiazole, benzaldehyde, undecanal, dodecanal, hexadecanal, 2-tridecanone and phenol), bactericidal Lipopeptides (Polymyxin E1 & E2) and antimicrobial Lipopeptides (Polymyxin B). It also produces iron chelating Siderophore (Hydroxamate) (Daud *et al.*, 2019).

Several works have shown that strains of *P. polymyx*a can produce different antimicrobial substances effective against fungi and bacteria (Dijksterhuis et al., 1999; Piuri et al., 1998; Rosado & Seldin, 1993), including human pathogenic bacteria and fungi (Seldin et al., 1999). Recently, *P. polymyxa* has shown antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* (El-Rahman et al., 2020).

ELD05 was predicted to contain BGCs for Trans-AT-PKS-NRPS, NRPS-Trans-AT-PKS-otherKS, Lassopeptide, NRPs, Siderophore, Trans-AT-PKS, Phosphonate and Bacteriocin. Phosphonate, not mentioned above, is a gene cluster responsible for bacterial degradation of phosphonates which releases biologically available phosphate. This is important as phosphate is a major limiting nutrient in soils and a large proportion of phosphate in soils is sequestered in mineral compounds. This has been shown as present in *P. polymyxa* (Eastman et al., 2014).

ELD08 was also predicted to contain BGCs for NRPs, Trans-AT-PKS, Trans-AT-PKS-NRPS, Siderophore, NRPS-Trans-AT-PKS-otherKS, Phosphonate, Bacteriocin and Lassopeptide. Additionally, it was predicted to contain a BGS

for a T1PKS-NRPs and a Lantipeptide. These have all been shown to occur in *Paenibacillus* spp.

3.4.3.3 Sporosarcina spp.

The genus *Sporosarcina* comprises 18 species (last update in November 2020) of Gram-positive, non-pathogenic, spore-forming, facultatively aerobic bacteria (Kluyver and van Niel., 1936; Yoon *et al.*, 2001). ELD06, identified as *Sporosarcina aquimarina*, inhibited VRE and *Candida albicans*. Research on the specific antibiotics or secondary metabolites produced by *Sporosarcina* spp is limited and would make a good target for future work. *Sporosarcina aquimarina* has been shown to produce the enzyme urease (Jiang et al., 2016). *Sporosarcina* spp have also been preliminarily proposed for use in probiotics for poultry (Priyodip and Balaji, 2019). A key component of validating an organism's probiotic potential is its ability to show antagonistic activity against enteric pathogens. The activity against Gram-variable enteric pathogens was hypothesised as the production of broad-spectrum bacteriocins, as seen in closely related *Bacillus* spp (Priyodip and Balaji, 2019). ELD06 was predicted to contain BGCs for Terpene and T3PKS. To my knowledge, these are clusters which have not yet been shown to be produced by *Sporosarcina* spp.

3.4.3.4 Pseudomonas spp.

The genus *Pseudomonas* comprises 302 species (last update in November 2020) of rod shaped, Gram-negative bacteria (Migula, 1894; Krieg, 1984). Members of this genus inhabit a wide variety of environments due to their metabolic capacity and broad potential for adaptation to different conditions (Moradali et al., 2017). ELD12 and 15, identified as Gram-negative *Pseudomonas vranovensis*, inhibited *Candida albicans*. ELD12 also inhibited MRSA. ELD12 was grown on AC, whilst ELD15 on LB. Both ELD12 and ELD15 were samples from Thetford. *Pseudomonas vranovensis* was proposed as a novel species by Tvrzová et al (2006) and described as Gram-negative, non-spore-forming rods. This species of *Pseudomonas*, along with its antibiotic and secondary metabolite production is not well published. Instead, I discuss the known secondary metabolite and antibiotic production of *Pseudomonas* spp.

Pseudomonas spp produce a spectrum of structurally diverse peptides. Many of these are synthesized by large, multifunctional proteins called non-ribosomal peptide synthases (NRPS). These include iron-chelating siderophores such as Pyochelin, Pseudomonine and Pyoverdines. It also includes lipopeptides (antifungal, antibacterial and antiviral), Safracin (anti-tumour) and Pyrrolnitrin (antifungal) (Gross and Loper, 2009).

Polyketides are a large class of structurally diverse natural products that include compounds with antibiotic, chemotherapeutic, and phytotoxic activities. Collectively, polyketide biosynthesis in *Pseudomonas* spp involves each of the three types of PKS. These are type I, type II and type III (Gross and Loper, 2009). *Pseudomonas* species are also known to produce hybrid NRPS-PKS compounds (Gross and Loper, 2009).

Other known compounds include Phenazines (antibiotic, antitumor, and antiparasitic activity), Quinolones (antibacterial) and Hydrogen Cyanide (extremely poisonous to most organisms) (Gross and Loper, 2009).

Genome-guided strategies have increased the appreciation for the metabolic potential of the Pseudomonads, especially once the prevalence of secondary metabolite gene clusters in their genomes was discovered. Notable compounds discovered in this way include the Orfamides (antifungal) and the Rhizoxins (phytotoxic, antifungal and antitumoral) (Gross and Loper, 2009).

ELD12 was predicted to contain gene clusters for production of NRPS and Aryl polyene. NRPS are common in Pseudomonads. Aryl polyenes, structurally similar to the well-known carotenoids and providing a protective function against reactive oxygen species (Schöner *et al.*, 2016), are highly abundant in bacteria and have been discovered in *Pseudomonas spp* (Trantas *et al.*, 2015; Schöner *et al.*, 2016). ELD15 was predicted to contain gene clusters for production of NRPS and Aryl polyene, but also for PpyS-KS. PpyS-KS, a PPY-like pyrone cluster has been found in other *Pseudomonas spp* using antimicrobial cluster mining (Steiner *et al.*, 2020). Known biological functions of PpyS-KS include intermediates and end products in primary metabolism and signalling molecules and molecules which are applied for defence against competitors and predators. The biological activities these compounds exhibit includes antimicrobial, antitumor and cytotoxic activities (Schäberle, 2016).

3.4.4 Future Work

With regards to taking a bioinformatic approach to analysis, there are several avenues for future work. In order to improve the read depth of genome sequences, all 15 isolates could be sent for further WGS. Calculating the gDNA required to obtain deeper read coverage proved successful on the three select isolates. Further, a method of sequencing which obtains longer reads than the 300 bp used by the Illumina MiSeq would assist in stitching together longer genome sequences (Rogers and Venter, 2005). Moss, Maghini and Bhatt (2020) used nanopore sequencing with read lengths on average 20 times longer than our Illumina reads to assemble seven genomes into single contigs. Often a mix of short and long read sequences can provide the optimum mix of nucleotide accuracy and read lengths.

Complete genomes would increase confidence in antiSMASH and phylogenetic data. Phylogenetic data could be further improved through comparison of other highly conserved genes. Research often compared *gyrB*, *rpoB* and *rpoD* genes as well as 16S rRNA. Yamamotot and Harayama (2017) used 16S rRNA, *gyrB* and *rpoD* genes to examine the phylogenetic relationships of *Pseudomonas putida* strains. It is also common to concatenate 16S rRNA, *gyrB*, *rpoD* and *rpoB* genes as shown by Wang *et al* (2020) to identify a novel species of *Pseudomonas*, *Pseudomonas laoshanensis*.

To compare the similarity of whole genomes, rather than specific conserved genes, one could also use Average Nucleotide Identify (ANI) to get a measure for genome sequence similarity (Lee *et al.*, 2016) or Genome-Genome Distance (GGD), an *in-silico* procedure to calculate the closeness between genomes (Srivastava *et al.*, 2020). Synteny, that is where in the genome is

conserved or divergent, can be measured with software such as BLAST Ring Image Generator (BRIG) (Alikhan *et al.*, 2011) and CGView Comparison Tool (CCT) (Grant et al., 2012).

Further culture-dependent approaches could also be explored with these isolates. A simple exploration would be to expand the bioassays against a broader spectrum of sensitive and drug resistant isolates of important clinical pathogens. One might start by testing against the bacteria labelled as of serious or urgent concern by the CDC (Table 1-3). For example, Ling *et al* (2015) tested the potency of their novel antibiotic Teixobactin against *Mycobacterium tuberculosis.*

Beyond bioassays, purification and identification of the secondary metabolites with antimicrobial activity needs to occur. Liquid Chromatography Mass Spectrometry (LC-MS) on culture's supernatants could be conducted to determine antibiotics produced by an isolate. Do *et al (*2020) used this approach to identify eight antibiotics in aquaculture and river water samples in Vietnam. Vikeli *et a*l (2020) used LC-MS to detect production of Kyamicin from sample extracts and downstream chemistry can purify active fractions and eventually active compounds. Further, in the discovery of a new *Streptomyces* species, *Streptomyces formicae*, Z. Qin *et al* (2017) used high-resolution LC-MS and metabolomics to identify the presence of novel metabolites in fractions exhibiting antibacterial and antifungal activities. They used high resolution Electrospray-tandem-mass spectrometry (ESI-MS) and Nuclear Magnetic Resonance (NMR) data to identify the structure of the novel metabolite (Z. Qin *et al.*, 2017). Once potentially novel metabolites have been identified, one could test for spontaneous resistance, for example against MRSA as it was in Z. Qin *et al* (2017).

If the isolate was genetically tractable, inducing a knock-out mutation in the BGC expected to code for antibiotic production would allow us to determine if the cluster in question was solely responsible for the antimicrobial potential of the isolate or whether multiple active compounds were being produced. If the isolate was not genetically tractable, you could instead clone the BGC believed responsible for the antibiotic production and see if you can bestow the killing potential in a recipient strain that produces no antimicrobials.

Whilst much of this work can begin on the three isolates sequenced with a greater read depth, there are many strains which could be brought forward to the level of these three.

3.4.5 Summary

Through a citizen science field study which successfully collected and analysed 454 soil samples, 15 partially sequenced genomes of eight species of bacteria have been determined (Figure 3-14, Figure 3-15): *Bacillus subtilis* (ELD01, 07, 09 and 11), *Bacillus altitudinis* ELD02, 04 and 14), *Bacillus velezensis* (ELD03), *Bacillus simplex* (ELD10), *Bacillus psychrodurans* (ELD13), *Paenibacillus peoriae* (ELD05 and 08), *Sporosarcina aquimarina* (ELD06) and *Pseudomonas vranovensis* (ELD12 and 15). Despite their geographical isolation and varied environments of collection, most of the predicted antibiotic and secondary metabolite clusters were highly conserved

between species. However, some of the predicted BGCs have not been reported previously. All isolates which showed inhibitory activity against one or more medically relevant pathogens are available for future study.

Furthermore, the antibiotic discovery pipeline optimised as part of this project provided a continuous source of media with which to engage interested citizen scientists. The contribution of this to the citizen science project is the focus of investigations presented in Chapter 5.

Chapter 4 Public understandings of and attitudes to antibiotics and antibiotic resistance: Findings from a citizen science project

4.1 Introduction

The increase in incidence of AMR has the potential to reverse arguably the single greatest health care advance in history (1.1). Individuals prescribed an antibiotic are more likely to develop bacterial resistance to that antibiotic for up to 12 months after treatment (Costelloe *et al.*, 2010). Furthermore, patient expectations for antibiotics are linked to higher prescribing (1.2.3). Therefore, in the comprehensive U.K Review on AMR by O'Neill (2016), one of the ten commandments was to launch a massive global public awareness campaign. Yet, despite continued Public Health campaigns aimed at improving public understanding of antibiotic resistance, global antibiotic use has grown 66% since 2000 (Klein *et al.*, 2018). In order to guide future Public Health campaigns, it is important to identify what the current public awareness of antibiotics and resistance looks like. In chapter 1.3, a review of key research examining the public discourse of antibiotics and antibiotic resistance was undertaken. From this, six key themes emerged.

When discussing antibiotics and their side effects, there was an awareness that antibiotics are not a trouble-free solution and can cause side effects. Sensible use and taking the full course were believed to minimise the effects. Researchers concluded that negative consequences of antibiotic use can be a powerful motivator in discouraging antibiotic use (1.3.2).

The public often mistook antibiotic resistance as a property of the human body, rather than of an infectious agent. This concept was coined the resistant human body theory. In either case, the logical conclusion to be drawn is that overuse of antibiotics can limit their efficacy. This is a misunderstanding that likely does not affect the outcome of sensible use (1.3.3).

The public blame the irresponsible other rather than consider their own contribution to the problem of antibiotic resistance. Researchers concluded that campaigns should be aimed at convincing individuals to take personal responsibility for the spread of resistance (1.3.4).

Factual knowledge and awareness of Public Health narratives has been positively and negative associated with sensible use of antibiotics. Individuals with lower levels of education were less likely to understand antibiotic resistance but were more likely to be positively influenced to change their behaviour when provided information. Researchers concluded that this should be a target for future public health campaigns (1.3.5).

Public awareness of antibiotics and antibiotic resistance is limited, and where awareness is present, it is often superficial and does not reflect a deep understanding of terms like antibiotic resistance. Public Health campaigns focused on antibiotics seemed not to be reaching the public, improving awareness or engendering behavioural change (1.3.6).

Finally, in understanding why users take antibiotics, evidence suggests that there has been an increase in awareness surrounding the ineffectiveness of antibiotics on viral infections. This knowledge seemed to be limited to viruses, rather than specific examples of viral infections (1.3.7).

I aimed to investigate these key themes, other less frequent themes and the public's conceptions of research using Brew's (2001) framework (Table 2-5). I also aimed to apply an adaptation of research by Biza et al (2018), which uses changes in consistency and specificity as a measure for learning. Finally, codes which emerged from the analyses themselves were incorporated into the analysis.

In Chapter 2, Part two, I laid out the theoretical perspectives which underpin my position as a researcher in the field of citizen science. These theoretical perspectives guided the selection of methods for collection and analysis of data on public discourse surrounding antibiotics and antibiotic resistance.

To facilitate the data collection a pop-up stand was used (2.6). For data analysis, a semi-structured interview was designed (2.7.1). The semi-structured interview included closed-ended yes/no questions to allow quantitative analysis built upon the positivistic approach to measuring phenomenon (Cohen, Manion and Morrison, 2007). The semi-structured interview also included open-ended questions to allow qualitative discourse analysis.

In 1998, Sfard (1998) discussed two metaphors for learning; the participation metaphor which frames learning a subject as a process of becoming a member of a community, and the acquisition metaphor which frames learning as a knowledge transfer from teacher to student. The acquisition metaphor sits well within the positivist epistemological framework, that there is an objective truth and therefore student misunderstandings are errors which can and should be corrected. The participation metaphor sits well within the non-positivist epistemological framework, as it views participant misunderstandings as a normal part of participating in a community of subjective views. Of crucial importance to the ontological and epistemological underpinning of this project was Sfard's recommendation that one metaphor is not enough.

> *"Acquisitionists and participationists might admit that the difference between them is not a matter of differing opinions but rather of participating in different, mutually complementing discourses" (Sfard, 1998, p. 11).*

These metaphors assume that the learner engages with an unfamiliar discourse. To successfully engage in an unfamiliar discourse a participant must agree to an unwritten learning-teaching agreement (Sfard, 2015). This simply states that the learner will be exposed to communicational conflict. To turn this conflict into a lever for learning rather than an obstacle, the learner must be in one mind regarding:

1. Whose discourse is to be eventually shared;
2. Who needs to act as the teacher and who as a learner; and
3. What is the expected form, mechanism and pace of the learning process?

To measure the extent to which a participant engaged with an unfamiliar discourse and undertook learning, Sfard (2008) put forward the phrase 'thinking as communicating'. Sfard (2008) suggested that interpersonal

communication and individual thinking are two facets of the same phenomenon, coining this *commognition*, that is a combination of *communication* and *cognition*. That is to say that by analysing an individual's interpersonal communication (discourse), be it speaking, writing or some other medium, one can attempt to understand what a person thinks about an issue (cognition). Sfard defines discourse as communication consisting of four key characteristics; word use, visual mediators, routines and endorsed narratives (Sfard, 2007).

In the following chapter, I descriptively analyse closed ended, yes/no questions. I then apply a coding schedule to transcripts of 32 participant interviews. In doing so, participant discourse is taken to reflect their internal thought processes surrounding antibiotics and antibiotic resistance. In the analysis that follows, participant is used as the person who takes part in the project, interviewee as the person who is interviewed, citizen scientist as a member of the public who collects and analyses data as part of this project and volunteer as a person who freely offers to take part in helping run our project.

4.2 Results: a descriptive analysis

4.2.1 Public understanding of soil microbiota's role in medicine

Participants were asked "Do you think most antibiotics are made by…?"

- Bacteria
- Fungi
- Human
- Plants
- Viruses

and could respond to each of five categories with either a yes, no or unsure response (Figure 4-1). Participants correctly identified that bacteria (88%) and fungi (72%) can produce antibiotics. 53% of participants identified humans as being able to make antibiotics. In 3/17 cases where participants answered yes, they clarified it was the bacteria on or in us that produced the antibiotic. In a further 5/17 cases the participant directly stated that they are only answering yes if they can clarify that they mean in a laboratory setting. There was a noticeably high yes response to plants (84%). Viruses received the fewest yes responses (45%).

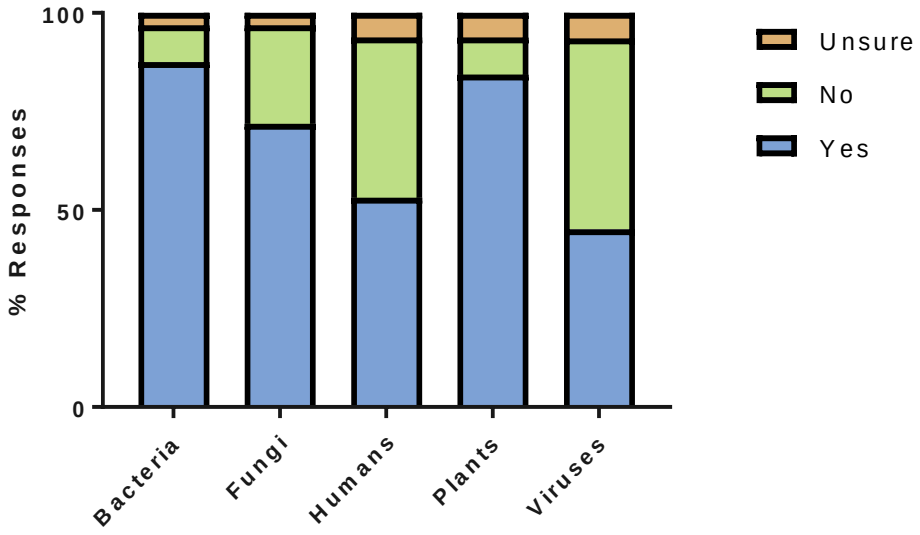


Figure 4-1. Participants see plants in a medicinal light. Participant responses (N=32) to the closed-ended question "Do you think most antibiotics are made by…?" Responses of either yes, no or unsure were noted for each of five categories: Bacteria, Fungi, Humans, Plants and Viruses. Responses are shown as percentages of total responses for each category.

4.2.2 Public understanding of antibiotic resistance

Participants were asked "Can you tell me which of the following can become resistant to antibiotics?"

- Bacteria
- Fungi
- Humans
- Plants
- Viruses

and could respond to each of five categories with either a yes, no or unsure response (Figure 4-2). 96.875% of participants responded yes to bacteria being able to become resistant to antibiotics. 87.5% of participants responded yes to humans. Fungi received less yes responses, 78.125%. Plants received a yes response in 62.5% of cases, whilst viruses received the lowest number of yes responses, with 56.25%.

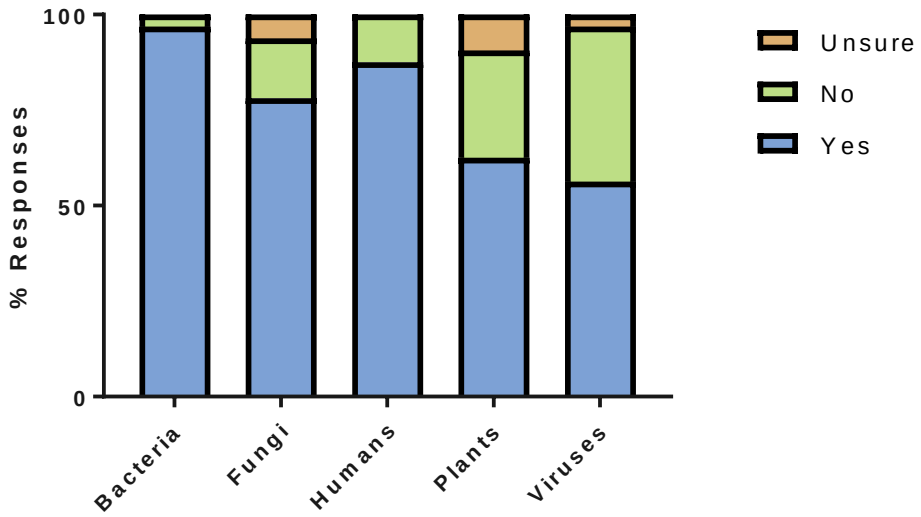


Figure 4-2. Members of the public align with the resistant body theory. Participant responses (N=32) to the closed-ended question "Which of the following can become resistant to antibiotics?" Responses of either yes, no or unsure were noted for each of five categories: Bacteria, Fungi, Humans, Plants and Viruses. Responses are shown as percentages of total responses for each category.

4.2.3 Public understanding of use of antibiotics in a medical setting

Participants were asked "We often refer to infections as bacterial, fungal or viral, so of those which do you think we can treat with antibiotics?"

- Bacterial
- Fungal
- Viral

and could respond to each of five categories with either a yes, no or unsure response (Figure 4-3). Participants responded that antibiotics could be used to treat bacterial infections in 97% of cases, more than any other category. 75% of interviews produced a yes response to the treatment of fungal infections with antibiotics. Viral infections received the fewest yes responses, with 22%.

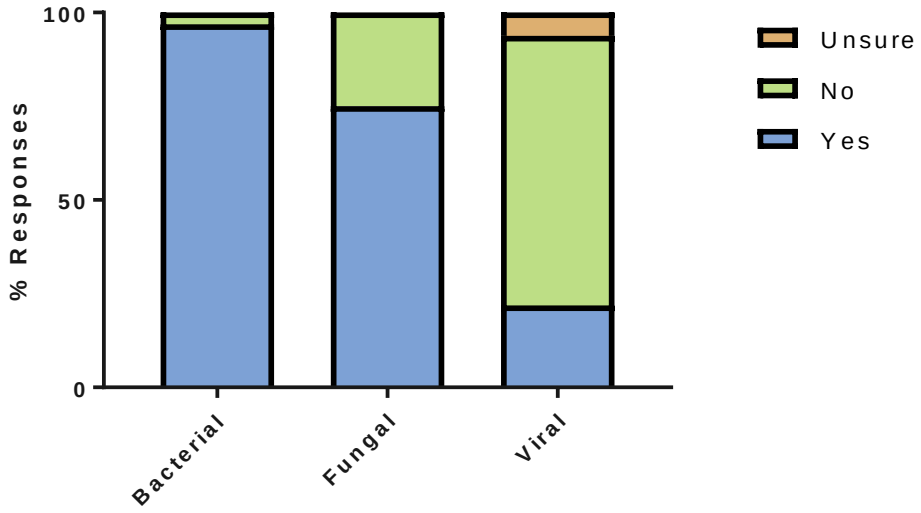


Figure 4-3. Members of the public are aware that viral infections are treated differently than bacterial or fungal infections. Participant responses (N=32) to the closed-ended question "Which of the following infections do you think are treatable with antibiotics?" Responses of either yes, no or unsure were noted for each of three categories: Bacterial, Fungal and Viral. Responses are shown as percentages of total responses for each category.

4.2.4 Public understanding of antibiotic discovery

Participants were asked "Have you ever heard of teixobactin?". They could respond with either a yes, no or unsure response (Figure 4-4). Of the 32 interviews, 97% (31/32) responded no, they had not heard of teixobactin.

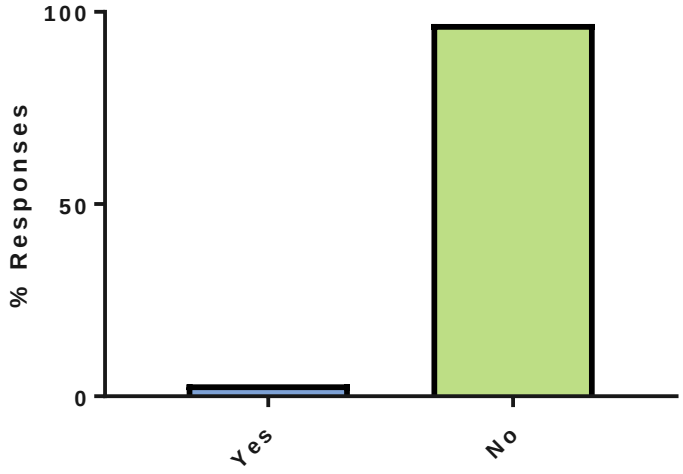


Figure 4-4. Members of the public had not heard of Teixobactin, a novel antibiotic discovered in January 2015. Participant responses (N=32) to the question "Have you heard of Teixobactin?" Responses of either yes, no or unsure were noted. Responses are shown as percentages of total responses.

4.3 Results: a thematic analysis

In 4.2, a descriptive account of the responses to closed-ended questions asked in the face-to-face interviews has been given. I now use a coded analysis of participant interview transcripts and factual summaries to identify

what the participants think about antibiotics and antibiotic resistance in a discourse analysis approach (Sfard, 2008). Participant consistency and specificity is also examined (Biza et al., 2018). As described in chapter (2.7.1), codes were reduced to major themes through ongoing discussion between researchers and re-reading of factual summaries and transcripts. Here, I present the eight key themes which emerged from coding of the interviews:

1. The correct use of scientific terminology
2. Using personal experiences in ritualised ways to participant in a new discourse
3. Public Health Narratives
4. Human Health Narrative
5. Who is to blame?
6. What are scientists after?
7. Do people need a hero?
8. Do people make bad decisions in the face of better knowledge?

4.3.1 The correct use of scientific terminology

One part of organising new experiences in terms of those with which an individual is already familiar requires using familiar words following pre-existing rules that seem in agreement with the new context (Sfard., 2008). However, when a new word is metaphorically introduced in this way, there is no guarantee that the word's meaning will be correct in its new context. This ambiguity can threaten the effectiveness of communication. In order to investigate this phenomenon, I developed the code "Scientific Word Use [SWU]" (105 references from 26 sources) to capture each time an interviewee used a scientific word or phrase, either correctly or incorrectly.

A reoccurring example was the familiar word "*virus*" or "*viral*" (ten references from eight sources). This word was used in a way not consistent with the scientific use of the word in the literate discourse. I note, for example, the following exchange between myself and interviewee 01. When asked what they knew about antibiotic resistance, they responded:

> *01: I know that err they're becoming, there are strains of viruses erm that are becoming immune to what we have developed.*

In the above, the metaphorical use of *strains of viruses* does not match the operationalised, scientific version. Of the ten references, interviewee 01 was responsible for three, and in each case used the word incorrectly in the scientific context. This misuse of the word virus did not preclude interviewee 01 from understanding that bacteria are the focus of antibiotic resistance discussion. Later in the interview whilst discussing the last time new antibiotics were discovered; interviewee 01 said:

> *01: …everything seems to sort of be able to sort of um beat us now if that makes sense, like MRSA and all that and what's the other one, C. diffa something or other.*

Interviewee 01 correctly identified two species of human pathogenic bacteria relevant to the topic of antibiotic resistance. This highlights that this individual engaged with scientific word use, however not consistently.

Further to how an individual might explicitly use the keywords of a discourse, I also examined how an individual might react to keywords when used by myself. In section 4.2, participants gave less yes responses to viruses (Figure 4-1, Figure 4-2 and Figure 4-3). Looking at the level of confidence of these answers, participants tended to answer more confidently when viruses were mentioned. The following example shows interviewee 01 responding when asked if viruses produce antibiotics:

01: Definitely yes.

Interviewee 01 used a qualifier. Qualifications made by participants were captured using the code "Confidence [CON]" (212 references from 30 sources). Interviewee 01 did respond yes to each of the five options presented to them during this question, however upon hearing viruses they inserted the positive qualifier '*definitely*'.

The following example is interviewee 23 responding when asked what the biggest issue would be if antibiotic resistance were to spread:

23: And also it means that err, that kind of, mild things are still gonna be as mild as ever when there is particularly bad, well I was about to say viruses but that's entirely wrong, but particularly bad erm bacterial infection, which might be pandemic, which might be epidemics etc, none of the normal things work.

Interviewee 23 had a close relationship with a practicing Microbiologist and as such had prior access to the literate discourse on such topics. In this excerpt there is a moment of conflict when interviewee 23 used the familiar word viruses in the incorrect context. They noticed their mistake and corrected themselves to say bacteria. This participant had a solid understanding of the topic, however had to actively correct themselves mid-sentence as their discourse slipped back into the colloquial.

This thematic analysis supports the notion that individuals bring familiar words to a new discourse as they begin to engage with it. The incorrect use of these words, which have specific operationalised meanings in the literate discourse, can cause ambiguity. This does not mean that the individual does not understand the key concepts of the discourse.

4.3.2 Using personal experiences in ritualised ways to participate in a new discourse

Sfard (2008) considers routines as repetition-generated patterns of our actions. When a participant engages with a new discourse, routines are initially implemented as rituals (process-oriented routines). Rituals simply mimic or replicate, sometimes not very critically, what has been bequeathed by previous users, for example out of respect for authority. This theme examines more broadly the concept of engaging with new experiences in terms of those with which an individual is already familiar. It examines how participants use habits, often based upon personal experiences, to engage with a new discourse and how this shapes the words they use, narratives they endorse and routines they

follow. The following except was captured by the code “What Users Take Antibiotics For [UTA]” (43 references from 20 sources). The following response was from interviewee 3a and 3b, when asked what kinds of infection they might take antibiotics for:

03a: I took antibiotics after my op against MRSA.

…

03a: Yeah I have used antibiotics loads!

Interviewee 03a used the experience of having had an operation to introduce the term “MRSA”, an infectious agent that can be treated with antibiotics. In the same interview, the code “Role of Doctors [ROD]” (48 references from 23 sources) captured another example of interviewee 03a repeating their own experience to engage with the discourse, this time when asked the ways in which they thought antibiotics were used:

03a: Cos our doctor won’t give anything for viral things will he…

R: No.

03a: If you’ve got a viral sore throat then ‘no you’re not having it’ but if you got septic pus-y throat then you have the antibiotics.

Interviewee 03a correctly distinguished between viral and bacterial infections because of conversations they had with their own doctor. They went as far as to directly quote their doctor in the last sentence.

Whilst participant 03a recalled personal experience, the ability to imitate extends to experiences which one is indirectly aware of. An example of this was captured by the code “UTA”. Interviewee 04 recalls their Mum’s experience with antibiotics when asked which kind of infections are treatable with antibiotics. I first draw attention to my wording of the question:

R: Okay, we take antibiotics to combat infections, which kind of infections do you think, or if you know anyone who has had an infection that was treatable with antibiotics, or?

By interview 04, I had seemingly noticed that participants often drew on personal experiences to answer this question and as such expanded the question to include “***if you know anyone…***” to prompt participant engagement with the discourse. Interviewee 04 responded:

04: Err my mum takes antibiotics all the time at the moment because she is not very well, but I have no idea what sort of thing, I should probably know more about it, but ye erm

Interviewee 04 recalled their mother’s experience with antibiotics to engage with the question. In this case the participant could not remember the specific details of the experience. Just like interviewee 03a, interviewee 04 used the ritual bequeathed by their doctor when asked if you can treat viral infections with antibiotics:

*04: *Whispers viral*. So when you have a virus does the doctor say you can have it or you shouldn't have it? Hahaha, I can't remember!*

Interestingly when comparing the two interviewees, it is noticeable that 03a, who more accurately recalled their own experiences, engaged more with the literate discourse than 04, that is used scientific terms correctly and expressed the expected narratives regarding viruses. Finally, the code UTA, captured the following statement from interviewee 05 when asked which types of infection to take antibiotics for:

05: I am just more familiar with viral infections, I am from having the kids. Everything they have seems to be viral.

Interviewee 05 stated explicitly that they were using their familiarity with viral infections due to personal experience to answer the question.

This thematic analysis highlights how individuals used rituals or recall their own or other's experiences to engage with a new discourse. Whilst these experiences were not always relevant or specific, they did facilitate engagement. In some instances, these experiences assisted individuals with utilising operationalised terms, as discussed in 4.3.1.

4.3.3 Public Health Narratives

In 4.3.2, I showed how in the process of engaging with a new discourse, a participant might use rituals or experiences. Public Health narratives are rituals bequeathed by authorities familiar with the literate discourse. These rituals are meant to be imitated and encourage behaviour considered sensible by a given community. Not imitating these rituals makes one an outsider. With regards to antibiotics and antibiotic resistance in the U.K, the authority is Public Health England. In order to identify moments where a participant implemented these ritualised public health narratives, the code "Public Health Narrative [PHN]" (54 references from 27 sources) was applied. The 54 references were divided into six key public health narratives. Four of these were mentioned frequently.

The first narrative is that antibiotics are taken for everything (overuse), and that they should not be (misuse). Misuse in these instances was linked to taking antibiotics for the common cold, coughs, viral infections, giving antibiotics to dogs and using antibacterial hand gel. One example came from interviewee 20, when answering whether they can personally make a difference to the spread of AMR:

20: Err ye, so only taking antibiotics when I absolutely need them. Erm, obviously antibiotics can be used for all manners of things but sometimes yano illnesses can just take their natural courses. I mean humans are very efficient at dealing with bacterial infection and even viral infection. So there's no, there's not always requirement to take antibiotics.

The participant drew on the narrative that antibiotics should only be taken when necessary. This included the narrative that some illness, both viral and bacterial, should be allowed to run their course.

The public health narrative surrounding overuse states that people should not be taking antibiotics as often, and that if they do, they no longer work. Participants thoughts on overuse were often captured by the code “The Resistant Body Theory [ReB]” (44 references from 19 sources). Individuals made the leap that the reason antibiotics no longer work when you take too many is that our own bodies develop immunity to antibiotics, rather than the infectious agent:

03: I know that if you have antibiotics for the same things, your immune system yano you handle them better so they aren’t as effective.

Interviewee 22 gave an insight in to the reason the Resistant Body Theory occurs so frequently, when asked whether humans, and then plants, can become resistant to antibiotics:

22: Well you kind of say humans, but there’s things in us which are resistant but yet, ye”.

and

22: Well they get bacteria still and bacterial infections, ye, they’re living,

Interviewee 22 highlighted how an individual may say that humans can become resistant to antibiotics, without qualifying that this is because of the bacteria living inside of them.

The second narrative is that an individual should take their full course of antibiotics once prescribed. Within this theme I included the need for shorter courses mentioned by one interviewee, and the returning of unused antibiotics to the doctor. Interviewee 28 provided a clear example, correctly mimicking the ritual that one must take their full course:

28: Erm, and to erm carry on taking your entire course of antibiotics, don’t stop halfway through.

The third narrative is that antibiotics do not work on viruses. In the closed-ended questions, interviewees seemed to understand that viral infections are to be treated differently from bacterial or fungal infections (Figure 4-3). This was expanded upon in the open-ended questions, where interviewee 08 said:

08: Well not viral, because you can’t use antibiotics for a viral infection, it’s gotta go its course.

This linked elements of the first narrative, that infections should run their natural course, with the third, that antibiotics do not work on viral infections.

The fourth narrative is the need for better personal hygiene: Within this theme, participants touched on washing hands, covering mouths and enforcing a self-quarantine. On one occasion an interviewee recommended only the use of your own towel. Interviewee 14b, when asked how you could personally make a difference to the spread of AMR, said:

14b: And by the way in which you behaved, you know, you can spread it and people who sneeze all over the place and get their hands filthy dirty and don't wash them, it's easy, it's by better hygiene and being careful.

I note that question 11 in particular, "How does antibiotic resistance spread?" targeted this public hygiene narrative by specifically asking participants about the environmental and genetic spread of bacteria. The code "PHN" was used during question 11 (4 references from 4 sources). Excerpts that were coded for usually referred to individual hygiene. Participants were much more likely to understand the concept of environmental spread of resistance than genetic ("SKO" 14/40 references):

22: Well, erm it's spread, well it's mainly close proximity contacts and airborne, plus, oh no bacteria, ye no it's close and bodily fluids isn't it bacteria, rather than viruses are airborne.

The common knowledge of two major routes of transmission; close contact and contact with bodily fluids (airborne), fed in well to the subsequent idea that you must take care not to spread infection via these routes, through the application of sensible personal hygiene measures:

31a: Erm its spread when you sneeze on the bus and when you shake hands and you haven't washed your hands properly.

The fifth narrative is the need for individuals to stay healthy, including the concept of remaining active as you grow older. Interviewee 16 touched on this when asked how they can help to limit the spread of AMR:

16: But we keep active and stuff like that. I certainly my jaw's very active yano.

The final narrative, mentioned by only one participant, is antibiotic stewardship. Interviewee 30, who was beginning a PhD project looking at discovering new antibiotics, answered in response to what they can do to make a difference to the spread of AMR:

30: Well antibiotic stewardship.

Most participants (27/32) ritualised at least one public health narrative as they engaged with the literate discourse. Some participants (5/32) did not draw on a public health narrative. Of these five, one participant offered an opposing view to the narrative that antibiotics are being misused. When asked whether antibiotics were being misused, interviewee 10 said:

10: Cos they are helping us so why, why would you be misusing them?

I note that interview 10 lasted below the average time for an interview in Breccon Beacons (8 minutes, average was 10 minutes 16 seconds from 13 interviews). Commenting generally on the mood of the interview, I also note that there was little engagement with the questions asked.

This thematic analysis has identified six key public health narratives which are, on the most part, being ritualised by members of the public as they engage with a new discourse. It has also touched on the frequency with which each of these narratives is drawn upon. Finally, it has highlighted that not all individuals are willing to mimic these rituals or engage fully with the discourse.

4.3.4 Human Health Narrative

In 4.3.3, I discussed how individuals use public health narratives to engage with a new discourse. In this section I examine how individuals engaged with the common rituals surrounding human health, and whether this was influenced by their perspective of science. In question 12, I asked interviewees what the biggest problem for humans would be if antibiotic resistance were to spread. Most responses (31/32 responses) focused on human health to some extent. 17 responses provided a more detailed response and so I could determine that the extent to which human health would be affected was linked to two narratives, coded "Utopian [UTO]" (10 references from 10 sources) and "Dystopian [DYS]" (7 references from 7 sources). Utopian and Dystopian narratives might be considered as part of a more general outlook, that is whether a participant is an optimistic or pessimistic person. Interviewee 05 provided a utopian narrative:

05: Not having anything up to date to help fight, when we become immune to things, to the antibiotics, and we are still getting ill, what are we going to do then?

…

05: So we need people like you then.

They presented the risk to human health, that antibiotics will no longer be able to fight infections, and then revealed their opinion of scientists. This was one of optimism, that scientists like me can help solve this problem. Interviewee 18 provided a utopian narrative of science:

18: Things like basic operations yano, just wouldn't be able to do them would ya. Infections you would be dying of like minor ailments wouldn't ya,

…

18: Or what they call minor ailments now.

They mentioned how in the future, basic operations would become difficult and spoke of what we now call minor ailments. I interpret this as the participant being aware that science and medicine have successfully minimised the risk of death by infectious disease. Interviewee 22 provided a dystopian narrative:

22: The biggest problem, well there's too many of us living in this planet in close proximity and we're just gonna spread it. And, erm it's one way of thinning down the population isn't it!

…

22: But yano, these things happen don't they haha, this always happens.

Interviewee 22 stated that overpopulation will be to blame for the spread of AMR and that the result will be reduction in the population. They then stated that these things always happen, which suggests science and scientists are powerless to overcome this. Interviewee 01, when asked how many antibiotics have been discovered since 1962 said:

01: …something is going to kill us all, or kill 90% of the planet.

This dystopian narrative, that between 90-100% of the planet will die from an infectious disease, is again an example in which science and scientists are powerless to stop it. This followed discussion about the common cold or flu, which I note is difficult to vaccinate against due to its ability to evolve, however not deadly enough to cause this level of death. The link between amount of faith placed in science and scientists and a utopian or dystopian narrative, that is an optimistic or pessimistic outlook, seemed particularly clear in interviewee 05's answer when asked how many antibiotics have been produced since 1962:

05: Mmm, I don't know, I would say you'd think it'd be more because obviously everything is more advanced now, but would it be less because we haven't had the need to create, cos like you said we thought we were safe so why would they have continued to waste money and time on testing. So I'd say, can I say less?

They acknowledged the success of science in advancing technology, which has increased the number of antibiotics taken to clinical trial (optimistic), before suggesting science and scientists may have become complacent with their successes and cautious with their funds, resulting in fewer numbers of antibiotics taken to clinical trials (pessimistic).

This thematic analysis highlights the different narratives an individual might bring to a new discourse and how this affects the way they engage with it. Specifically, it seems that faith in science and scientists is linked to a utopian narrative and optimistic outlook, whilst lack of faith in science and scientists is linked to a dystopian narrative and pessimistic outlook.

4.3.5 Who is to blame?

In 4.3.4, I highlighted how trust or faith in science or scientists seems linked to an individual's narrative of dystopian or utopian. In this section, I investigate participants narratives of blame. It is important to know who the public blame for the current antibiotic crisis, described in 1.3.4, to repair the faith and trust that is necessary to work together to resolve this issue.

The dedicated code "Role of Doctors [ROD]" (48 references from 23 sources) aimed to capture the level of blame attributed to doctors. A narrative which arose in seven interviews was that doctors are better now than they were back then:

16b: Well the doctors don't want to prescribe it as much as they, I mean they used to just write out a prescription but nowadays they are saying unless you really think you need it, don't use it if you go for an antibiotic.

There are few explanations from the interviewees as to why this might be the case. One interviewee mentioned updated guidelines from the British Medical Association; another touched on increased awareness of the problem of resistance. One interviewee provided the opposite opinion, that in fact doctors back then were more likely to offer bed rest as an antidote:

18: I think it's very easy for a doctor to just prescribe antibiotics as opposed to prescribe bed rest and water and good food, Ye, ye which is what it would've been wouldn't it years ago. So ye I do, I think, but I think what we do here is just the tip of the iceberg.

However, despite most participants thinking doctors now prescribe fewer antibiotics than doctors in the past, doctors are still taking a large portion of the blame for antibiotic misuse:

04: Haha, erm only having antibiotics when you, when well I was going to say when the doctor prescribes it but actually sometimes they prescribe it all the time when you don't even need it.

This lack of trust in GPs to know when it is best to hand out antibiotics could affect current public health messages which encourage you to trust your GP rather than demand a certain prescription. This is not to say that participants are not sympathetic with the predicament doctors are facing. One example from interview 14, interviewee 14b was:

14b: Oh yes, I think it's, I think an awful lot of doctors have people come to see them who expect a prescription. And a lot of doctors are so overworked they haven't got time, and so they write a prescription. You know they are trying to stop people doing that aren't they.

Interviewees 14a and 14b perceived doctors as being overworked and on top of that, having a patient demand for prescriptions. This inevitably leads to prescriptions being written unnecessarily. Interviewee 13 hinted at a potential avenue through which to repair trust and encourage faith in doctors moving forward:

13: They do tell you that now when you get it, they say you must finish it, it's really important. But they never say why do they!

Faith in GPs encourages individuals to want to work together with them to contribute to resolving the issue of AMR, as explained by interviewee 31b:

31b: I think they've got to explain like all of the side effects, and all the things that are gonna happen to you if you take antibiotics. It's gonna destroy the bad bacteria but it's also gonna destroy your

good bacteria, so you might not recover, you might have a lower immune system or whatever.

…

31b: Ye exactly, ye, cos I mean there's no point in erm, in taking something that has side effects and the side effect is going to lead you back to your original state. Yano, what's the point.

The code "Kept in the Dark [KID]" (11 references from 8 sources) captured the level of blame attributed to figures of authority who hold power. Interviewees identified a similar feeling of not being listened to or cared for:

22: Unfortunately, it's the people at the top, don't listen, and the people with the money isn't it, that's what you are fighting against a lot of it.

Finally, the code "Irresponsible Other [IRO]" (70 references from 26 sources) captured the level of blame attributed to other members of the public, often therefore not attributed to oneself. When interviewee 05 was asked whether they had an opinion on whether antibiotics are being misused, they answered:

05: I think they do be misused by certain people like erm I suffer for example with tonsillitis, I can't have them taken out, because I don't have it the 6 times within the 12 months. But I don't go and get antibiotics for it because there's no point me taking it all the time. They won't do anything because I have it so often. But I think a lot of people are quick to be like, no let's just go and get it, get rid of it, don't leave our body fight it on its own. So I think maybe, they can be misused.

Interviewee 05 suggested that other people are quick to take antibiotics, unlike themselves. Often this theme manifests in the use of the word *people*, when discussing the issue. When asked whether antimicrobials are being misused, interviewee 08 says:

08: Well they probably are, because people will drop in to the doctors when they have a cold, and erm the doctors will prescribe them antibiotics.

In this instance, it is the fault of other people for going to the doctors, and the doctors for being willing to prescribe in this case. When a person talks about themselves, using words like *I* or *we*, the story changes and often details how many ways a person might be doing their bit to solve the problem. When asked if they can make a difference to the spread of antibiotic resistance, interviewees 09a and 09b responded by saying:

09a: From a limited knowledge, only in as much as we know that, so ye we don't, we try to avoid using antibiotics or trying to, we don't go to the doctor and get them unless we need to (we think), and if we do have

09b: keep reasonably healthy so you don't need to

09a: Ye and if we do happen to have them we'll finish the course or return them to the doctors

This is of course not to say that our interviewees do not all practice sensible behaviour when it comes to the prescription of antibiotics. Interestingly the same interviewees later went on to admit that they could be part of the problem. Not because of any bad practices however, but because of their contribution to the total population of humans in general:

09a: Erm I think we'd like to say, particularly based on the discussion we've had, that they are clearly being misused but then we appreciate we are part of the problem of that as well. So, because we are just more people so erm err.

This thematic analysis shows that blame is mostly directed at doctors, whilst also being directed at people in power and other irresponsible individuals. The general feeling is that doctors and people in power do not listen to individuals and this could be an important part of repairing faith and trust in experts. This faith may be key when asking the public to work together with experts to solve the problem of AMR. Further, analysis of participants word use suggests that *people* are irresponsible, yet *I*, *we* or *you* are responsible. This is a cause for concern if individuals do not perceive a reason to modify their own antibiotic use.

4.3.6 What are scientists after?

In 4.3.5, I examined the attribution of blame to certain groups and discussed how this may provide a barrier to working together to develop a solution to the crisis that is AMR. Building on public perspectives of science and scientists in 4.3.4, I now apply Brew's (2001) framework which presents four qualitatively different ways in which research is understood. Fundamentally, Trading and Domino are product focused perspectives, whilst Journey and Layer are process focused. Furthermore, Trading and Journey are perspectives which include the researcher in their awareness, whilst Domino and Layer do not (Table 4-1).

Table 4-1. Relationships between conceptions of research (Brew., 2001)

	Researcher present to, or the focus of awareness	**Researcher absent from, or incidental to awareness**
External Products	Trading	Domino
Internal Processes	Journey	Layer

These four conceptions of research became codes for my framework, namely: "Domino [DOM]", "Trading [TRA]", "Layer [LAY]" and "Journey [JOU]". The definitions of each of these themes provided by Brew were not easily adapted to help designate codes to the interview transcripts. Instead, I relied heavily on utilising the more simplistic Table 4-1, asking first whether the excerpt was

product or process focused and then whether the researcher was present or absent in the awareness.

Interviewee 23, during the after-interview discussion where the participants could talk to me about anything they wanted to, said:

> *23: Err nah, I think it's all intriguing stuff, working out, and also cos. So once you've, I was having a look at some of the plates in there, and you've got the areas of exclusion where the bacteria is clearly winning in some form. How would you go about finding out kind of why it's winning?*

Their question focused on the internal processes of research "*go about finding out*" whilst including me, as the researcher, in their awareness "*how would you*". This is coded for as Journey. Brew's definition of Journey is that that research informs, and is informed by, life issues (Brew & Lucas., 2009) where the researcher grows or is transformed by a personal journey of discovery (Brew et al., 2016).

Unlike Journey, trading is external-product orientated where the intention is to produce an outcome, whilst still having the researcher present in the awareness. Interviewee 03 provided an example during our after-interview discussion:

> *03: And then obviously if you do find any antibiotics, we'll have a cut!*

Trading is defined as researchers undertaking research in order to trade for other things such as promotion and recognition (Brew & Lucas., 2009).

Layer is focused on internal processes, however, does not include the researcher in the awareness. Interviewee 07, when asked whether antibiotics are being overused, said:

> *07: Overused, yes, it has improved, definitely because obviously there's been so much highlighted, we are trying to get that down but they are still being overprescribed.*

Interviewee 07 talked about how information has been highlighted and how that has helped to reduce overuse of antibiotics. This matched well with the definition of Layer which says research is bringing to light the ideas, explanations and truths lying in the background by illuminating or uncovering the underlying layer of knowledge (Brew, 2001).

Finally, domino is product focused when the researcher is absent from awareness. Interviewee 32a provided an example during our after-interview discussion:

> *32a: Yes, I do agree, I mean the other thing of course is that part of the way science works is to dispute the consensus and to challenge it and to, and to err, I can't think of what the term is but really try and disprove,*

They talked about research with the researcher absent from awareness. They talked about how the outcome of science is to dispute the consensus and disprove, later mentioning this is based on evidence. Domino is defined as research viewed as a series of separate tasks, events, things, activities, problems, techniques, experiments, issues, ideas or questions, each of which is presented as distinct (Brew, 2001).

This thematic analysis has provided examples of each of Brew's (2001) concepts of research, qualitatively distinct from each other. Participants view research through the lens of these four concepts. The perspective they take influences their opinions of research and researchers. This ties in well with section 4.3.4 and 4.3.5, as understanding how researchers are perceived could provide useful in regaining the public's trust.

4.3.7 Do people need a hero?

Whilst analysing the interviews, I noticed occasions where a participant recalled names of famous scientists or responded positively when I recalled famous discoveries. Whilst this has some overlap with rituals (4.3.2), I thought this distinct enough to warrant developing a new code to capture this phenomenon. This code was "Science as a Story [SAS]" (14 references from 10 sources). Interviewee 08, when asked the last time we discovered new antibiotics said:

08: Erm… Fleming?

R: Okay. So that's

08: Hahaha, Marie Curie?

R: A long time ago.

08: Yes, a long time ago. Not Marie Curie, she was cancer!

By using names of famous scientists as a direct answer to the question, there was an assumption that the name could be paired with a discovery and a timeline which answers the question. Interviewee 23 used the name of a famous scientist, Fleming, to attempt to engage with a landmark in scientific history:

23: So do we have, so if that was 50 years ago and the first one was Fleming what 200 years ago now?

I note that Flemings discovery of Penicillin, often considered one of the first major antibiotic discoveries, was in 1928, less than 100 years ago. Other interviewees who could not remember Fleming by name instead remembered the name of the antibiotic that he discovered. When asked the last time we discovered a new antibiotic, interviewee 18 said:

18: No, but I do know the story about penicillin, that was quite an interesting story.

Interviewee 18 could have responded no, but instead chose to engage with the new discourse by recalling the story of penicillin. Whilst this was not

explained further, it was an attempt at a show of knowledge relevant to the discussion. When interviewee 29 was asked when a new antibiotic was last discovered, they were stuck until Penicillin was mentioned. Penicillin instantly conjured up a date that they had associated with its discovery:

29: Oo, I don't know, quite a while ago I would say.

R: So penicillin is an antibiotic, things like that.

29: Oh well that was 1800's or something isn't it?

This was the second interviewee who thought that Fleming's discovery of penicillin took place somewhere in the 1800's, almost 100 years before the actual discovery was made in 1928. Even when participants have no idea about the answer to the question, they might try to bring in a name of someone who they think is related to the topic at hand. When asked the last time we discovered a new antibiotic, interviewee 27 said:

27: Couldn't tell you no. There's only the one piece of history that sort of most people know about. Err was it Florence nightingale, I think that's what I'm gonna go with.

This thematic analysis shows that individuals view the evolution of science as the succession of work of some very clever people. Names of famous scientists or scientific discoveries help paint a picture of scientific history, even if not quite accurate. This in turn helps participants engage with the new discourse, providing some familiarity.

4.3.8 Do people make bad decisions in the face of better knowledge?

In the literature review (1.5), I discussed how citizen science represents the move away from public understanding and towards public engagement. Public engagement follows the principle that provision of objective facts to improve public understanding is not the way to effect change in the general public. Instead the public should be engaged. I also presented literature which showed that better factual knowledge did not always lead to individuals making better decisions with regards to antibiotics and antibiotic resistance. To capture the relationship between a participant's factual knowledge and an individual's actions with regards to antibiotics and antibiotic resistance, I developed the code "Better objective knowledge [BKM]" (6 references from 5 sources). Interviewee 29 said:

29: I think perhaps people rely on them, it's like a wonder drug. I mean, it's not nice getting a sore throat is it, but what you're saying to us all is you should try and fight it, or let your body fight it in other ways rather than relying on antibiotics. Having said that if I had a sore throat I'd probably be down there the doctors asking for some penicillin. So then perhaps it's the doctors sort of, they are trying to say no, they are trying not to prescribe it, they are.

They represented the general theme emerging from this code; that whilst participants were aware that they should not ask for antibiotics and that they

needed to use less, if they or their immediate family were sick, they would ignore that advice.

The code "Paternal and Maternal instincts [PMI]" (3 references from 3 sources) was developed upon analysing interview data for the relationship between objective knowledge and resulting decisions. Interviewees 09a and 09b, when asked which infections are treated with antibiotics against, said:

09b: When you have a child yano you do loads of, she's had, not loads, but she's had at least four courses already.

09a: The only time in our lives so far really

The interviewees stated that their child had been provided with four courses of antibiotics prior to which they had not personally taken antibiotics at any point in their lives. These interviewees then go on to state:

09a: She's a primary teacher, she should know something, but anyway.

…

09b: Ye, I teach a lot of antibiotics.

It is clear then that this was an individual who self-reported their own education on antibiotics. This phenomenon was also seen from interviewee 17 when asked whether people are misusing antibiotics:

*17: Erm, mmm I don't know really, because I know with the kids obviously my immediate reaction is well, must be something, *child's name* has got a really bad cough at the moment, so my immediate reaction was there must be some kind of antibiotic, but then at the other side of my brain is going well yano, maybe she shouldn't get them so often, yano so I dunno, I am on the fence really.*

…

17: Ye, it's like oh it's my kid I want it but everybody else no.

Interviewee 17 was aware that their child should not have antibiotics often but stated than when it is your child, you want to do what you can to help. This contradicted the irresponsible other theme that emerged in 4.3.5, that other people are to blame for overuse of antibiotics. Interviewee 32a, who was with their son during the interview, said during our after-interview discussion:

*32a: And even people that know about the subject, if it was my, if it was *son's name* here, and he had a really bad skin infection and the doctors said well antibiotics could help but there's a problem cos err, I'd say 'give him the antibiotics, he looks terrible'… So, and I would say I was a fairly sort of conscious person around the issue, until it really bites I suppose.*

This again saw interviewee 32a self-reporting their knowledge around the topic of antibiotic prescription and resistance (made clear over the course of the interview). They then said that if it came to seeking treatment for their son when they were sick, they would ask for antibiotics.

The code "Body as a temple [BAT]" (19 references, 12 sources) also highlights the relationship between factual knowledge and the resulting decisions individuals make. This code was developed in response to literature which reported that individuals prefer to let their body take care of the illness, a general aversion to antibiotics (more generally, medicine) partly based on the knowledge that antibiotics kill both good and bad bacteria (Norris *et al.*, 2013). The narrative that the body can look after itself, at its extreme, can lead to a distrust of modern medicine such as antibiotics. Interviewee's 19a and 19b discussed this when asked if they could personally make a difference to the spread of resistance:

19a: Err yes, yes. By my attitude towards antibiotics. I hate them, I will not take them.

19b: Build up your own immune system really.

19a: Yep, I just wanna fight my all through my life mostly my immune system has fought any infections I've had. Erm and I took them after an operation and quite honestly I stopped taking them cos I hated them, I just don't like antibiotics.

Interviewees 19a and 19b's expressed their belief that the immune system could be built up and could fight any infections, whilst 19a expressed their hatred of antibiotics. In a separate interview, interviewee 06 expressed their preference of using herbal medicine rather than antibiotics:

06: Err, for anything really. They pass them out too often. I mean there's other ways and means of using… erm other things like tea tree and eucalyptus, without using pills.

This preference to rely on homeopathy was also represented in interview 31, as interviewee 31a and 31b discussed the biggest problem for humans if antibiotic resistance were to spread. I note that the two interviewees clearly began with a different stance on this matter:

31a: Erm the struggle of finding a new antibiotic, which was, which could combat this one, and then again the struggle of that becoming resistant to that, and then eventually we could all end up with something as serious as MRSA and it could be fatal.

31b: We could refer to other treatments, I don't think it's the end of the world if antibiotic resistance becomes, like if all bacteria are antibiotic resistant. I think they'll erm…

31a: What could they do though if there was, if we run out of antibiotics?

31b: Other treatments, like there are loads of treatments that like…

31a: Really?

…

31b: There's homeopathy for a lot of things, my mum did homeopathy so I erm, there are things that can

31a: Is that like ginseng?

31b: Ye like health things I dunno, like if you've got I dunno, if you've got erm something like erm tuberculosis or something you'd go and do stuff like inhaling stuff, inhale mints.

31a: Oh right?

31b: Like there's always something else.

31a: I believe in it to be honest.

31b: Ye. I mean it's not only medicine, you've only got to take medication or else we'll die.

Interviewee 31a began with the narrative that resistant bacteria pose a serious threat to human health if they become resistant to antibiotics. 31b expressed their belief that antibiotics are not the only medicine that can be used for illnesses and over the course of the discussion, 31a changed their stance. This shows that despite understanding the risk posed by antibiotic resistance, one can be convinced to forego antibiotic treatment in exchange for homeopathic remedies. This distrust or move away from modern medicine, medicine which has been the foundation for improved life expectancy and quality of life (1.3.5) is a decision not recommended in the literate discourse.

This thematic analysis shows that participants would generally make bad decisions in the face of their own objective knowledge. This was through the need to feel like they were actively taking something to get better, the need to help their children get better or the distrust of modern medicine through either trust in your own immune system or a preference for homeopathic remedies.

4.4 Discussion and Future Work

4.4.1 Pop-up stands and interviews are part of a potent design for a citizen science project

Pop-up stands were used at five different events in different parts of the U.K to collect 454 soil samples and 32 interviews (Table 2-3). Four of the events were held in forests, with one at a science festival. These utilised neutral 'third places' that allow parties to meet on equal terms, rather than a hierarchical environment such as work or home (Dowell., 2017). Pop-up stands facilitated transparent, fully consensual and enriched active participation of citizens; however such practices and methodologies are far from being a well-established standard tool for citizen science research (Sagarra et al., 2016).

The success of the interviews was the result of a carefully constructed set of interview questions adapted from existing instruments (Creswell, 2012). These instruments were prior public discourse research, utilising themes emerging from the literature to guide the interview (Table 2-5). The complete list of 32 codes taken from public discourse literature and added to during analysis of the interview data represents a methodological contribution to the field. The development of eight key themes emerging from the interview data is a contribution to the public discourse literature surrounding antibiotics and antibiotic resistance.

4.4.2 Descriptive analysis reveals that our participants' scientific understanding resonates with participants in other research

Quantitative analysis suggests that participants are aware that bacteria and fungi produce antibiotics. Many antibiotics which have proven highly valuable in clinical practice are natural products (Wright, 2014, Table 1) and some of the most famous examples of these have been isolated from soil microbes, namely bacteria and fungi (Bowater, 2017). Participants incorrectly identified plants as producing antibiotics, however plants do have a key role in the production of pharmaceuticals other than antibiotics (Cowan, 1999). Fewer participants believed humans and viruses to produce antibiotics. Whilst research has suggested that most members of the public do not understand the distinction between bacteria and viruses (European Commission., 2013; McNulty et al., 2007; Norris et al., 2013) my findings challenge this.

Participants were aware that bacteria become resistant to antibiotics. Concurrent with literature and coined the 'resistant body', participants believed humans could also become resistant to antibiotics (Brookes-Howell et al., 2012; Brooks et al., 2008; Hawking et al., 2017; Hawkings et al., 2007). This literature separates the perspective of resistant body from resistant bacterium; however, my findings suggest participants think both bacteria and humans can develop resistance to antibiotics. Further, most participants believed fungi and plants could develop resistance. Participants were less likely to suggest viruses can become resistant, echoing the earlier finding that members of the public can distinguish between bacteria and viruses.

Participants were aware that bacterial infections are to be treated with antibiotics whilst viral infections are not. This echoes the increase in percentage of members of the public that understand antibiotics cannot kill viruses (Dyar et al., 2018; European Commission, 2013; Filipetto et al., 2008; McNulty et al., 2007, 2016) and could well be a result of exposure to media campaigns (Gonzales *et al.*, 2008). Most participants believed fungal infections could be treated with antibiotics. This may suggest some confusion between the term antibiotic and antifungal or antimicrobial (Mendelson *et al.*, 2017).

Participants were unaware of Teixobactin; a high-profile novel class of antibiotics discovered in 2015 (Ling *et al.*, 2015) which received substantial coverage by U.K main stream media (Sample, 2015). My findings are in line with survey data, which demonstrate members of the public receive limited exposure to scientific literature or major scientific discoveries (European Commission, 2010).

To develop the rigour of these analyses, statistical tests would be carried out to understand whether the differences in responses were significant. However, the quantity of responses would need to be increased and questions would need to be more specific. The current quantitative interview data does not allow for rigorous statistical analysis.

4.4.3 Thematic analysis reveals key themes in public understanding of, and attitudes towards, antibiotics

Qualitative analysis suggests that participants use both familiar words and familiar rituals as they attempt to engage with a new discourse. This finding is in line with research that suggests we organise new experiences in terms of those with which we are already familiar (Sfard., 2008).

My findings suggest that participants engage with the literate discourse through the ritualising of public health narratives. Six public health narratives were mimicked by participants: overuse/misuse, taking the full course, antibiotics do not work on viruses, need for personal hygiene, need to stay healthy and antibiotic stewardship. Literature suggests that rather than public health campaigns which don't reach the public (McNulty *et al.*, 2007b; Brooks *et al.*, 2008; European Commission, 2010, 2013, 2016) it is likely that this information is bequeathed by doctors (European Commission., 2013; Filipetto et al., 2008; Gudnadottir et al., 2013; McNulty et al., 2016), Television (Hawkings et al., 2007) or the internet (Gudnadottir *et al.*, 2013). My findings show that the resistant body theory (Brookes-Howell et al., 2012; Norris et al., 2013; Hawking et al., 2017) is a common misconception that emerges when participants mimic the ritual of overuse of antibiotics. The tendency for members of the public to imitate public health narratives is an important phenomenon for public health officials, particularly relevant in the face of the coronavirus pandemic where the mimicking of public health narratives like "hands, face, space" is saving lives (Department of Health and Social Care, 2020).

My findings suggest that participants who place faith in science and scientists harbour an optimistic outlook on antibiotic resistance. Scientific optimism, a favourable expectancy of the future of science, has increased and decreased over human history and is shaped by global events such as world wars (Onghena, 2011), whilst trust in science is affected by individual factors such as age, gender, political ideology, religiosity, education, income and science knowledge (Huber et al., 2019). Evidence suggests that people who are inherently optimistic, in that they hold positive expectations for the future, respond to difficulty and adversity in more adaptive ways than people who hold negative expectations (Carver et al., 2010). A positive attitude about science helps to increase support toward scientific research and industry, enabling political actors to legitimize relevant decisions and encourage public participation in scientific research projects (Huber et al., 2019). Huber et al (2019) posited that people who do not believe in anthropogenic climate change will see no need to take political action to slow its progress. I would posit the same about antibiotic resistance. One who does not believe in the threat of antibiotic resistance sees no need to follow sensible behaviours to mitigate the risk. This has been seen in the coronavirus pandemic with news reports

discussing individuals who refuse to wear masks (Morgan, 2020), despite scientific consensus agreeing that masks reduce the rate of transmission (Centers for Disease Control and Prevention, 2020). It is important that scientists interact with members of the public, engage them in their research and develop a level of trust so that the public actively engage with the narratives that help mitigate the effects of antibiotic resistance.

Many of my findings concerning participants' views of responsibility and blame for antibiotic resistance can largely be predicted by attribution theory (Brooks *et al.*, 2008). Attribution theory is concerned with people's explanation of events or behaviour by attributing causes. People offer one of two types of explanations about why things have happened: external attribution (causality attributed to an outside factor), or internal attribution (causality attributed to factors 'within the person'). In my findings, participants tended to make external attributions. They did not find themselves personally responsible but instead blamed outside factors like overprescribing by GPs, lack of care from people in power and irresponsible use of antibiotic by others. This concurs with other literature on the attribution of blame (Brooks et al., 2008; Butler et al., 1998; Dyar et al., 2018; Hawking et al., 2017; Hawkings et al., 2007). It is also reflected in the current political climate where, for example, the President of the U.S.A recently accused a pharmaceutical company of delaying announcement of a coronavirus vaccine until after an election for political gain (Ensor, 2020). Further, in line with my own findings, Brooks et al (2008) identified that participants wanted to present themselves and their own behaviour in a positive light. Applying these findings to clinical practice, one might encourage patients to consider why antibiotic resistance develops before assigning causality to the self rather than to others. My findings also show that participants believed doctors do not take time to explain why they refuse to prescribe antibiotics. Catering to the emotional side of an illness may alleviate the need to physically prescribe something (Butler et al., 1998; Macfarlane et al., 1997). Given that educated individuals in higher social grades are more likely to report being given information about antibiotics or caring for their infection (McNulty et al., 2016) it is even more important to not neglect those that are most vulnerable.

My findings suggest that participants consider research to be worthwhile despite deriving worth from different aspects of scientific process or outcome. In line with research by Brew (2001), I saw evidence of the domino, layer, trading and journey conceptions when participants discussed science. The trading concept, that there is money, fame or status to be had from scientific research, is a common thought pattern. Whilst it is true that productive academics are research-active (outcome focused); driven to grow social networks, gain reputation through going to conferences and collaborating with other researchers (Brew *et al*., 2016), this does not mean that they are 'in the pocket of big pharma'. Recent political attacks on researchers describe them as being motivated by financial and political outcomes (Alexander, 2020). This is a concept which undermines public trust in scientists and, as discussed above, can lead to individuals disengaging emotionally from major scientific crises. Applying these findings, it is important to engage participants in research. There they can explore the process focused mind of a scientist in

their own work. The extent to which a citizen science project can facilitate this is discussed in Chapter 5.

My findings suggest that participants relate historical figures to discoveries and timelines; the figures are the 'heroes' of scientific history. Humans are 'story-telling animals' and perceive facts, numbers and urgent appeals that surround global crises inherently as stories (Arnold, 2018). Climate change research has suggested that in order to make the fight against climate change a priority, climate advocates need to tell stories, to mobilise people and guide their actions (Shenhav, 2015). Within all stories are heroes, villains, victims, an object of struggle, a beginning, middle, end and morale of the narrative (Arnold, 2018). Scientific crises, such as antibiotic resistance, need to be explained as problems that are characterised by uncertainty over consequences, diverse and multiple engaged interests, conflicting knowledge and high stakes (Lazarus, 2009). In practice, utilising storytelling, within which you need scientific heroes, to frame antibiotic resistance in a way that encourages people to act is of importance to a joint, global solution.

My findings suggest that participants would make 'bad' decisions in the face of their factual knowledge, often when acting on an 'urgent' problem in the present is considered lower risk than the consequential resistance that might develop in the future. This links to the above, that facts alone do not encourage action and instead a narrative approach would be better used (Shenhav, 2015). Research on the effect of better factual knowledge is inconclusive. Better factual knowledge has been linked with bad decision making (McNulty et al., 2007) and good decision making (European Commission., 2013). Within science, there has been a move away from the provision of objective facts (public understanding) and towards joint negotiation for future science by scientists, laypeople and policy makers (public engagement) (Gregory and Lock, 2008; Schäfer, 2009). In chapter 5, I discuss how engaging members of the public in a citizen science project affects their decision making considering improved scientific literacy.

My findings also show that some members of the public consult unorthodox medicine, homeopathy. Whilst one might expect that unorthodox medicine would have diminished as a result of advances in medicine during the second half of the twentieth century, that does not seem to have been the case (Loudon, 2006). Whilst members of the public are being encouraged to only take antibiotics when necessary, it is important to note that the complete rejection of modern medicine in favour of homeopathy can be dangerous. In 2018, a total of 1.5 million people died from tuberculosis and TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Furthermore, MDR-TB remains a public health crisis and a health security threat, whilst modern medicine is estimated to have saved 58 million lives through TB diagnosis and treatment between 2000 and 2018 (World Health Organisation, 2020).

4.5 Summary

One of the aims of my study was to explore if codes and frameworks identified in the literature review which describe various topics of discourse of antibiotics and antibiotic resistance (Table 2-5), or research conducted on conceptions of

research (Brew, 2001), are fit for purpose when investigating the public's experiences and understandings of antibiotics, antibiotic resistance and scientific research. My analyses of participant interviews indicate that there was no one code or framework that enabled the mapping of all themes emerging from the study. Instead, a combined use of codes and frameworks was necessary. My analyses show that from both quantitative and qualitative analysis of the interview data, eight key themes emerge.

My study set out to explore how participants perceive the issue of antibiotic discovery and antibiotic resistance, as well as how they perceive scientific research. Participants use familiar words and familiar rituals as they attempt to engage with a new discourse. Of these rituals, participants frequently mimic public health narratives to facilitate their engagement with the discourse. Mimicking existing narratives without an understanding of why those narratives exist is an essential part of engaging with a new discourse, but leads to misunderstandings, such as the belief that the human body becomes resistant to antibiotics, rather than the infectious agent. These misunderstandings don't always produce a negative behavioural outcome as shown by this misunderstanding, which still leads to the avoidance of antibiotic overuse. Campaigns should first target those misunderstandings that do produce negative behavioural outcomes.

Individuals who place faith in science and scientists and show scientific optimism are more likely to actively help mitigate their own behaviours which contribute to antibiotic resistance. Scientists should consider this when deciding on whether to engage the public with their research. Participants generally consider research to be worthwhile, however derive this worth from different aspects of the scientific process or outcomes. As well as scientists, those who receive the most blame from the public: doctors and people in power, should strive to rebuild trust with the public. This trust provides the belief that working together to achieve the same goal is possible and is crucial in the effort to tackle this global problem. Further, individuals should be encouraged to consider why antibiotic resistance develops before assigning causality to the self rather than to others. Each of these groups could benefit from the use of storytelling to engage the public, including the use of heroes and villains. This use of storytelling might dissuade individuals from acting against their factual knowledge in the present to resolve an urgent problem, neglecting the increased risk of an apocalyptic outcome in the future.

In Chapter 5, I aim to use medium- and long-term data collection methods to understand the effect engaging with a citizen science project has on participants discourse surrounding these key themes. I trace discursive transformations (Sfard, 2015) by examining the expanding set of endorsed narratives familiar to participants (object level learning) and the way these new narratives contradict previous narratives (meta level learning).

Chapter 5 Medium- and long-term study of public understandings of and attitudes to antibiotics and antibiotic resistance: Findings from a citizen science project

5.1 Introduction

Raising public awareness of antibiotic resistance is a critical step in tackling the global crisis (1.2.1) In 1.4, I discussed public health campaigns and their role in provoking behavioural change to combat significant public health issues such as antibiotic resistance. Analysis of participant interviews highlighted current public awareness with regards to key topics surrounding the issues of antibiotic and resistance. Specifically, interviews indicated that there are still misconceptions about the definitions, causes, implications and solutions to this global concern. Extrapolating from this finding, the results in Chapter 4 suggest that public health campaigns aimed at raising public awareness about this issue are having limited effect.

In Chapter 1 and Chapter 4, I described how my analysis of the literature provided a list of key topics emerging from research on the public discourse of antibiotics and antibiotic resistance and the subsequence coded analysis resulted in the emergence of eight key themes. These themes are outlined in 4.3. Participants were found to use familiar words (4.3.1) and familiar rituals (4.3.2) as they engaged with a new discourse, concurrent with research by Sfard (2008). Common rituals included, for example, public health narratives, bequeathed by Public Health England through a variety of public health campaigns (4.3.3). Participants who placed faith in science and scientists harboured an optimistic outlook on antibiotic resistance (4.3.4), which has been shown to increase support for scientific research and industry (Huber et al., 2019). Participants largely externally attributed blame for the antibiotic resistance crisis to GPs, people in power and irresponsible others (4.3.5). This concurred with previous research on attribution of blame (Brooks et al., 2008; Butler et al., 1998; Dyar et al., 2018; Hawking et al., 2017; Hawkings et al., 2007). Participants considered scientific research to be mostly worthwhile, however concepts of research were diverse; research was both product and outcome focused and with researchers both absent and present in awareness (4.3.6). This mapped to the concepts of research as held by researchers, presented by Brew (2001). Participants also used historical scientific ‘heroes’ and major scientific discoveries to navigate the landscape and timeline of antibiotic discovery (4.3.7). Finally, participants reported that they would act against better knowledge if they deemed the short-term gain to be greater than the longer-term risk (4.3.8). Research on the effect of better knowledge on improving the uptake of ‘sensible practice’ is not conclusive (McNulty *et al.*, 2007a; European Commission, 2013).

In Chapter 1.5, criticisms of citizen science were discussed; notably the lack of evaluation which determines scientific or participant outcomes. Separately, public discourse research summarised in Table 1-6 used mostly short-term data collection tools such as surveys and semi-structured interviews. My analysis showed that of the 20 articles examined, only one used portfolios (Norris *et al.*, 2013). These portfolios were used to detail medicine taking or contact with medication through avenues such as advertisement, however the length of time participants made entries for was not reported. The need to measure several dimensions of project outcomes (1.5.6) and the potential of medium- and long-term tools for data collection were gaps in the literary landscape which this chapter aims to close.

In this project, medium-term data collection was based upon data obtained from participant engagement on social media, notably Facebook (2.7.2). To analyse this medium-term data, web analytics and discourse analysis were used. Web analytics looks at the use of a website or parts of a website to find which sections are visited most often (Phillips *et al.*, 2014). This was useful for understanding and optimising web usage and trends in those that visited the Antibiotics Unearthed Facebook page. Participants from across the globe could engage with the project and this was a low-cost method (Phillips *et al.*, 2014). However, web analytics only have limited utility for summative evaluations and information on users' choices cannot be obtained (Phillips *et al.*, 2014). To mitigate these limitations, in 5.2 only trends are reported. Discourse analysis looks at recording forms of discourse for later analysis. Social media is ideal as comments are already transcribed and timestamped, however as with all discourse analysis, issues may be oversimplified when taken out of context (Phillips *et al.*, 2014). In 5.3, I explore the extent to which social media facilitated enough discourse to be analysed.

In this project, I recognised the potential that portfolios may have for revealing the perspectives of the writer over a period of extended time. Longer-term data collection was based upon data obtained from participant portfolios (2.7.3). Portfolios can provide a personal recording of ideas, thoughts, or activities over a time span, revealing the perspective of the writer (Phillips *et al.*, 2014). They provide feedback on a specific topic on a particular day and can provide an understanding of thought evolution over time (Phillips *et al.*, 2014). However, they can be narrowly restricted to a specific question, require consistency and determination from the participant and are labour intensive to review (Phillips *et al.*, 2014). To manage these limitations, as part of this project I designed and developed a document with a wide range of different topics that provided food for thought on antibiotic resistance. This document was not designed to be prescriptive but to act as a prompt if required by the participant. This document can be found in Appendix G-1.

Using medium-and long-term data, I aimed to understand user trends when visiting the Antibiotics Unearthed Facebook page (5.2) and conduct discourse analysis on Facebook comments (5.3) and portfolios (5.4 and 5.5). The discourse analysis aimed to build on the work undertaken in Chapter 4, to track longer-term evolution of public understandings and attitudes to antibiotics and antibiotic resistance through discursive transformations (Sfard, 2015). Discursive development is studied by identifying transformations in given discursive characteristics at different levels of learning: object and meta.

Object-level development expands what is already known about an existing universe of objects. On an individual level, object-level development occurs when a learner increases the set of endorsed narratives with which they are familiar (Sfard, 2015). An example of this would be an individual who learns that viruses, with which they were already familiar, cannot be treated by antibiotics, with which they were unfamiliar.

Meta-level developments are those that change the rules of a discursive game, when objects contradict previously endorsed narratives. At the level of an individual, meta-level development cannot be attained by pure logic, instead the learner must try to participate, to engage in reflective imitation of an expert

(Sfard, 2015). An example of this would be a member of the public's realisation that there is relative truth and uncertainty in science. This would result in a more nuanced perspective of what science is and what scientists do. This process involves changes in more than just narratives, but in vocabulary (word use), visual mediators and discursive routines as well; the entire discourse.

5.2 Results: A descriptive analysis of key metrics for the Antibiotics Unearthed Facebook Page

Nowadays, the public increasingly gets science news online, particularly via social media such as Twitter, Facebook, or YouTube (Brossard, 2013). Social media has the potential to promote user engagement with science, especially when content is posted by trusted social contacts (Huber et al., 2019). In order to achieve this however, users must want to interact with science content. In the following section, I examined web analytics from the Antibiotics Unearthed Facebook page, the chosen social media platform for engaging participants in this project (2.7.2). What follows is a descriptive analysis of analytics which examines how successfully this study attracted users to a science-focused Facebook page, before discussing which content best reached or engaged the user.

5.2.1 How to attract participants to a Facebook Page

I examined the total number of people who liked the Facebook page over time (Lifetime Total Likes, Figure 5-1). Data collection began on the 17th May 2016 with lifetime 'total likes' at 476. The Antibiotics Unearthed Facebook page was created by the Microbiology Society's Communication Team and had been used prior to the 17th May 2016 to advertise other parts of the Microbiology Society's Antibiotics Unearthed Mission (1.6.2). From the 17th May 2016, it was used solely for the purpose of the PhD study. By the 5th July 2016 [Post 1], lifetime 'total likes' had reached 581. This number increased steadily and at the time of the final post on 21st February 2018 stood at 1,795. Web analytics were downloaded on the 22nd May 2018, at the conclusion of the social media data collection. On this date, the number of lifetime 'total likes' had plateaued and stood at 1,798. A linear trend-line shows that lifetime 'total likes' increased in a linear fashion until Jan/Feb 2018, where the lifetime 'total likes' plateaued (Figure 5-1). My findings suggest that lifetime 'total likes' increase in a steady, linear fashion for as long as content is being published. Detailed descriptions of the Facebook posts can be found in Table 2-6, whilst links to the news articles can be found in Appendix F-1. Following the completion of data collection for the Antibiotics Unearthed Facebook Page, the Microbiology Society Facebook Page was taken down by the Microbiology Society and is no longer available to members of the public. Therefore links to the Facebook page posts cannot be provided.

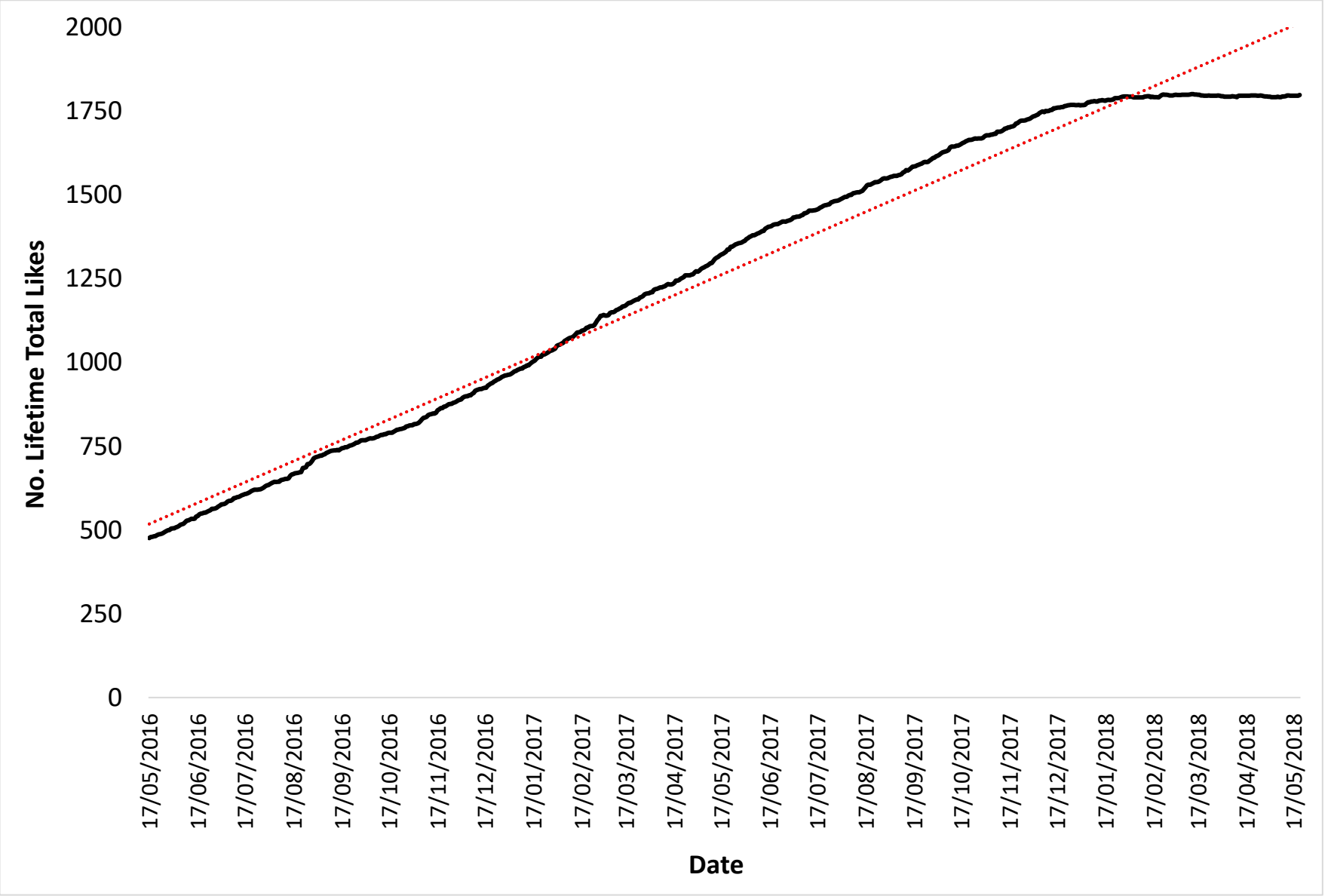


Figure 5-1. Lifetime total likes of Antibiotics Unearthed Facebook Page. The Y axis displays the total number of people who have liked the Facebook Page (unique users). The X axis displays dates from 2016-2018. The solid black line shows the number of lifetime total likes from 2016-2018. The dotted red line is a linear trendline used to show a steady rate of increase or decrease in values over time.

5.2.2 Understanding how to best reach, impress upon and engage participants

Whilst number of lifetime 'total likes' increased steadily with the release of any new Facebook content, I hypothesised that certain types of content would lead to greater engagement from users, specifically media in which the participants had a stake, for example images of bacteria they had collected personally. Over the course of the study, 47 different posts were released. For these analyses I consider all sample images released on the same day as one post. Of these 47 posts, starting on the 5th July 2016 [Post 1] and ending on the 21st February 2018 [Post 47], 21 web links, two event links, four images, two videos, one photo and 17 images of samples provided by members of the public were posted (Table 2-6).

Three key metrics were used to identify the level of engagement of posts. These were the number of people for whom any of our Facebook content entered their screens (Reach of Posts), the number of times content entered peoples screens (Total Impressions) and the number of people who engaged with the Antibiotics Unearthed Page, either through clicking or creating a story, which included commenting on posts or liking posts (Total Page Engaged Users). These metrics were visible daily, weekly or 28-daily. Table 5-1 shows the daily figures. Daily figures were chosen as some posts were released within a week of each other, and so a weekly or 28-daily average did not give the resolution necessary to distinguish between separate media types.

Of the 21 web-links posted to Facebook, the lowest number of engaged users, reach and impressions was three, 36 and 58 respectively. Each of these was on the 18th July 2016. The smallest number of engaged users over the course of the study was one, on the 25th July 2016 in response to an event link for the pop-up stand at Brecon Beacons. The same event link was responsible for the smallest reach, at 31. The smallest impression was in response to the release of a web link detailing upcoming pop-up stands at the Brecon Beacons and Kielder Castle on the 18th July 2016, at 58 (Table 5-1). The highest number was 197, 2,580 and 3,537 respectively on the 21st February 2018 (Table 5-1).

Of the 17 images of samples provided by participants posted to Facebook, the lowest number of engaged users, reach and impressions was 15, 146 and 388 respectively. The lowest engaged users and reach occurred on the 5th December 2017 one month after the Norwich Science Festival and just before Christmas, whilst the lowest impressions occurred on the 16th May 2017, eight months after the pop event in Glasgow Botanic Gardens. The highest number was 43, 15,106 and 80,552. The highest engaged users occurred on the 27th July 2016, after the first pop up event at Brecon Beacons, whilst the highest reach and impressions occurred on the 9th November 2017 straight after the pop up event at the Norwich Science Festival (Table 5-1).

The largest number of engaged users over the course of the study was 197, on the 21st February 2018 in response to a web link to a BBC news article about a new family of antibiotics found in dirt. The largest reach was 15,106, in response to sample images on the 9th November 2017. The largest impression was on the same date, at 80,552 (Table 5-1).

As would be expected, the data show posts released earlier on in the study had a lesser reach, lesser total impressions and lesser page engaged users. Inversely, posts released later in the study had a greater reach, greater total impressions and greater page engaged users. The three posts which reached and impressed upon the greatest number of users were sample images, however these fell between the 6th November and 20th November 2017. This is significant because Antibiotic Awareness Week spanned from the 13th to 19th November and as such, may have driven the sharp increase in these numbers. In addition, these occurred immediately after the pop-up event that took place at the Norwich Science Festival. The greatest number of page engaged users occurred not during this week, but instead in response to the last post released, a web link. This suggests that reach and impressions do not drive engagement. Perhaps instead, engagement is driven by total number of likes of the Facebook Page.

Table 5-1. Level of engagement of types of relevant Facebook content. Table shows the release date and media type of each of the 47 Antibiotics Unearthed Facebook posts. For each release, the daily page engaged users (number of people who engaged with your Page, that is any click or story created), the daily reach (number of people for whom any content from your Page entered their screen) and the daily total impressions (number of times that any content from your Page entered a person's screen) are shown.

Date	Media Type	Daily Page Engaged Users	Daily Reach	Daily Total Impressions
05/07/2016	Web Link	7	38	83
18/07/2016	Web Link	3	36	58
25/07/2016	Event Link	1	31	78
10/08/2016	Image	19	559	1039
16/08/2016	Web Link	82	1049	1768
18/08/2016	Video	23	254	407
23/08/2016	Web Link/Photo	37	409	794
24/08/2016	Image and Sample Images	60	743	4297
22/09/2016	Web Link	17	92	157
23/09/2016	Sample Images	39	420	2768
04/10/2016	Web Link	6	86	155
03/11/2016	Web Link	14	144	262
14/11/2016	Web Link	22	148	244
16/11/2016	Video	13	63	117
23/11/2016	Sample Images	23	439	2169
21/12/2016	Sample Images/Web Link	34	531	1358
09/01/2017	Sample Images	23	531	1378

12/01/2017	Web Link	8	151	263
23/01/2017	Web Link	37	788	1153
26/01/2017	Web Link	18	220	337
09/02/2017	Web Link	18	280	460
22/02/2017	Web Link	65	786	1248
27/02/2017	Web Link	13	170	334
03/03/2017	Web Link	18	158	243
24/03/2017	Web Link	16	220	306
05/04/2017	Image	25	290	499
16/05/2017	Sample Images/Event Link	19	176	388
30/05/2017	Sample Images	38	612	2735
27/06/2017	Sample Images	43	682	4075
31/07/2017	Web Link	5	77	107
10/08/2017	Sample Images	34	1396	3697
19/09/2017	Web Link	11	110	138
02/10/2017	Sample Images	38	352	1088
10/10/2017	Web Link/Sample Images	62	1335	3125
06/11/2017	Sample Images	20	13634	32020
09/11/2017	Sample Images	22	15106	80552
15/11/2017	Image	49	915	1400
20/11/2017	Sample Images	20	8129	37122
05/12/2017	Sample Images	15	146	401
06/12/2017	Sample Images	23	186	954
15/12/2017	Sample Images	33	177	536
21/02/2018	Web Link	197	2580	3537

5.3 Results: A coded analysis of Facebook Comments

Facebook comments were hypothesised to be a tool through which discourse analysis could be conducted to identify discursive transformations in participants across the eight key themes highlighted in Chapter 4. I hypothesised that participants would use resources made available on the Facebook page in order to conduct data analysis of their own samples. I also hypothesised that in doing so, participants would become researchers and adopt process focused perspectives of research.

However, upon examining the data set at the end of data collection 21st May 2018, it became clear that the medium-term engagement did not provide a rich enough pool of data from which to draw these conclusions. Instead, the coding schedule used for interview analysis (Table 2-5) was applied to each of the user comments. In doing so, user comments, as part of participant discourse, were taken to reflect their internal thought processes surrounding antibiotics and antibiotic resistance.

Twenty-six users commented on Facebook content over the course of the study, amounting to a total of 39 comments. Four participants posted twice, either on separate samples or tagging somebody else before asking a question. Five participants engaged in conversation with me. Three of the five participants asked a question, and then proceeded to reply to my response. This took the form of enthusiastic remarks about the information provided, good lucks or compliments regarding the images and took place over a range of two to five days. One participant engaged in comments and responses over the course of a month. One participant sent five comments in response to two images over the course of 11 days. In total, excerpts were captured by 11 codes over 52 references. In the next sections, I examine the key themes emerging from these data.

5.3.1 Participants did not feel confident utilising resources provided to analyse data

Facebook comments, unlike the face-to-face interviews, offered participants a chance to engage with the project by leading the questions. In doing so, it highlighted participants thought processes upon examining images for the first time:

FB01: This is my sample. What does it mean?

Captured by the code “Confidence [CON]” (14 references from 14 sources), participants viewed their images and asked for my help in analysing the data in front of them. In order to assist with this process, a diagram was uploaded [Post 9, 24th Aug 2016] which could be used to describe colony morphology (Figure 5-2). This diagram was also pinned on the Facebook page, so that it would be the first thing users saw as they entered the page. Despite this, participants rarely used this tool, instead opting to ask for my analysis.

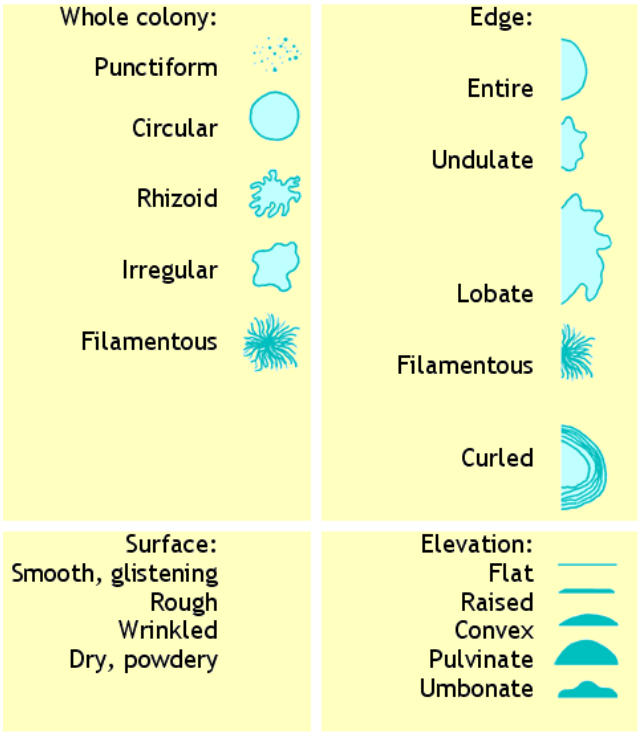


Figure 5-2. Terms used to describe bacterial colony morphology. Whole colony, often described as colony shape, describes the shape of the colony. Edge, often described as margin, describes the edge of the colony. Surface describes the way the surface looks. Elevation describes the 3D shape of the colony growing away from the 2D nutrient source. Taken from Wikipedia, free of license, adapted and redrawn from Seeley & Vandemark (1962). This was posted on the Facebook page on the 24/8/16.

For user FB10, confidence increased as they engaged in conversation about how to analyse their sample data:

FB10: I don't think my sample is showing much.

...

FB10: Wow, is that really all it takes? I think I see another slightly up and to the left of the bottom red circle and on the right hand side opposite the left circle. I took this sample from the stream.

...

FB10: You are spot on with the two I saw Ethan. I'm glad I wasn't just imagining things. So fascinating to learn more about this so thanks for replying to my comments.

They began cautiously, suggesting their plate of bacteria was not showing much. In response, I pointed out examples of inhibition. Once they had seen this done, they began analysing their own plate, spotting different examples of inhibition. I highlighted these examples for them to finalise the analysis, for which they thanked me for engaging them.

Not all participants felt confident enough to try this, as shown by FB11:

*FB11: So what does that mean have we found something ha I'll show *son's name* later xx*

…

FB11: That's sounds great! Very interesting…good luck

In response to their request for help analysing their sample data, I provided examples of inhibition. Unlike FB10, they chose to thank me for the information but then took their leave.

This analysis suggests that when participants were given an opportunity to conduct self-driven data analysis, confidence was a limiting factor. By working in partnership with a researcher, this confidence can be fostered, however only when the participant is willing.

5.3.2 The correct use of scientific terminology

Like with the interview data, I used the code "Scientific Word Use [SWU]" (14 references from 14 sources) to capture each time an interviewee used a scientific word or phrase, either correctly or incorrectly. Within this code, I developed the code "Drug Resistant Infection [DRI]" (2 references from 2 sources). This code was developed in response to research which highlighted the failure of experts to use simple, clear and consistent language in the face of antibiotic resistance, and that this risked undermining the global response (Mendelson et al., 2017). This research recommended that the term *drug-resistant infection* be used to describe infections caused by organisms that are resistant to treatment, including those caused by bacteria that do not respond to antibiotics. User FB12 and user FB19 had excerpts captured by these codes:

FB19: …Do you think mine shows any signs of antibiotic resistance?

and

FB12: …We think there is some resistance activity over on the left hand side but not certain…

Both used the term resistance incorrectly, where the appropriate term would have been antibiotic activity.

This analysis suggests that individuals use familiar words following pre-existing rules when engaging with a new discourse (Sfard 2008). This is in line with my own findings in 4.3.1.

5.3.3 A comment on Medium-Term Facebook data for identifying discursive transformation in participants

During development of methods for this study, Facebook was identified as a relatively low-cost way of engaging participants after the initial interaction at pop-up stands. The low number of comments received, combined with the lack of users who came back to post on several occasions prevented analysis of discursive transformations. Coded analysis has shown that, just like in 4.3.1, participants use familiar terminology, sometimes incorrectly, when engaging with a new discourse. My results suggest that when participants are given an opportunity to conduct self-driven data analysis, confidence is a limiting factor.

However, a limitation of discourse analysis is that issues may be oversimplified when taken out of context (Phillips *et al.*, 2014). Due to the lack of context provided by the small number of Facebook comments, these themes are not added to the following thematic analysis in 5.4. Instead, Facebook comments are used, along with the interview transcripts, to add to the long-term data providing further detail when assessing the evolution of participant learning over the course of project engagement.

5.4 Results: Detailed thematic analysis of Portfolio Participant 01 (PP1)

So far, I have provided a descriptive and thematic account of Facebook interaction and engagement. Now, I present the detailed thematic analysis of one participant portfolio. Portfolios are defined generally as a purposeful collection of work that illustrates efforts, progress, and achievement in one or more areas over time (Stiggins, 1994). Portfolios in education are seen as a means of student assessment that capture the learning process (Barrett, 2005). Herman & Winters (1994) wrote that well-designed educational portfolios represent important, contextualized learning that requires complex thinking and expressive skills and went on to suggest that portfolios encourage focus on important student outcomes, student achievement, and inform policy and practice at every level of the educational system. In understanding the potential that portfolios might have to evaluate the effectiveness of citizen science projects, they were selected as a method of tracing participants' discursive development as they engaged with the topic of antibiotics and antibiotic resistance, as well as initialising the practices and the processes of research.

Participant PP1 took part in an interview on the 4th May 2017, as interviewee 24b [Day 1]. Therefore in Chapter 4, Participant PP1 would have been referred to as 24b. This participant was the first participant to agree to complete a portfolio (PP1) and was the 24th participant to be interviewed across the five pop-up events (24). They were annotated 24b as they interviewed with their partner, who spoke first, and was thus annotated 24a. They made their first portfolio entry on the same day as they attended the pop-up event at Thetford Forest. They submitted their first and only social media comment as FB16 on 26th May 2017 [Day 23], by which point they had made 13 portfolio entries. In total, they submitted 39 portfolio entries until their last entry on the 19th November 2017 [Day 200]. The portfolio contained 9,052 words over 40 pages and was the lengthiest and most substantial portfolio submitted.

With a focus on continuity and coherency, I examine evidence of discursive shifts at the object (what has the participant learnt about science) and meta (what the participant now sees scientists as doing and how they work) level over the course of 200 days. I do so by applying the eight key themes presented in Chapter 4 to PP1's discourse throughout the portfolio and the course of the study. In doing so I present the story of a participant who was willing to engage long-term with the project. I note that in the portfolio, the act of selecting something to write about is already evidence that the participant has encountered something they relate to the project. There are critical

moments when they see or hear something and decide to add it to the portfolio. In the following sections, I analyse the portfolio whilst referring to relevant interview and social media data provided by this participant.

5.4.1 The correct use of scientific terminology

As one engages with a new discourse, one uses familiar words following pre-existing rules that seem in agreement with a new context, regardless of whether others are using this word in the same way (Theme 1) (Sfard, 2008).

In the interview [Day 1], in response to a question about what might be causing antibiotic resistance, PP1 said:

> *24b: Everything's evolving, improving themselves ain't they to fight. [Interview, Q10]*

They drew on a familiar word 'evolving'. It is true that bacteria evolve antibiotic resistance, however I would expect to specifically see the use of the term bacteria. Whilst 'everything' may be evolving, that is not strictly an accurate response to the question at hand. This is pertinent because of the five questions asked to the participant prior to this response to a question about what we take antibiotics for, PP1 says:

> *24b: Flu?*
>
> *R: Okay.*
>
> *24b: You get flu and colds. [Interview, Q3]*

In this instance colds and flu were used incorrectly as these are two diseases not treatable with antibiotics.

On the 5th May [Entry 3, Day 2], PP1 included a reflection of what they had remembered or learned from their interview. I note that during the interviews, I avoided providing facts that might affect participant's proceeding answers, however after the interviews I would answer any questions the participants had and provide detail on topics of questioning. This included information on where antibiotics came from, the drying up of the antibiotic discovery pipeline, the spread of resistant bacteria through genetic and environmental means and which types of infection are treated with antibiotics, among others. PP1 wrote:

> *PP1: The shortage of new antibiotics: no new antibiotics for roughly 30 years & who would have thought about antibiotics being found in the soil – I definitely didn't know that. I didn't know that there's lots of bad germs in soil, if you cut yourself & get soil or dirt in the wound you need to have a tetanus jab. I've never really thought about antibiotics only that they can given to patients in tablet form*

Whilst PP1 reflected on multiple topics and self-reported learning, a key feature was the use of the term 'bad germs'. Early excerpts from interviews and portfolio entries give a baseline that this participant was not familiar with scientific terminology and instead chose to use colloquial terms to facilitate their engagement with the literate discourse. However, the participant was

already expanding their knowledge of one discursive object, bacteria, and provided an example of object level change. Bacteria became of interest not just because of their threat to humans, but also that they can be found in the soil.

As the time spent engaging with the project increased, word use transitioned from colloquial to literate discourse. On the 15th May [Entry 9, Day 12], PP1 discusses what type of infection cold and flu are:

> *PP1: Cold & flu are viral infections & antibiotics are not effective against them. [Entry 9]*

PP1 showed object level change in relation to a new discursive object, viral infections. They now appreciated that viral infections cannot be treated with antibiotics.

On the 19th May [Entry 12, Day 16] PP1 analysed their soil sample which had been uploaded to social media:

> *PP1: When I first saw my soil sample I thought it was just a thick mass & I wouldn't be able to see anything from it, but once I got looking at it more closely I could see zones of inhibitions; circular; irregular & filamentous colonies & the edges were entire; undulate; filamentous & curled. Fascinating really to see all the different shapes. I only know these terms because I saw the definitions also on the microbiology facebook page. [Entry 12]*

Not only did the participant display the correct use of scientific terminology like filamentous and undulate, the participant gave an insight as to how they acquired the use of these words. They reported that they noticed the terms on the Facebook page (Figure 5-2) and then used them to describe their own experiences. The change in ways in which this participant chose to learn about science is a meta level change, which includes their experimenting with new sources of information. I note their correct use of scientific terminology such as zones of inhibition and fili.

On the 31st May [Entry 14, Day 28], the participant examined the difference between a bacterial, fungal and viral infection. In the interview [Day 1, Q3], the participant suggested that all three types of infection could be treated with antibiotics. In the portfolio Entry 28 days later, they stated:

> *PP1: Antibiotics which are used to treat bacterial infections will not work against fungal infections & vice versa. [Entry 14]*

I observe here a realisation by PP1 that not all infections can be treated with antibiotics as well as correct use of the terms bacterial and fungal infections. This evidences an increasing familiarity with the discursive object antibiotics showing object level change.

During the interview [Day 1, Q8], participants were asked what they knew about antibiotic resistance. PP1 responded:

> *24b: I don't really know nothing about it.*

and

24b: Well it's gotta be how your body reacts to fight the antibiotics what you're trying to give us. [Interview]

This would have been captured by the code "The Resistant Body [REB" in Chapter 4.3.3 as part of the Public Health Narrative theme, which I go on to discuss in 5.4.3. On the 6th June [Entry 16, Day 34], the participant made a portfolio entry which set out to answer the question: What is antibiotic resistance?

PP1: Antibiotic resistance is the ability of microbes to resist the effects of drugs. [Entry 16]

PP1 confused 'antimicrobial resistance' and 'antibiotic resistance' when using the term 'microbes' rather than the correct 'bacteria', however they showed a new awareness that resistance is a product of an infectious agent, not the human body. This is an example of object level change. At the end of the entry, the participant added a diagram which explained the process of antibiotic resistance, which specifically mentioned antibiotics and bacteria (Figure 5-3). This evidences object level change as the participant had cemented their knowledge that antibiotics can only kill bacteria.

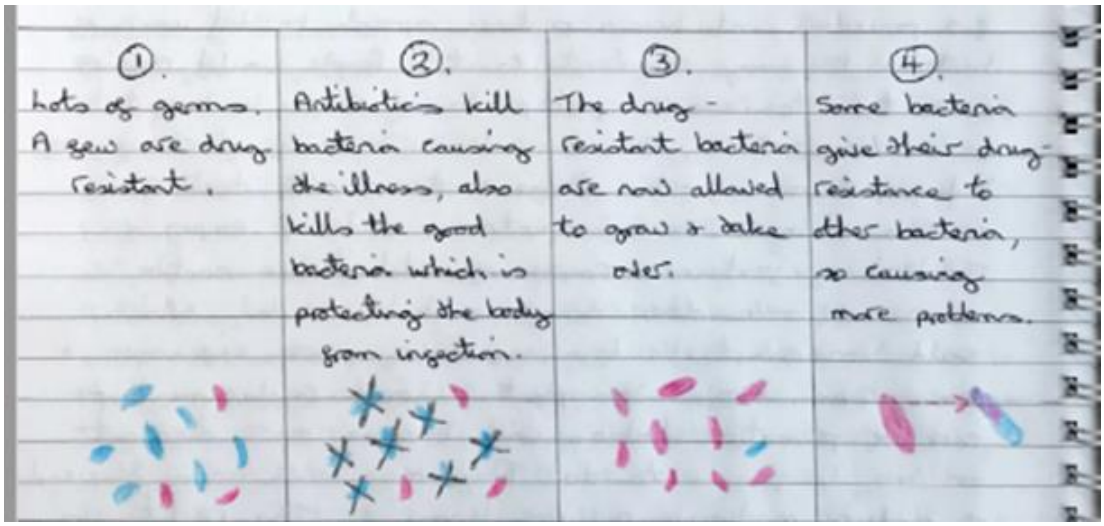


Figure 5-3. What is antibiotic resistance?' an image created by PP1. The entry breaks resistance down in to four steps. Firstly, there are a mix of sensitive and drug resistant germs. We then face a scenario where antibiotics are added, killing the sensitive bacteria, before explaining that this leaves the drug-resistant bacteria to grow and take over. Finally, we have a scenario where drug-resistance is shared between bacteria.**On the 26th June [Entry 22, Day 54] the participant asked the question:**

PP1: "Is all bacteria bad for us?". [Entry 22]

Posing this question itself highlighted the move from general terminology like germs and microbes to the more specific terminology bacteria. I also see the participant begin to break bacteria down into beneficial and potentially harmful organisms:

PP1: This is why we must use antibiotics only when really necessary because if they can harm the dangerous bacteria, they can also harm the friendly good bacteria as well. [Entry 22]

This highlighted a new familiarity with this discursive object, that the word 'bacteria' is not an all-encompassing word but is instead nuanced, another example of object level change. I note that it is at this point of the portfolio that PP1 begins to ask specific questions and use scientific terminology with a greater consistency.

The next few entries from the 4th July [Entry 25, Day 62] to the 25th July [Entry 29, Day 83] are focused on the specifics of antibiotics:

PP1: When a person is admitted into hospital with something wrong with then & the doctor needs to prescribe antibiotics, a broad spectrum of antibiotics is used. [Entry 25]

and

PP1: Since the discovery of penicillin over 100 new antibiotics have been created; each capable of fighting different bacterial infections. Mostly antibiotics are produced naturally in living organisms such as fungi & bacteria. Some of the modern antibiotics are produced artificially. [Entry 26]

and

PP1: Each antibiotic is only effective against certain types of infections., Your GP assess your needs & match them with the drugs available. Mostly a GP will choose an antibiotic based on the most likely cause of the infection. The number of doses & common side effects are also taken into consideration & patterns of infection in your community when choosing an antibiotic. [Entry 27]

and

PP1: Aminoglycosides – tend to be used in hospitals to treat very serious illnesses e.g. Septicaemia. As they can cause serious side effects; including kidney damage & hearing loss, they're usually given by injection. May be given as drops for some ear & eye infections. [Entry 29]

These four excerpts taken from the portfolio entries over this 22-day period highlighted a developing use of scientific terminology. The participant talked of broad-spectrum antibiotics, bacterial infections, doses & common side effects. They also delved into specific antibiotic classes such as aminoglycosides. As well as a notable lack of colloquial terminology, this showed an increasing level of specificity in knowledge they were gaining. It is as this point that I see the participant realised that each discursive object, like antibiotics and bacteria has a history. Rather than a word that can be used to facilitate discourse, they were

exploring the nuance of these words. These excerpts evidence object level change with regards to the object *antibiotics*.

On the 4th August [Entry 32, Day 93] the participant adapted data they saw in a newspaper article identifying average prescription rates per child in the first year of life by country (Slezak, 2017) and provided a personal view of data presented. This is an example of their journey into becoming a scientist and is an example of meta level change. They then commented:

> *PP1: Surely the best way to try to keep babies healthy is by making sure they are vaccinated; this will cut down on some of the life threatening infections. Having too many antibiotics must change the good bacteria which is present in our stomach & skin. [Entry 32]*

This comment showed an increased level of confidence and specificity. The phrase 'surely the best way' is a confident qualifier. PP1, in increasing their understanding of antibiotics, began to realise that they were not always the correct solution and suggested vaccines to reduce overuse. This a further example of object level change.

Towards the end of the portfolio entries on the 12th September [Entry 36, Day 132], PP1 reflected on a piece in the New Scientist. They noted:

> *PP1: It said that antibiotics can save your life, but they can also mess up your microbiome. But now they've formulated some charcoal which could protect your body from the side effects of antibiotics & perhaps even help fight against antibiotic resistance. By killing too many of the good bacteria in our guts, it's making way for the harmful, drug resistant bacteria like C. difficile, which is responsible for about 30,000 deaths in the U.S.A. [Entry 36]*

I note that when comparing the word use in this entry to those during the interview, I see more specific, scientific terminology used in a more confident manner. These words included but were not limited to 'microbiome', 'antibiotic resistance' and 'drug resistant bacteria' and evidenced an object level shift with regards to each of these discursive objects. That this information was taken from The New Scientist highlights that not only was the participant seeking out new information, but they were finding new sources of information from which to do so. The participant often relied on newspapers at the beginning of their journey, however towards the end they began exploring more technical sources. This is an example of meta level change, as the participant changed the ways in which they were learning about science.

This thematic analysis shows that as part of engaging with this study, PP1 experienced discursive transformations. They became more familiar with discursive objects such as bacteria, antibiotics and antibiotic resistance in examples of object level change. They changed the ways in which they conducted scientific data analysis and gathered new information about science in examples of meta level change. These shifts were combined with an increasing consistency and specificity. These shifts were most dramatic in the period between Entry 22 [Day 54] and Entry 25 [Day 83].

5.4.2 Using personal experiences in ritualised ways to participant in a new discourse

Sfard (2008) says that in initial encounters with a new discourse, learners participate in routinised ways, using rituals. Some rituals are more exploratory and have more agency, for example if an individual were to form a hypothesis and this led to experiments being set up to test this. Some rituals simply mimic or replicate, sometimes not very critically, what has been provided by previous users, for example out of respect for authority (Theme 2). Examples of this might include following rituals prescribed by Public Health England, such as handwashing and social distancing. As a society, we are expected to adhere to these rituals and often we do so without knowing the scientific reasons behind them. There is an assumption that when you belong to a community of practice, some of your actions may be rituals or ritualised engagements with what the community would do. If you don't follow these rituals, you will be an outlier. These rituals are process-oriented routines, where routines are repetition-generated patterns of our actions. Whilst an individual may not know why they must take the whole course of antibiotics, beginning to understand the underlying principles is what helps individuals transition into part of the discursive community. As rituals underpin many of our participants actions, I discern these across the remaining sections.

5.4.3 Public Health Narratives

Public Health Narratives are rituals bequeathed by Public Health England. As a member of the U.K public, you are expected to mimic these (Theme 3). In Chapter 4.3.3, I highlighted six public health narratives that had become part of people's everyday practice when engaging with the discourse on antibiotics and antibiotic resistance:

1. Antibiotics are taken for everything (overuse) but shouldn't be (misuse)
2. You should run the full course with prescriptions
3. Antibiotics do not work on viruses
4. Practice better hygiene
5. Stay healthy, including staying active
6. Practice antibiotic stewardship

During their interview [Day 1], when asked about how to make a difference to the spread of antibiotic resistance, PP1 said:

24b: Make sure you wash your hands. [Interview, Q13]

and

24b: Hygiene. [Interview, Q13]

Coded using Public Health Narrative [PHN], this was a direct reference to the ritual of practicing better personal hygiene (4). This was the only public health narrative that PP1 referred to throughout the 13 questions asked in their interview. In their portfolio, PP1 ritualised or discussed four of the six public health narratives across their 39 entries. In the following, I cover the two narratives most frequently referred to over the course of the 200 days of

engagement: overuse/misuse of antibiotics (1) and not taking antibiotics for viral infections (3).

The narrative that PP1 most frequently referred to was that of overuse and misuse. In their seventh entry [Day 8], PP1 tackled a question I had posed in my portfolio factsheet; what causes antibiotic resistance? They focused on overuse as the main, but not only, cause of antibiotic resistance:

> *PP1: Antibiotic resistance results from the overuse of antibiotics, so when you do become ill the antibiotic may not work to over-power the germ bacteria & may led to you being sick for a longer time. [Entry 7]*

In this entry, the participant explained the narrative in the simplest terms, that misuse of antibiotics prevents them working. The next portfolio entry [Entry 8, Day 10] provided a bullet point list of how we use (surgeries and fighting infection) and misuse (over prescription by doctors, self-prescribing, farming) antibiotics.

Their 13th portfolio entry [Day 23] touched on how antibiotic awareness campaigns reduce antibiotic use:

> *PP1: Read today that the number of antibiotics prescribed in England has fallen from 37.3 million in 2014-2015 to 34.3 million in 2015-2016. Antibiotic awareness campaigns help to reduce antibiotic use. [Entry 13]*

In this entry, the participant began to expand on their knowledge of the narrative, specifically quantifying the scale of the problem whilst noting the reduction of prescriptions year on year in the light of antibiotic awareness campaigns. This is an object level change.

The 16th entry [Day 34] tackled what antibiotic resistance is, how it happens, where infections happen and how to fight resistance. When detailing core actions to fight resistance, they wrote:

> *PP1: Only use antibiotics when necessary & use them properly as instructed by the doctor. [Entry 16]*

and

> *PP1: Improving antibiotic use. In order to slow down the development & spread of antibiotic resistant infections, must change the way in which they are used. Antibiotics are given unnecessarily to people & animals – (up to half). They should only be used when needed to treat a disease & the correct antibiotic used & in the proper way. [Entry 16]*

PP1 expanded the narrative, primarily aimed at humans, to include animals. They also detailed how 'the correct antibiotic' should be used 'in the proper way' to treat disease. This shows an increased understanding of antibiotics through the form of specificity. In understanding that there are different types

of antibiotics and different ways in which to use them, they have expanded their understanding of what specifically constitutes overuse/misuse. This is an example of object level change.

In their 20th entry [Day 48], they discussed an internet article about the misuse of antibiotics causing harm in and of itself:

> *PP1: Over a 6-year period this lady received 400 injections, took 20 pills a day & had a lung removed. The drugs were so toxic that they changed the colour of her skin, damaged her hearing & vision, caused excruciating joint pain, triggered bouts of psychosis, left her constantly nauseous & unable to eat. [Entry 20]*

This entry coupled with Entry 22 [Day 54] asking if all bacteria are bad for us, discussed in 5.4.1, showed an expanded understanding of antibiotics as the discursive object in relation to the narrative of misuse/overuse. Instead of seeing misuse as bad only for reducing efficacy of medication when sick, they discovered that misuse can be bad due to the severe side effects of antibiotics themselves. This is an example of object level change.

Over the last few entries, PP1 diverged from the common health narrative, that overuse/misuse of antibiotics can lead to antibiotic resistance and explored other risks present with the abuse of antibiotics in examples of object level change. This learning was facilitated by the participant's active reading on this topic; the collection of new information which expanded their understanding of the discursive object. The active seeking of knowledge is an example of a meta level change. I note that much like in 5.4.1, the participant experienced this significant shift at around the two-month mark.

Their 23rd entry [Day 57] focused on alternatives to antibiotics. The participant began to explore antibiotics as an interesting standalone topic, rather than focusing only on their role in the narrative of overuse/misuse. The participant noted ways in which we can reduce misuse with novel medical techniques, an example of object level change. These techniques included the use of maggots to remove infection & dead tissue and orthopaedic implants which resist infection.

In their 30th portfolio entry [Day 85], they wrote:

> *PP1: Growing resistance to antibiotics is an increasing problem around the world. They are becoming less effective because we take so many of them & this means that deadly infections spread more easily. [Entry 30]*

The participant, after expanding their knowledge of antibiotics over the last few entries, circles back to update their definition of the narrative of misuse/overuse. Their level of specificity had improved since their earlier definition [Entry 7] as had their consistent use of scientific terminology.

In Entry 34 [Day 97], PP1 took a more critical view of the narrative, based on newspaper articles surrounding a debate between scientists about completing the full course:

It is true that by reducing the use of antibiotics should help with the spread of superbugs, but you do have to be careful because a person could be feeling better, but still got the infection in their bodies, so causing either a relapse or treatment failure. By coming up with new ideas, it does leave patients wondering what advice to believe. Prescribing antibiotics in such a readily way & in such large volumes does need to change, because we will run out of antibiotics if we don't reduce the usage, also the resistance problem will worsen. [Entry 34]

This is the first entry where PP1 actively discussed both sides of the underlying aspect of the narrative surrounding overuse and misuse. This is not to say they hadn't considered this, but this was the first time they felt it important enough to make a note of it, an example of object level learning. It is also an example of increased specificity when compared to earlier entries [Entry 7] which suggested that misuse is bad as antibiotics will stop working.

In their 36th entry [Day 132], they discussed another solution to overuse of antibiotics. They begin by stating another critical argument behind taking antibiotics; that antibiotics can both save your life and ruin your microbiome. The use of the term 'microbiome' is an example of object level learning. They then discussed activated charcoal which had been used in drug overdoses to soak up the excess drug:

PP1: The biotech company in Paris have evidence that a modified version could do the same for antibiotics. Apparently there are plans to start testing the charcoal in people taking antibiotics to treat infections next year. [Entry 36]

In Entry 38 [Day 163], PP1 read a newspaper headline (Donnelly, 2017) which reported the U.K Chief Medical Officer talking about a potential antibiotic apocalypse. They rounded off the topic of overuse/misuse by saying:

PP1: Antibiotics should be used more sparingly, apparently patients think that GP's were being "mean" when the refuse to give them antibiotics, but this is to cut out the needless use & to conserve the antibiotics for use when they are really needed. [Entry 38]

They considered criticism levelled at GPs for being mean in context of all the information they had gathered over the previous 163 days. They concluded that rather than being mean, it was action taken in consideration of the narrative of overuse/misuse. PP1 has transformed from an individual who mimicked the ritual of overuse/misuse bequeathed by Public Health England, to an individual who critically assessed behaviour of experts considering this narrative.

The second most frequent narrative referred to is that one should not take antibiotics for a viral infection. PP1 wrote in their seventh entry [Day 8]:

PP1: The more antibiotics are used in excess to treat colds; flu & other viral infections, the more they become ineffective against bacterial viruses. [Entry 7]

At this early stage, it is clear PP1 was still getting to grips with the scientific terminology. Instead of using the term 'bacteria' they used the term 'bacterial viruses'. This mistake is one that is not repeated in further portfolio entries as they continued to engage with the project, an example of object level learning. However, object level learning has taken place with regards to overuse of antibiotics and the resultant reduction in efficacy. They did accurately highlight the narrative that antibiotics should not be used for viral infections.

Their ninth entry [Day 12] answers what type of infection are cold and flu. They first noted that these are viruses and so antibiotics are of no effect. They then described a viral infection their husband had at the time, which culminated in the participant believing the pharmacist had prescribed antibiotics. On this, they wrote:

PP1: Why were these prescribed though as apparently antibiotics are no longer routinely used for sore throats? Surely this is an example of the misuse of antibiotics! [Entry 9]

This shows PP1 imitating the ritual that we should not take antibiotics for viral infections. Perhaps more notably, it also shows PP1 questioning the advice given by an authority figure, in this instance a pharmacist, through conducting their own research. This is an example of meta level change as it shows the way in which the participant views authority changing.

Their 14th entry [Day 28] covered the difference between a bacterial, fungal and viral infection. One excerpt from this was:

PP1: Viruses are smaller than bacteria. They require living hosts to multiple; if not they do not survive. They reproduce by infecting a host & using the DNA from the host they can make copies of themselves. [Entry 14]

Interestingly, the focus on the discursive objects (bacteria, viruses and fungi) switched from being in the lens of antibiotics and antibiotic resistance, and more towards the distinct physical differences between the three organisms. This development of interest in the organisms themselves, rather than just the focus that I imparted on the participant, feels particularly noteworthy and is an example of object level change.

Skipping forward to Entry 33 [Day 94], the participant continued to express interest in the discursive objects themselves as they stuck in several Scanning Electron Microscope (SEM) images of bacteria, viruses and fungi:

PP1: I am quite fascinated with the different shapes of bacteria. So today I thought I'd find some photos which have been taken with a "Scanning Electron Microscope". The detail on the photos is unbelievable, you wouldn't think that bacterial infections could look so pretty… The colours have been added to the photos in order to

make them more impressive & to stand out, well it certainly did this to me! I feel quite silly really & a bit disappointed, but this is something else that I have learnt since doing this project. [Entry 33]

In exploring this newly developed interest in the discursive object, the participant self-identified as having learnt something new, object level change. This is an important reflection for public engagement and speaks to the power of encouraging self-driven research based on personal interests, rather than the presentation of facts as is popular in public understanding. The fact that the participant was curious enough to seek out of new information is an example of meta level change.

Entry 27 [Day 70], 28 [Day 76] and 29 [Day 83] covered different types of antibiotics and the specific infections these can treat. PP1 increased their specificity of knowledge around antibiotics during these 14 days, delving beyond the narrative that antibiotics are for bacterial infections and instead asked which specific types of antibiotics treat which specific types of bacterial infections. In Entry 27, they noted:

PP1: Antibiotics are not needed for viral infections e.g. cough & colds, flu, sore throats or acute sinus. Usually your own immune system will kick in & fight the virus off. [Entry 27]

This goes beyond viral infections as a general term and provides specific examples of viral infections you would not take antibiotics for, an example of object level change. Further, in Entry 29 they note:

PP1: Cephalosporins – used to treat a wide range of infections; also effective for treating more serious infections e.g. septicaemia & meningitis. [Entry 29]

PP1 focused with far greater specificity than they had done previously on the use of individual antibiotic classes in the treatment of bacterial infections. This is an example of an object level shift, whereby the participant was looking deeper into the object itself, rather than using it as part of a predisposed narrative. It is also of note that they were using the term bacteria and virus correctly in a consistent and specific fashion at this stage of their engagement.

During their engagement with this study, PP1 used scientific terminology more consistently and specifically. They experienced object level learning as they examined in closer detail ‘bacteria’, ‘viruses’, and ‘fungi’ but also as they improved their understanding of ‘antibiotics’ outside of the role they play in overuse/misuse. In examples of meta level learning, they challenged authority and sought new information on topics that were of interest to them. The combination of these shifts resulted in discursive transformation, whereby the participant developed a more evidence based, critical approach to the rituals adopted by the literate discourse.

5.4.4 Human Health Narrative

In chapter 4.3.4 I showed that participants who placed faith in science and scientists had a more positive outlook on antibiotic resistance (Theme 4). This

science optimism was linked to a likelihood to act to help mitigate effects of global crises. In this section, I examine the extent of PP1s science optimism and whether this changes over the course of the study.

Whilst PP1 presented a more dystopian perspective of antibiotic discovery and the threat of antibiotic resistance in their interview [Day 1], they expressed science optimism from the outset in their portfolio. In PP1's second portfolio entry [Day 1], they wrote:

> *PP1: Today I took a soil sample from Thetford Forest. Who knows, perhaps a new antibiotic will be discovered in my sample! [Entry 2]*

It was made clear to PP1 that finding new antibiotics was a very difficult task Yet still, PP1 showed faith in my ability as a scientist, as well as a utopian perspective that a cure is out there to be found, that underpinned this utterance.

Rather than provide quotes for each time the participant expressed positive or negative emotions, utopian or dystopian perspectives or optimistic and pessimistic outlooks, I describe my general feeling from the data analysis. Reading through the 39 entries over a 200-day period, there is a continued sense of science optimism.

The most noticeable transformation of PP1 captured by this theme was their development of the understanding of what a scientist is, facilitated by their role playing as a scientist or researcher. In their earlier entries, such as Entry 5 [Day 5] PP1 said:

> *PP1: It appears to represent a new class of antibiotics & seems hopeful that the new isolation techniques could lead to further antibiotic discoveries. Which would be would great news as 30 years is a long, long time to wait since the last antibiotic was discovered. [Entry 5]*

The final sentence suggests a sense of relief that scientists may have discovered a new class of antibiotics, there is emotion attached to the factual account of this novel drug. As the entries continue, the participant began to divorce themselves from emotion and instead presented factual summaries of the discursive objects they were researching. Entry eight [Day 10] is an example:

> *PP1: There are many used for antibiotics – heart surgery; organ transplants, joint replacements, severe burns, chemotherapy, blood transfusions, chest infections – to name but a few. By using antibiotics they have helped to increase our life expectancy. If antibiotics lose their effectiveness then key medical procedures as mentioned above could become too dangerous to perform. [Entry 8]*

Here there is no emotion attached to their writing. Whereas before they expressed happiness, they now presented key facts on behaviours which may help us or hinder us. I note that this is a key part of becoming a scientist who

must objectively analyse the facts and attempt to remove emotion from the discussion. This suggests a meta level change, that the participant was developing their understanding of what a scientist is as they themselves engaged with their role of becoming a citizen scientist and a researcher.

This thematic analysis suggests that PP1's level of science optimism did not change over the course of their engagement. They showed science optimism from the first portfolio entry. Instead a meta level shift occurred with regards to the emotion they used in their writing. They developed from including their emotions in their writing to writing objectively about the facts they were researching.

5.4.5 Who is to blame?

In Chapter 4, I found that individuals would often blame external factors, that is other people, as responsible for the worsening antibiotic resistance crisis (Theme 5). Specifically, participants blamed doctors, people at the top and irresponsible others. Very rarely would an individual acknowledge their own contribution to antibiotic resistance. In this section, I examine the apportion of blame by PP1 and whether their perspective changed as they engaged with the project.

In their interview [Day 1], when asked whether antibiotics were misused, PP1 suggested that they were, placing the blame mainly on doctors:

> *24b: Ye probably ye, I'm gonna say perhaps, perhaps they're prescribing them, or giving them to you a bit too much. [Interview, Q15]*

In Entry 3 [Day 2], the participant wrote:

> *PP1: I was rather surprised that so many people said "No"! [Entry 3]*

I note that the question to which the participant believed people answered no was whether they wanted to take part in our project. Most people asked during the pop-up stand were enthusiastic. Whilst done in the passive voice, the participant revealed an elevation of their own level of responsibility compared to others; whilst others said no, they took the time to learn about antibiotics, something that they didn't know before. In the same entry, PP1 wrote:

> *PP1: I'm hardly ever ill, apart from coughs & colds, just minor illnesses. [Entry 3]*

This again divorced their own actions regarding misuse of antibiotics from that of the general public: if they are not ill, then they are not the person asking for medicine. Whilst this may be accurate, the fact they felt it important enough to write suggests that they are proud of their own behaviour. In Entry 4 [Day 4], PP1 focused on the contents of their own medicine cabinet:

PP1: I don't believe I've got much in the medicine cabinet, but it was surprising what I found once I started to take things out & make a note of them! [Entry 4]

Participation in the study provided an opportunity for reflection that otherwise the participant may not have had. I note that none of the medicines the participant had contained antibiotics. In Entry 7 [Day 8], the participant talked about a bug which is resistant to all antibiotics and killed a woman (Gallagher, 2017). They said:

PP1: The lack of new drugs & over prescribing antibiotics have all led to bacteria becoming more & more resistant to modern medicines. [Entry 7]

This entry infers the pharmaceutical industry may be to blame for not producing new drugs and the doctors that are prescribing too many of the current drugs.

In Entry 8 [Day 10], the participant discussed how we use and misuse antibiotics. Naturally this entire section attributes blame. During this entry, PP1 attributed blame to doctors, people who self-medicate and farmers whilst suggesting that antibiotics used for surgery are used properly, increasing our life-expectancy:

PP1: As well as doctors overprescribing antibiotics, you can buy them without prescription on the internet. This is encouraging self-medication & a low quality care, as you're probably taking the incorrect antibiotics. [Entry 8]

Whilst this is still an example of external attribution, there was a move from 'they' as large groups of individuals to 'they' as individuals. However, this almost pardons the individual by suggesting they are encouraged to self-medicate, perhaps placing blame at those who are selling the antibiotics online.

In Entry 9 [Day 12], discussing what type of infection is cold and flu, the participant noted an infection that their husband had been suffering. The final part of this entry was discussed in 5.4.3 as a meta level change in their perspective of authority. The first part of the entry described the experience of going to a chemist belonging to a well-known pharmacy chain to receive over the counter medication to manage the symptoms, they noted:

PP1: She said to help with his throat "Tyrozets[6]" would be what she recommended; along with paracetamols. These tablets are dual action, containing "Benzocaine" & "Tyrothricin". Apparently an anaesthetic to quickly numb throat pain & an antibiotic to help fight throat infection. [Entry 9]

The use of objective facts to make real life decisions is an example of a meta level change. The participant began to imitate the behaviour of a scientist as

they engaged with the study. With regards to apportioning blame, whilst the participant suggested the prescription of an antibiotic for a likely viral infection is misuse by the pharmacist, they personally assumed the responsibility to ensure this medication was suitable. This is another example of a meta level change, where they are moving from the external attribution of blame to the internal attribution of blame.

Entry 20 [Day 48] covered a newspaper article about misuse of antibiotics leading to dangerous resistance (Picard, 2017). The article discussed a woman who had MDR TB and her resultant treatment plan which left her very sick:

> *PP1: The problem was that she never had diagnostic tests to identify the drugs to which her TB was resistant. The drugs were prescribed on a hit-or-miss basis & actually made her more ill. [Entry 20]*

Whilst the participant used the passive voice in saying 'were prescribed' rather than 'the doctor prescribed', I assume with some confidence that the participant was alluding to practitioners of the medical profession.

Entry 21 [Day 48] about TB in Britain included the following:

> *PP1: Experts want all immigrants & long-stay visitors from countries rife with TB in Asia, Africa & South America to be screened on entry to Britain[7]. [Entry 21]*

> *PP1: TB is making a comeback because of complacency & lack of medical staff to trace victims & offer prompt treatment. [Entry 21]*

> *PP1: Multi drug-resistant strains of TB are becoming more common because patients fail to take their treatment correctly & so this allows the bacteria to fight back. [Entry 21]*

> *PP1: In the 1970's we were close to wiping out TB, but once again development of new drugs did not come about & the disease has re-established itself in Britain. [Entry 21]*

These four consecutive sentences have been split to highlight the four key places in which blame was attributed. Whilst the participant appealed to experts, they were not taking a critical stance on what they had read and so we can assume that they agreed. Thus, there was blame for immigrants and long-stay visitors from countries rife with TB, tracing staff and those responsible for employing medical staff, individuals who fail to take their treatments and finally the pharmaceutical companies who are not developing new drugs. Based on the specifics of these entries and their factual accuracy, I confidently assert that PP1 explored a diverse range of sources to investigate

a topic of interest sparked by a previous entry. This is an example of meta level change.

Entry 22 [Day 54], previously discussed in 5.4.1, explored whether all bacteria are bad for us:

> *PP1: …That's why it's important to do exactly what the label says regarding hours between taking the pills. Always make sure that the full course of antibiotics prescribed are taken. [Entry 22]*

PP1 adopted personal responsibility, which notably coincided with the more prominent use of public health narratives in the participants writing. This meta level change, the imitation of experts narrative that we must all be personally responsible, is facilitated by object level change, the objects being 'bacteria' and 'antibiotics', discussed in detail in 5.4.3.

In Entry 25 [Day 62], PP1 discussed the use of broad-spectrum antibiotics by doctors for recently admitted patients:

> *PP1: When an antibiotic is used in this way to cover against everything; it is known as "carpet bombing" but actually what is needed is more precision targeting. So this is why DNA sequencing could be used as it would be more accurate & diagnosis would be quicker. [Entry 25]*

This entry is markedly different from those attributing blame earlier on. Firstly, the participant was more specific with their language and used researched facts to explain a practice used by doctors that they did not agree with. They then went on to suggest the solution to this problem. This fact-based criticism followed by the provision of a solution is something the participant continues to do across the following entries and is an example of meta level change.

Entry 30 [Day 85], discussed in 5.4.3, documented a news report on the debate between the British Medical Portfolio and longstanding Public Health Narratives; that one should complete their full course. In this entry, the participant used the phrase 'we take so many of them'. In doing so, the participant had stopped divorcing themselves from actions taken by individuals of others. This is increasingly noteworthy given that scientists and doctors are questioning each other in this debate, and so it would have been very easy to pick a side and blame one or the other. This continued transition from external to internal attribution over the course of the study is further example of meta level learning.

Entry 32 [Day 93] focused on a news article (Slezak, 2017) which discussed antibiotic prescription rates per child in the first year of life in different countries. This was discussed earlier in 5.4.1. PP1, instead of attributing blame to doctors as would have been understandable, presented a potential solution to overprescribing (vaccinations) and presented information to back up reducing the use of antibiotics at an individual level.

In Entry 38 [Day 163], discussed in 5.4.3, PP1 read a newspaper headline which reported the U.K Chief Medical Officer talking about a potential antibiotic apocalypse (Donnelly, 2017). They reflected on antibiotic use as a whole and

for the first time, actively defended doctors. PP1 suggested doctors had a reason to be acting with prudence when considering antibiotic prescriptions. In the same entry, PP1 wrote about a GP from Manchester who went on to a Television breakfast news show to talk about misuse of antibiotics:

> *PP1: She was only on for about 4 minutes if that, surely to get people's understanding of antibiotics more time should be taken to explain things, instead of trying to cram more & more subjects – which don't amount to anything into the breakfast news. [Entry 38]*

The participant again defended a doctor seen as trying to educate the public and criticised the news programme for not allowing the doctor enough time to do so. As the participant had increased their knowledge of the discursive object 'antibiotic resistance', they began to understand the roles doctors play. This facilitated a change in attitude towards doctors, in which the participant started to see public education as a noble cause that should be defended. This is another example of meta level change.

This thematic analysis shows that over the course of engagement with the project, the participant experienced meta level learning. They moved from only external to a mix of external and internal attribution of blame for antibiotic resistance. They began to seek out new information from a wide variety of new sources. They began to think like an objective scientist, using facts to back up their opinions or base their decisions and they developed a growing appreciation for the role experts play in communicating with the public.

5.4.6 What are scientists after?

Brew (2001) presented four qualitatively different ways in which research is understood (Theme 6). These were labelled Domino, Trading, Layer and Journey (Table 4-1). In Chapter 4, I provided examples of participants who perceived research through each of these four different lenses. This section focuses on PP1's perception of research and how engaging with this project changes this perception of science and scientists.

During PP1's interview [Day 1], when coding for Domino, Truth, Journey or Layer, there were no examples registered. This is likely a reflection of the lack of targeted questions based around the concept of research. In the portfolio Entry 1 [Day 1], the participant wrote:

> *PP1: Today I was asked if I'd like to be part of a scientific experiment. I was very excited!! Me & *Husband's name* only went for a walk around, or so we thought! Never expected to be asked to take a soil sample. We were happy to do so. Something you don't get asked to do every day! [Entry 1]*

The participant expressed their excitement at the prospect of being part of a scientific experiment, that was exploring their role as a citizen scientist. I have touched slightly on PP1's growing understanding of what is it to be a scientist in the previous sections. The participant was excited to be part of the experiment, without any mention of external products, an example of Journey.

In Entry 2 [Day 1], discussed earlier in section 5.4.4, the participant wondered whether a new antibiotic might be discovered in their sample and expressed their wonder at what the sample would look like once grown in the lab. This is a product focused perspective with the researcher absent from awareness, an example of Domino.

In Entry 3 [Day 2] the participant said:

> *PP1: I was very happy to be involved with the soil samples, & I'm interested in learning about things that I don't know about... [Entry 3]*

The participant adopted the internal process focused nature of a scientist, that is to learn about things they didn't know about. As a scientist, I suggest that much of the excitement surrounding experimentation is driven by the possibility of discovering things that weren't previously known. The products generated along the way are a pleasant consequence but are not the underlying motivation. This mindset is a continuation of Journey, seen in Entry 1. In the same entry, they wrote:

> *PP1: By you having information on stands & getting out in the community & talking about things, this must make people aware of what's happening about antibiotics, even if it's only a few people at a time! [Entry 3]*

This excerpt was written with the researcher absent from awareness. The focus was on how the information itself must increase awareness; a process focused perspective and as such is coded by Layer.

In Entry 5 [Day 5] the participant detailed their day to day workings, how they were going about conducting research:

> *PP1: I decided today to try to find out the answer to the above question. I put the computer on & went onto the Wikipedia site & there I found out that… [Entry 5]*

This entry is coded by Journey. The participant discussed their role as a researcher, that is the internal processes of searching for new information to help answer a question of interest. This is also an example of meta level learning.

In Entry 6 [Day 6], the participant again discussed the process they undertook to discover new information, providing a source, a date and their overall feeling about the information:

> *PP1: Today I read an article on line from the mail (08/03/17) which I found very interesting & fascinating. [Entry 6]*

I will not highlight every example, but I note that the participant did this often. In each case, this would be coded by Journey. The participant was fulfilling their role as a citizen scientist and was going through the process of research to discover information that they were previously unaware of. When writing about their sources of information, the participant began by saying Wikipedia.

As entries progressed, they added a date. In later entries, they provided the title of the article, the date they read it and the information they deemed important. This was an increasingly structured and mature method of data entry and is an example of meta level learning.

In Entry 12 [Day 16], the participant noticed their soil samples had been uploaded to Facebook. They wrote:

> *PP1: Today I've noticed that our soil samples are on the Facebook page, wonder what they'll look like, only hope my sample hasn't failed! I'll be really disappointed if there's no results. [Entry 12]*

The participant was no longer worrying specifically about whether they discovered a new antibiotic [Entry 2] but was generally hopeful that there would be results. It is a common feeling as a scientist to be invested in your experiment, hoping that the time spent will produce an outcome. Often outcomes are then used to justify undertaking new processes of research. As researchers are absent from the awareness, this is coded as Domino. The participant went on to describe the results of their sample, as discussed in 5.4.1. The participant discussed their data, together with the hidden meanings. That is, they had their sample, and using a tool provided by the Antibiotics Unearthed Facebook Page, they uncovered the truth of the results of their sample. They then detailed their fascination with this process. This is coded for by Layer.

In Entry 16 [Day 34], in response to the question "Where do infections happen?" the participant talked about tracking:

> *PP1: CDC (Centers for Disease Control and Prevention) gather data on antibiotic resistant infections, the causes of infection & whether there's a particular reason why some people are more susceptible to getting a resistant infection. With this information, experts can develop strategies to prevent these infections & prevent the resistant bacteria from spreading. [Entry 16]*

The participant discussed data collected by the researchers at the CDC, focusing on the separate issues which come together to produce meaningful strategies to fight antibiotic resistance (Domino*)*.

In Entry 17 [Day 36] the participant had a realisation:

> *PP1: It wasn't until I was reading a bit of information on the computer about the history of antibiotics, that I realised penicillin (the first commercialized antibiotic) was discovered in 1928 by Alexander Fleming (which I have mentioned previously in this diary). But it wasn't until 1945 when it became used for the general public, so that had taken 17 years before it was processed into a proper commercial drug. [Entry 17]*

Like Entry 12, the participant uncovered the hidden truth behind the data they were examining (Layer). In this instance the data was information about the history of antibiotics, and the hidden meaning was that the process of turning

a novel drug into a commercial product takes a substantial amount of time. This is an example of object level learning as the participants knowledge about antibiotics had increased. It also examples meta level learning, as the participant expressed a change in understanding that science takes time, something highlighted a few more times throughout the portfolio.

In Entry 18 [Day 37] PP1's development as a data analyst was highlighted. An email alerted the participant to further sample data having been uploaded to Facebook. Like the first analyses [Entry 12, day 16], they used the tools provided to describe the morphology of their sample. However, they went further:

> *PP1: This time I actually compared my husbands soil sample with mine (our samples were from the same hole but his were deeper). The same grown in the all culture media looked totally different to my soil sample, looked to be different shades of yellowy/orangey – a lot lighter shade from mine. He still had filamentous shapes, but his circular shapes looked larger & not all of them were circular but irregular. Could also see zones of inhibitions. The sample grown in the brain-heart infusion media looked to be the same colour as my sample. He did have bigger circular growths & a few more irregular ones along with filamentous. Looked at the other soil samples which other people took, surprising how different they looked, very diverse. [Entry 18]*

Over the course of six entries, 21 days apart, the participant had gone from a descriptive to a comparative account of their data. Descriptive account describes the process by which a scientist would describe their data as what they could see, for example when the participant said 'I could see zones of inhibitions; circular; irregular & filamentous colonies & the edges were entire; undulate; filamentous & curled' [Entry 12]. Comparative account describes the process by which a scientist would take the description of their data and compare it to other data. This would often be other data in the literature, but PP1 made use of the other samples collected at the pop-up event 'This time I actually compared my husband's soil sample with mine' and 'Looked at the other soil samples which other people took, surprising how different they looked, very diverse'. Examining this entry technically, there were several typographic errors and spelling mistakes. This entry is an example of Domino, where separate elements are synthesised so that questions are opened.

In Entry 19 [Day 44] the participant watched a BBC2 programme called Britain's Greatest Invention (BBC TWO, 2017). Whilst the public voted, the participant documented the history and then future of antibiotic discovery:

> *PP1: This study opens new avenues of research & possibly a new antibiotic. Very fascinating, something else knew I have learnt since I began doing this project. [Entry 19]*

The participant discussed a study about antibiotics found on the skeleton of ants, before writing that how hidden beneath this study were new avenues of research (Layer).

In Entry 21 [Day 48] at the end of a discussion about cases of TB in Britain, the participant said:

> *PP1: Researchers in Oxford & Birmingham have managed to isolate different strains of the disease, using a process called "genome sequencing". This means patients who may have waited months to get the right drugs for the treatment of TB can now be diagnosed in just a few days, so they have a greater chance of recovery. [Entry 21]*

This entry is coded by Domino. The participant described the synthesis of research which used genome sequencing which ended up solving the problem of rapid diagnosis of TB.

Entry 23 [Day 27] is an example which highlighted how the media depiction of science and scientists influences public perspectives of science. The media is overwhelmingly outcome focused:

> *PP1: A method to treat the surface of titanium orthopaedic implants, hip & knee joints, plates & screws; so that they resist bacterial infection, which often developed following surgery, without the use of antibiotics. [Entry 23]*

When discussing news report throughout the portfolio, PP1 mimicked this perception of science and took an outcome focused perspective. Media often creates a discovery focused image of science and scientists, whereby the trials and tribulations are not reported unless they are juicy, for example Penicillin being discovered by accident because Fleming's bench was not tidied away before a holiday. Discussion of products with the scientist in the perspective is coded for by Trading.

In Entry 24 [Day 60], whilst watching a BBC 2 programme about milk production, PP1 wrote:

> *PP1: The man who works at "Aria" said that they didn't want antibiotics being present in the milk because it would make people become immune to the antibiotics. I would have said the same before I started learning more about antibiotics, but actually that statement is wrong! It's not people but the bacteria inside of us that actually becomes resistant to the antibiotics. This really does show that getting science communication out to the general public & making people aware of things is very important!! [Entry 24]*

The participant, through their own exploration, self-reported their personal journey of discovery. This is coded for by Journey. The participant corrected the suggestion that the human body becomes resistant to antibiotics, a perception that PP1 shared at the beginning of their portfolio and was discussed in 5.4.1. This is evidence of object level learning. They correctly identified why this was the incorrect view and went on to suggest the importance of science communication in making people aware of key 'things'. Meta level learning is evidenced through the participants new appreciation of

the need for science communication. Their own learning was highlighted when forced to reflect on how much knowledge they had gained since taking part in the study. This facilitated the realisation that their own exploration of discursive objects had empowered them.

Entry 25 [Day 62] talked about scientists moving from a broad stroke when prescribing antibiotics to a more targeted approach where the correct antibiotic is prescribed for the bacterial agent:

> *PP1: DNA sequencing is being done but as in all scientific research it takes time to get it right & the "100,000 Genomes Project" has been extended from 2017 until the end of 2018. [Entry 25].*

The participant was further exploring the notion that scientific research takes time, first exampled in Entry 17. In this instance, rather than discussion about the historical development of penicillin, the participant discussed the difficulty of active research and the need to take time to 'get it right'. Scientific rigour is a key foundation of all research projects. This evidences meta level learning and is coded by Journey.

In Entry 29 [Day 85], the participant wrote about a scientific study by the British Medical Portfolio:

> *PP1: Doctors have always said that you should always finish a course of antibiotics, even if you do feel better. This is being challenged now by a group of leading medical experts writing in a British medical journal. It is being argued that taking antibiotics for longer than necessary can raise the risk of developing a resistance to the drugs. However Englands Chief Medical Officer says that people shouldn't change their behaviour because of one study. He has said that the evidence will be reviewed, but for now the message remains that you should stick to prescriptions & always follow the doctors advice. [Entry 29]*

This focused on the outcome of a culmination of scientific research, without the researcher present in the awareness (Domino). This excerpt is of further interest as it was the first time that the participant encountered a scenario where scientific findings disagreed, an important part of scientific study. Conflicting studies can bring in to question how true the findings from any scientific studies are. Scientists are aware that it is the overwhelming consensus of multiple studies which support hypotheses and lead to the generation of theories. In imitating the Chief Medical officer's narrative, that behaviour should not change based on one study, PP1 experienced meta level learning.

In Entry 32 [Day 94] the participant glued in images taken of Bacteria, Fungi and Viruses under a Scanning Electron Microscope. They provided descriptive analysis of each, as well as a summary paragraph. This was discussed in 5.4.3. This is an example of Journey, object and meta level change. The participant, in their role as a citizen scientist, conducted the internal process of exploring a topic which fascinated them, as the researcher. They increased

their knowledge of the discursive objects ‘bacteria’, ‘viruses’ and ‘fungi’, as well as ‘Scanning Electron Microscopes’. In being a researcher, their perspective of what data was changed. They self-reported disappointment and embarrassment when they realised the data was not what they expected; that the colours added to these photos are superimposed. They reported this to be learning. Further, this excerpt is an example of increased specificity, both in their chosen topic of exploration and the resultant technical language they used when describing their findings.

In Entry 33 [Day 97], the participant wrote about the BMJ debate discussed earlier in Entry 29:

> *PP1: By coming up with new ideas, it does leave patients wondering what advice to believe. [Entry 33]*

This example is coded by Journey. As scientific theory is constantly evolving, the narratives released to the scientific community and the public might only be true at the time. This can cause confusion. The participant was beginning to understand the difficulty of engaging with the public, that the narratives provided by experts are subject to change. This is an example of meta level learning, understanding some of the complexities of being a scientist and sharing results with the general public. Giving credit to the power of this citizen science project, the object and meta level learnings experienced up until this point gave the participant the ability and confidence to decide where they stood in the debate between the BMJ and the narrative that one should complete the full course.

Entry 35 [Day 100] covered an antibiotic working against Gonorrhoea:

> *PP1: A new class of antibiotic has been found to work in the lab against the STD gonorrhoea, which can cause infertility & damage to babies & it is fast becoming resistant to all existing drugs. Successful lab tests of “closthioamide” show potential as an effective new treatment, it’s early days as yet as the antibiotic has yet to be tested in animals or humans. [Entry 35]*

This entry is captured by Domino, considering product generation with the researcher absent from awareness. This was an important milestone in the participants shifting perspectives towards science and scientists when reflecting upon media releases. They presented the outcome focused discovery of a new antibiotic before qualifying this with the fact it was early days and further experiments would need to be undertaken. This is an example of meta level learning.

In Entry 38 [Day 163] the participant talked about a GP from Manchester on Breakfast Television. This entry was discussed earlier in section 5.4.5. This is captured by Journey. Like in Entry 33, the participant identified the complexities involved in being a scientist who shared results with the general public. Further to Entry 33 however, Entry 38 was a reflection on the way scientists were communicating with members of the public and how it wasn’t working. This was a substantial comment on science communication at present, especially coming from a member of the public whom this broadcast

would likely have been aimed at. It is also of interest in the light of the movement away from public understanding towards public engagement; a negative review for this example of a provision of facts from a participant who had been engaging with the literate discourse and had self-reported learning on several occasions.

This thematic analysis shows object level learning. The participant provides evidence of learning about 'antibiotics' and the process through which scientific findings are reached. It also shows meta level learning. PP1 sought out information to explore new topics of interest and matured in their reporting of the sources from which they gained their information. They developed an understanding that science takes time. They learnt that scientific narratives are built upon many studies, rather than just one, and that these narratives are subject to change as the literature expands. Finally, they discussed the importance of communicating with the public based on their own learning experiences engaging with this study, and critically assessed one form of science communication as not achieving its aims. Over the course of the study, the participant showed examples of perceiving science through each of the four lenses described by Brew (2001). Toward the end of the study, the participant was often captured by Journey. In the middle of the study, Domino and Layer were more common. Trading occurred when the participant presented information from news articles.

5.4.7 Do people need a hero?

In 4.3.7, I presented evidence that suggests individuals use elements of storytelling, notably heroes, to engage with a new discourse (Theme 7). Whilst the discoveries and historical timelines attributed to these heroes (famous scientists or scientific discoveries) were not always accurate, they helped the learner picture a historical timeline in which a succession of discoveries were made by particularly clever individuals. In this section, I examine PP1's use of heroes when discussing their thoughts about antibiotics and antibiotic resistance and whether this use changes over the course of engagement.

There were no examples of PP1 using heroes during their interview. In Entry Five [Day 5], the participant discussed what Teixobactin is. In Entry 11 [Day 15], PP1 provided an update on Teixobactin:

> *PP1: Scientists are still waiting on the development of teixobactin as a drug. It's been 2 years since it was discovered, but still it's only at the stage of development in a laboratory. This just shows how long a process the development of a new antibiotic takes. All the time; dedicated effort by the scientists & of cause the cost of it!!*
> *[Entry 11]*

Teixobactin is discussed as an important discovery and the scientists working on it modern heroes. Just like with the historical penicillin, Teixobactin is an important antibiotic discovery. Unlike the discussion of penicillin by interviewees in 4.3.7, and PP1's own discussion of teixobactin in Entry 5 (5.4.4), PP1 now provided nuance when talking about Teixobactin. Whereby the team of scientists who helped develop Penicillin might be boiled down to

Fleming, Teixobactin was considered in the light of scientists. This is an example of meta level learning in which the participant begins to appreciate the way in which teams of scientists work together to collaborate on scientific discoveries, as well as the time requirement and the significant level of financial investment required to get the scientist's research into the clinic.

In Entry 15 [Day 33] the participant mentioned Alexander Fleming:

> *PP1: Predicted figures are that by 2050 10 million people a year will die! That's not many years away only 33. (considering when Alexander Fleming won the Nobel Prize for penicillin, at his acceptance speech he did warn of bacteria becoming resistant to it!) [Entry 15]*

Alexander Fleming is a hero in the story of antibiotic discovery. The participant used Fleming as an authority to emphasise their point, that we likely should have been aware antibiotic resistance was going to happen. They also hinted at the length of time that has passed since Fleming's Nobel Prize to infer that 33 years is not very far in the future. The participant's increased awareness of the facts surrounding the threat of antibiotic resistance to human health is an example of object level learning. There is also a growing realisation about the scale of the issue with the addition of the number of possible deaths from antibiotic resistant infections.

In Entry 17 [Day 36], the participant wrote about the history of Penicillin, from its initial discovery to its use in World War 2 to treat Gonorrhoea. They used Penicillin, Fleming, World War 2 and Churchill as anchor points for this story as familiar historical figures who were influential in creating the antibiotic landscape. This entry was discussed in section 5.4.6. The participant, in exploring the heroic discovery of Penicillin by Fleming, began to uncover the successive sequence of events that resulted in the testing of Penicillin in World War 2. It was at this time that they realised how long it takes to develop antibiotics into commercial products usable by the general public, a meta level change, through improved object level understanding, specifically 17 years. These are examples of learning about the antibiotic discovery pipeline facilitated by the exploration of a famous historical discovery.

In Entry 19 [Day 44], the participant wrote a descriptive history of antibiotics as reported by the BBC TWO programme "Britain's Greatest Invention". This was a long entry (645 words), that began with antibiotics saving human lives and ended with new studies which are continuing to probe novel antibiotic discovery. In this entry, PP1s repertoire of historical heroes expanded:

> *PP1: It wasn't until 10 years later in a laboratory in Oxford, that Howard Florey & a team of brilliant minds, turned Flemings unexpected discovery into a miracle cure. [Entry 19]*

and

> *PP1: Dr Ian Bedford (Head of Entomology at John Innes Centre) along with other scientists are working on inventing a brand new antibiotic by studying South American Ants. [Entry 19]*

In order to construct this story about the history of antibiotics, the BBC TWO programme and then the participant used famous scientists as heroes. The result of this storytelling was antibiotics being voted Britain's Greatest Invention by the British public. The participant ended this entry, as discussed in 5.4.6, self-reporting their fascination and learning. This is further evidence that the use of storytelling can encourage engagement and object level learning. The use of historical discoveries also builds a base upon which the importance of modern science is elevated.

In Entry 23 [Day 57], the participant again mentioned penicillin, this time considering the use of alternative medicines to fight infection:

> *PP1: This sounds really horrible but actually throughout 1930's & 1940's maggot therapy blossomed until the discovery of penicillin. Maggots are really good at removing infection & dead tissue in wounds. [Entry 23]*

The discovery of Penicillin is used as a historical anchor on which other scientific discoveries were explored. This included historical maggot therapy discussed above and a modern technique used by Indian researchers which removed the need for antibiotics in Orthopaedic implants, discussed in 5.4.6. These are examples of object level learning.

In Entry 29 [Day 83], PP1 talked about types of antibiotics and what they treat:

> *PP1: Penicillins – Widley used to treat a variety of infections e.g. chest; skin & urinary tract infections. [Entry 29]*

Penicillins, as well as Cephalosporins, Aminoglycosides, Tetracyclines, Macrolides and Fluoroquinolones/quinolones were mentioned and what they are used for was detailed. In this entry, the participant had moved away from the use of the discovery of Penicillin to track the history of science or discuss novel discoveries of antibiotics. Instead they began increasing their specificity and knowledge of 'antibiotics'. Penicillin was used as a jumping-off point, the first of six bullet points exploring the specifics of antibiotic classes and their uses. This improved understanding of 'antibiotics' as a discursive object highlights further object level learning.

In Entry 35 [Day 100], discussed in 5.4.6, the participant discussed a new class of antibiotics found to work against Gonorrhoea. They last discussed Gonorrhoea in the light of Penicillin in Entry 17 [Day 36]. Sixty-four days later they came back to this infection. When comparing the two entries, it becomes clear that the participant had increased their level of specificity. In the original, they called it "the clap" and focused on its treatment with Penicillin in WW2. In this entry, they went into detail about number of infections, its associated priority level and its incidence in the U.K. Penicillin was not mentioned in this entry. This shows object level learning with regards to new antibiotic discoveries and relevant resistant infections. They also actively explored topics of interest. The participant explored Gonorrhoea as its own interesting object, rather than through its relationship to Penicillin. The participant used the WHO as a source of information showing an increased diversity of sources from

which they were gaining object level knowledge. This is an example of meta level learning.

This thematic analysis shows that famous scientists and scientific discoveries provide familiar ground from which PP1 felt safe to explore novel objects. Object level learning included ‘Penicillin’, ‘Fleming’, ‘Gonorrhoea’, classes of antibiotics and their treatment options, the difficulties of the drug discovery pipeline and the threat of antibiotic resistance to human health. Throughout their engagement, the length of entries and the level of specificity increased. In exploring these discursive objects, the participant also showed meta level learning as well as an intellectual curiosity. They began to appreciate that scientific discoveries are made by teams of people, rather than the hero figures. They explored a diverse range of sources to allow the seeking of information on new topics of interest. Finally, they self-reported fascination and learning.

5.4.8 Do people make bad decisions in the face of better knowledge?

In 4.3.8 I found that participants would make incorrect decisions in the face of better factual knowledge, especially if they felt acting in the present was more critical than the risk of antibiotic resistance in the future (Theme 8). This was especially the case when acting on behalf of someone else, like a child. In this section, I highlight examples of PP1s decisions in the light of information gained or shared as the study progressed.

PP1’s interview [Day 1] had no examples of this theme, however, does provide a picture of the factual knowledge the participant had to begin with. PP1 was aware that bacteria and fungi produce antibiotics, however, was not aware that that plants, humans and viruses do not. They were not aware that colds and flu cannot be treated with antibiotics and were not aware that resistance is a result of an infectious agent, rather than the human body. They were aware that personal hygiene is a powerful tool in the battle against the spread of antibiotic resistance. They believed doctors to be key individuals when attributing blame for antibiotic resistance.

In Entry 3 [Day 2], discussed in 5.4.6, the participant self-reported their willingness to take new factual knowledge on board. In the last seven sections, I have highlighted evidence of increased knowledge of many discursive objects. Throughout the study, the participant’s factual knowledge increased.

One of the key early entries was Entry 9 [Day 12]. Parts of this entry have been discussed in 5.4.1, 5.4.3 and 5.4.5. The participant, based on the factual knowledge that colds and flu are viral infections not treatable with antibiotics, researched medication provided by a leading chain pharmacist. They decided that this medication was irresponsibly prescribed based on its containing an antibiotic. This is a notable example of an instance where better factual knowledge results in making better decisions, that is to be aware of and upset about being prescribed an antibiotic for a viral infection.

In Entry 34 [Day 97], discussed in 5.4.3, PP1 discussed a BMJ article that was circulating in mainstream news reports, the conclusions of which suggested people stop taking antibiotics when a person feels better. The participant

began by reinforcing the factual knowledge they had gained over the course of the 97 days. The participant talked about the confusion new factual knowledge can cause. This highlights that better factual knowledge does not necessarily mean a clearer picture of the best course of action. Instead, in this instance, better factual knowledge has led to two viewpoints which conflict with each other and, perhaps more importantly, the current public health narrative which advises individuals to take their full course. This could ultimately lead to decisions which go against the literate discourse.

This thematic analysis shows that better factual knowledge can lead to better decisions with regards to what the literate discourse considers sensible behaviour. However, it also highlights the risk of confusion that better factual knowledge can cause, especially when the discourse is not congruent.

5.5 Results: Selective thematic analysis of four Portfolios

In section 5.4, I presented the lengthiest and the most substantial of the five portfolios that were submitted. I thoroughly and extensively analysed this portfolio considering the eight themes that emerged from the coded analysis in the interviews. In the following section, my narrative accounts aim to highlight the discursive shifts at object and meta level of the participants in a summary. I make highly selective summaries of the contents of each of the other four portfolios and examine them using the eight themes. I present this information as a narrative account of the contents of each portfolio, providing examples and the themes that these elements relate to.

5.5.1 Analysis of Portfolio Glasgow 001 (PP2), Interview 030

Participant PP2 took part in an interview on the 7th September 2017, as interviewee 30 [Day 1]. I note that in Chapter 4, Participant PP2 would have been referred to as 30. This participant was so named as the 30th participant to be interviewed across the five pop-up events. They made their first portfolio entry the next day [Day 2]. In total, they submitted 14 portfolio entries until their last entry on the 13th February 2018 [Day 158]. The portfolio contained 1,902 words over 19 pages.

During the interview, I learnt that PP2 was about to embark on a PhD project looking at antibiotics and soil bacteria, having worked as a pharmacist previously. They were familiar with the literate discourse and answered my interview questions with specificity and detail. They were consistent in their use of accurate scientific language. They were the only participant to focus on antibiotic stewardship as a way we can individually make a difference to antibiotic resistance in their interview responses. They did suggest doctors should not hand out antibiotics willy-nilly and antibiotics should never be used prophylactically in animals. They suggested GPs and patient education had a role to play in reducing misuse of antibiotics and thought farmers should be sanctioned regarding misuse in animals.

In general, PP2 used technical language with a higher specificity and consistency than any other participant during the study. They also glued in articles taken from a Pharmaceutical journal, a source different to those utilised

by others which were more often the media, radio or educated friends (GPs). However, in this highly selective analysis I focus on their ability to compare and reflect on parts of their own life. This reflection provided new insights into several themes. In 5.4.6, I discussed PP1's move from descriptive to comparative accounts of data. PP2 utilised this comparative skill from the beginning of the portfolio and on several occasions following. For example, in Entry 4 [Day 12], the participant described the similarities and differences between their experience with antibiotics as both a patient, both in Nigeria and the U.K, and then in Entry 5 [Day 21], as a professional pharmacist.

In Entry 4 [Day 12], the PP2 wrote:

> *PP2: I've only had two courses of antibiotics in the last 14 years or so. Both courses were for seven days, which I completed on both occasions. I was issued more antibiotics than I needed on the second occasion, I returned extra tablets and capsules to my local pharmacy for destruction. My first course (about eight years ago) was monotherapy; while my second course (earlier this year) consisted of two types of antibiotics. [Entry 4]*

Not only does this highlight the participant's familiarity with the discursive objects in this field, using words like 'monotherapy' correctly (Theme 1), it also shows action guided by the current Public Health Narratives (Theme 3). Further, as I know the level of education and experience of this participant, I suggest that PP2 makes sensible decisions in the face of better factual knowledge (Theme 8). The participant went on to write:

> *PP2: Would I have completed the seven day course of treatment if I wasn't aware of the implications of not doing so, bearing in mind the pills tasted awful and made me feel sick, and I had no counselling whatsoever? [Entry 4]*

The participant expressed an inferred belief that individuals may not be following the narrative to complete the course, whilst highlighting side effects of antibiotics like nausea (Theme 3 and Theme 5). Based on their own experience, they express empathy and understanding in the reasons behind this noncompliance. Strengthening the evidence for Theme 8, the participant described a level of emotional commitment to the public health narratives; holding the importance of completing the course above their own physical and emotional comfort.

Entry 4 provided support for the use of public health narratives in the U.K:

> *PP2: Prior to the last 14 yrs, I lived in Nigeria. From what I can remember, I would have had at least a course of antibiotics every year. This is in sharp contrast to my more recent experience. [Entry 4]*

They went on to detail reasons why this might be, including government-led initiatives and existent antibiotic stewardship in the U.K, as well as the lack of public awareness of AMR and its consequences in Nigeria. Whilst this is not

an example of the participant following a Public Health Narrative, it is a participant statement which evidences their perceived importance of public health narratives and the vital role they play in reducing antibiotic prescription (Theme 3). It also provides awareness of the global approach that is required to tackle this issue, moving outside of the U.K. Further, antibiotic stewardship examples internal attribution of blame. Even if one were to be taking all measures to ensure they are following public health narratives, the responsibility is then to advise others of the need to do so (Theme 5).

In Entry 5 [Day 21] the participant wrote about their experience with antibiotics as a pharmacist:

> *PP2: Where dental prescriptions for antibiotics represent about 50% of all dental prescriptions issues during the week, at the weekend the proportion jumps to almost 90%. It would be interesting to investigate whether dental patients are more likely to present with acute infections at the weekend; or dentists are more inclined to prescribe antibiotics "just in case" during out of hours consultations. [Entry 5]*

The participant detailed a personal experience, formed a hypothesis and discussed the idea of testing it. This is an example of the Layer perception, the process-focused task of discovery without the researcher present in the awareness (Theme 6). The participant, as a scientist, has observed a phenomenon and wishes to understand the underlying factors which bring this about. This is the cornerstone of the natural sciences. Knowing PP2 was embarking on a PhD, this finding provides a window into the motives of a prospective scientist.

In the same entry, the participant wrote:

> *PP2: While I haven't seen a noticeable change in the volume of scripts issues for antibiotics as a whole, I think more patients are presenting at the pharmacy counter for advice on treating viral infections (especially colds and coughs) because the doctor "won't give them anything". [Entry 5]*

Their professional opinion is that doctors are changing their prescribing habits in line with public health narratives to not take antibiotics for viral infections (Theme 3 and Theme 5). The result of this is members of the public asking for advice on how to go about treating viral infections without the need for antibiotics. This means that individuals still receive treatment, but precious antibiotics are spared.

Finally, the participant again touched on the cultural differences of antibiotic use and antibiotic prescription:

> *PP2: I have had a few requests by people visiting the U.K to buy antibiotics over the counter. They are usually very surprised that antibiotics are prescription only medicines in the U.K. [Entry 5]*

During the interviews and now with PP2, there is a consensus that different countries are dealing with the threat of antibiotic resistance in different ways. PP2 suggests that the U.K is taking measures to tackle the problem where other countries may not be, or to a lesser extent. This reinforces Theme 3 in suggesting that the public health narratives are making a real, measurable difference to U.K prescription rates and to U.K perspectives of antibiotics.

This thematic analysis shows the specificity and consistency with which technical language is used by someone familiar with the discourse. It also highlights the use of routines to both objectively analyse and reflect, as well as to observe, hypothesise and experiment.

5.5.2 Analysis of Portfolio Norwich Science Festival 001 (PP3)

Participant PP3 visited the pop-up stand on the 19th October 2017. They made their first portfolio entry on the 8th November 2017 [Day 21]. In total, they submitted 16 portfolio entries until their last entry on the 1st March 2018 [Day 134]. The portfolio contained 1,087 words over nine pages.

In Entry 1 [Day 21], the participant provided a list of things they thought they knew and what they didn't know. They knew antibiotics don't work on viruses, the less often you take them the better, the more you take the more likely is antibiotic resistance, antibiotics destroy harmful as well as good bacteria, it takes a long time for good bacteria to return to pre-antibiotic levels and you should eat organic foods to avoid antibiotics in the food chain. They didn't know how antibiotics work. They went on to say:

> *PP3: Do I care? Not really: I don't want to understand how my car works. I just want to drive it. [Entry 1]*

This statement sets PP3 apart from the other participants in that there is an active admission that they don't care to fill in the gaps in knowledge they may have. This highlights an output-oriented focus rather than process oriented (Theme 6). They don't wish to explore how the process works, but instead are just happy that the outputs do work, and that they can benefit from this. This is an example of viewing research through the concept of Trading. This sets up the rest of the portfolio entries where most entries explore discursive objects that they already admitted to knowing, albeit whilst discussing these objects in greater detail or with a greater breadth.

Jumping to the very last entry, Entry 16 [Day 134], the participant wrote:

> *PP3: Thanks for allowing me to keep this diary and reading it. I've really enjoyed heightening my awareness for AB items in the media and I've learnt a lot. [Entry 16]*

Self-reported learning is important as part of our evaluation, but even more interesting considering the participant's own admission that they weren't necessarily interested in exploring new topics of information. The result seems to contrast with their statement in Entry 1. This highlights the potential importance of public engagement versus public understanding. By allowing the participant to explore their own topics of interest rather than a predetermined

set of topics that I deemed important, the participant continued to engage with the portfolio for 134 days and experienced object level learning as a result.

This point is further highlighted by the participant in Entry 12 [Day 101]:

> *PP3: Incentivisation: I find this really very patronising. It also points to an ethos I don't really like. [Entry 12[*

This excerpt is taken from writings the participant made about a Television Programme they watched called 'The truth about Hawaii'. In this play, only one antibiotic is left to which there is no resistance. Following the play was a talk provided by an academic from Bristol University. The participant felt patronised that the population might have to be incentivised. This is likely because the participant believed they were doing all the correct things and they did not need incentivisation to do so. This also expresses a narrative of discontent with the perceived elitism of the scientific community. This would be captured by the code 'Us vs Them [UVT]'. In the same entry, they asked:

> *PP3: Did she mean me or those poorer and less educated? [Entry 12]*

This statement highlights the external attribution of blame, an us vs them mentality (Theme 5). In this case, PP3 saw the academic providing the talk as 'them' but also sees the 'them' as being poorer and less educated members of society.

Still focusing on Entry 12, the participant wrote:

> *PP3: This was a difficult program and I gave up trying to understand it. [Entry 12]*

This excerpt was taken from writings made about a Radio 4 Programme 'The Infinite Monkey Cage' in which Brian Cox and Robin Ince looked at the history and future of antibiotics. The participant noted that this was too difficult to understand and spoke to the difference between public understanding and public engagement. It is important to note that the participant had been actively seeking out programmes that relate to the topic of antibiotics and antibiotic resistance. Exploring a diverse range of sources is an example of meta level learning. However, some of these programmes failed to meet the mark and were disengaging the participant. This is perhaps a criticism of public understanding, whereby you are talking to someone rather than discussing with someone.

Comparing this disengagement of public understanding to the public engagement approach used in this study, it is clear that the participant was engaged enough to spend the large majority (13/16 entries) of their 134-day engagement with this project exploring a topic which was of clear interest to them; the relationship between food, the microbiome, antibiotics and antibiotic resistance. Of the three entries in which they did not cover food or the microbiome in some fashion, one was Entry 12 where they felt patronised and struggled to understand, one where they were excited that a new antibiotic family had been discovered in dirt [Entry 13, Day 118] and their final entry where they thanked me for allowing them to keep the portfolio [Entry 16, Day

134]. Notably, as the entries progressed, the participant began to write more positively about science, showing an increased level of science optimism (Theme 4).

This thematic analysis shows that self-guided learning using the portfolio can engage individuals who may otherwise feel like science communication is not aimed at them. PP3 gave up listening to or felt patronised by media aimed at increasing public understanding of the issues of antibiotic resistance. Despite this, they spent 13 entries over 134 days increasing their knowledge of the discursive objects surrounding the human microbiome and self-reported learning by the end of the study. This participant experienced lots of object level learning, however a little less meta level. This could have been linked to their outcome focused, rather than process focused, perspective.

5.5.3 Analysis of Portfolio Norwich Science Festival 002 (PP4), Interview 032

Participant PP4 took part in an interview on the 19th October 2017, as interviewee 032b [Day 1]. I note that in Chapter 4, Participant PP4 would have been referred to as 32b. They were so named as the 32nd participant to be interviewed across the five forest events. They have been annotated 32b because they interviewed with their father, who spoke first, and was thus annotated 32a. Their first portfolio entry was not dated; however, the second portfolio entry was submitted on the 28th October [Day 10]. In total, they submitted four portfolio entries until their last entry on the 24th January 2018 [Day 98]. The portfolio contained 332 words over four pages.

During their interview, PP4 understood that there were bacteria in the soil which can become antibiotics and knew antibiotics were made by bacteria and fungi. They pointed out that humans can use bacteria and fungi to make antibiotics, but incorrectly suggested plants can too. They did say no to viruses. They knew bacterial infections were treatable by antibiotics and only bacterial. They thought that discovery of novel antibiotics was recent and had an optimistic guess about how many antibiotics scientists had taken to market. They knew that resistance is a property of an antibiotic losing effectiveness treating bacteria and attributed this to overuse.

In Entry 1 [No Date] they went through the questions provided in the supplemental pack (Appendix G-1) and answered each of them with short responses. Their responses, whilst short, were accurate in nearly all cases. They also mirrored their views presented in the interview, as described above. The remaining three entries detailed instances where PP4 heard or watched something which mentioned antibiotics or antibiotic resistance.

Entry 2 [Day 10] described the Radio 4 programme they had listened to:

> *PP4: Heard a radio programme on Radio 4 about the dangers of antibiotic resistance spreading, and what the situation might be like if antibiotics were no longer to work [Entry 2]*

Entry 3 [Day 97] described a Television drama about a future world where antibiotic resistance rendered antibiotics useless:

PP4: It explains how A.R. occurs and follows the story of a girl who has a scratch in her knee which becomes infected. She cannot be treated and her leg is at risk of being amputated. [Entry 3]

Finally, Entry 4 [Day 98] described an episode of Countryfile about surfers ingesting antibiotic resistant superbugs which made their way into rivers after being excreted by animals. Unlike the previous entries, PP4 took time to write the takeaway messages of this programme:

PP4: Antibacterial cleaning products should only be used if necessary, not routinely. Plus AR bacteria enters rivers through treated waste water from water treatment works. The message was to take personal responsibility for antibiotic use. They also advocated use of UV in water treatment works. [Entry 4]

These excerpts were largely representative of Public Health Narratives (Theme 3). They detail misuse/overuse, a theme which this participant frequented through the course of their engagement with this project. In their final excerpt, they also discussed who is to blame (Theme 5), suggesting that the message of a Television programme they watched was that people should internally attribute blame and responsibility for the antibiotic resistance crisis. Further, in exploring the topic of antibiotics and antibiotic resistance, the participant sought out diverse sources of information in an example of meta level learning.

This thematic analysis shows that even in a small number of entries, a participant can begin to provide greater depth of description and some analysis in explaining key messages provided by varying sources of information. Their exploration of new sources of knowledge is an example of meta level learning. This portfolio also highlights the pervasiveness of public health narratives. Within an 88-day period the participant found three different sources of information on antibiotics or antibiotic resistance, one radio, one a Television drama and one a Television nature programme.

5.5.4 Analysis of Portfolio Norwich Science Festival 003 (PP5)

Participant PP5 visited the pop-up stand on the 19th October 2017. Their first portfolio entry was not dated; however, their second and final portfolio entry was submitted on the 2nd April 2018 [Day 166]. In total, they submitted two portfolio entries. The portfolio contained 246 words over three pages. As a child, PP5's experience with antibiotics and antibiotic resistance was kept to their inner circle, that is describing experiences of family members (Dad, Sister, Mum, Sister's Boyfriend, Aunt). On only one occasion did they mention a source outside of family, a newspaper. In general, their focus was on describing times where people they knew had to take antibiotics or had an association with antibiotics.

In Entry 1 [No Date], PP5 described that antibiotics made them sick when they were younger:

PP5: When I was little I had tonsillitis and I took Antibiotics. Every time I had them, I was sick so, I hate to have banana flavour instead. [Entry 1]

This participant provided a child's perspective of the issues of antibiotics and antibiotic resistance and hinted at the fact antibiotics can produce side effects such as nausea. PP5 had taken antibiotics enough to know the flavour they preferred. This links to the theme surrounding better factual knowledge (Theme 8), in which parents often suggest the only time they have antibiotics is for their child.

In Entry 1 [No Date], the participant wrote:

PP5: I think my sister is growing bacteria (possible Antibiotics) in her old tea cups that she leaves in her room. My Mum had a sore tooth and she has to take Antibiotics (called Amoxicillin 500 mg Capsules) for a week, (one per day). My sisters boyfriend took antibiotics for a chest infection. [Entry 1]*

This shows that the participant was aware of bacteria and that they can produce antibiotics. They describe the antibiotic by its technical name and the dose. They are descriptive in their writing, with little elaboration. The participant reports that their sister's boyfriend took antibiotics for a chest infection, perhaps contrary to the public health narrative of not taking antibiotics for viral infections, as chest infections are often viral.

In Entry 2 [Day 166], the participant wrote:

PP5: My Aunt took Antibiotics because she had a sinus infection. [Entry 2]

This continues the theme of descriptive writing; however, this was the only text in Entry 2 and shows no signs of discursive shifts at the object or the meta level. It is another example of antibiotics being taken for infections which are likely to be viral.

In their first entry, PP5 identified viral infections as not treatable with antibiotics:

PP5: My Dad told me that Antibiotics don't work on viruses, they only work on bacterial infections. [Entry 1]

As they did not actively state that they thought their family should not have taken antibiotics for viral infections, I assume that they were unaware. This highlights the issue with the application of public health narratives. Whilst the public may now be aware that antibiotics are not to be taken for viral infections, they might not know which infections are viral. This increases the need for doctors to advise as such.

This thematic analysis shows that even in the portfolio with the fewest words and fewest entries, some form of learning can take place. In this case, it was object level learning gained from asking family members about their knowledge of antibiotics.

5.6 Discussion and Future Work

5.6.1 Descriptive analysis of social media data reveals how to best attract and engage participants with relevant content

Quantitative analysis of social media data suggests that in order to increase total users on a Facebook page, relevant content must be released regularly. The type of content seemed to have little effect. The more users who have liked the Facebook page, the more users are engaged by content released. Content released during Antibiotic awareness week was seen by the most people, however the level of engagement did not increase. In clinical practice, I recommend that significant dates in calendars such as antibiotic awareness week are exploited to increase reach and are a good time to disseminate key information, such as public health posters, rather than to drive engagement.

5.6.2 Coded analysis of social media data reveals key themes aligning with interview research

Social media did not drive the level of medium- to long-term engagement that I expected. Thematic analysis of comments that were posted suggest that participants often lack confidence to analyse their own data, even when the tools to do so are made readily available. Participants, like in the interviews, use familiar words following pre-existing rules when engaging with a new discourse. In the clinical setting, I suggest that researchers must go as far as possible to help foster confidence in participants. I discuss the portfolios ability to foster confidence in the next section. I also suggest, in concurrence with Mendelson *et a*l (2017), that in order to help participants engage with literate word use, the community of experts might choose less ambiguous terminology and apply it consistently in both academic writing and in media releases.

5.6.3 Selective thematic analysis of four Portfolio Participants highlights emerging themes.

Selectively applying thematic analysis to the most unique aspects of four portfolios provided interesting topics for more detailed analysis. In the case of PP2, thematic analysis highlighted the specificity and consistency used by an individual who is familiar with the literate discourse. It highlighted the use of scientific routines which allowed the critical observation of external and internal phenomenon. It also highlighted that discursive transformation is less evident when the participant is already familiar with the literate discourse.

In the case of PP3, selective thematic analysis highlighted the importance of the portfolio in facilitating self-guided learning. The participant made clear that they felt that messages delivered in pursuit of public understanding were not aimed at them and felt patronised when subjected to this information. Instead, they explored themes related to antibiotics and antibiotic resistance that were of interest to themselves. Doing so, they experienced object level learning surrounding the human microbiome and self-reported learning at the end of the study.

Both PP4 and PP5 highlight how without the continued contribution of participants, portfolios do not hold the power to evidence discursive transformation. Despite this, the eight themes captured key excerpts of fewer and less lengthy entries, showing their use for discourse analysis. For PP4, the themes highlighted their increase in descriptive detail and level of analysis of information they chose to write about. For PP5, the themes highlighted that for a child, their main source of information is experiences of family members and the narratives bequeathed by their inner circle.

5.6.4 Thematic analysis of Portfolio Participant reveals portfolio facilitated discursive transformation

In Chapter 4, participants used familiar words as they engaged with a new discourse (Sfard 2008). In this chapter, my findings suggest that the portfolio facilitated object level and meta level changes with regards to the participant's use of scientific terminology. At the object level, the participant became more familiar with discursive objects such as bacteria, viruses, antibiotics, and antibiotic resistance, among others. This increasing familiarity was evidenced through a more specific and consistent usage of the relevant terminology. This concurs with research conducted by Biza *et al* (2018). At the meta level, the participant changed the ways in which they viewed certain objects. The participant changed the sources from which they gained object level knowledge utilising increasingly varied and technical sources of information. Furthermore, they developed the confidence to take the data from these sources and adapt it, presenting it in a way which suited their portfolio entry.

In Chapter 4, participants organised new experiences in terms of those with which they were already familiar. In this chapter, my findings are consistent with this. However, more specific thematic analyses are required in order to pick apart this phenomenon and provide insights into the nuances of this concept: scaffolding new experiences on to familiar knowledge and experiences.

In Chapter 4, participants engaged with the literate discourse through the ritualising of six key public health narratives. Participants mimicked narratives bequeathed to them by Public Health England. In this chapter, my findings suggest that the portfolio facilitated object and meta level change with regards to the participant's ritualised use of public health narratives. At the object level, the participant expanded their knowledge of what was already known about bacteria, viruses, and fungi, as well as their understanding of antibiotics outside of the role they play in overuse/misuse. This object level learning resulted in increased specificity and consistency when using terms familiar to public health narratives. At the meta level, the participant, rather than mimicking narratives passed down from authority, began to challenge authority when the evidence they had researched contradicted the narratives they were provided. They also developed an interest in topics they were not initially familiar with as a result of research into public health narratives and went about exploring these topics in greater detail.

In Chapter 4, participants who placed faith in science and scientists harboured an optimistic outlook on antibiotic resistance. Research has shown that

individuals who are inherently optimistic are more likely to take action to avoid crises like antibiotic resistance (Huber et al., 2019). In this chapter, my findings suggest that the portfolio did not facilitate change in the level of optimism held by the participant. Instead, the participant experienced a meta level change in the way they wrote about the information they were researching. At the beginning of the portfolio they would include their emotional reaction to topics they wrote about. At the end of the study, they discussed information objectively examining only the relevant facts.

In Chapter 4, participants made external attributions of blame, not finding themselves personally responsible. This was congruent with previous literature which showed the same (Brooks et al., 2008; Butler et al., 1998; Dyar et al., 2018; Hawking et al., 2017; Hawkings et al., 2007). In this chapter, my findings suggest that the portfolio facilitated both object and meta level changes with regards to who is to blame for antibiotic resistance. The participant experienced object level learning with regards to antibiotics and antibiotic resistance, as well as on specific topics such as antibiotic prescription. This object level learning was further evidenced by an increase in specificity and consistency over the course of the study. Increased knowledge of these discursive objects preceded meta level learning. Understanding the role of doctors, the participant contradicted their earlier blaming of doctors and began to defend doctors by the end of the study. The participant began to use the word ‘we’ when describing actions that needed to be taken, placing responsibility on themselves to help mitigate the effects of antibiotics. Like in the other themes, the participant sought information to explore this theme from new sources. They also developed their ability to think like an objective researcher as they began to base decisions and opinions on the growing basis of facts they accumulated. Finally, they showed an appreciation for the role experts, like doctors, play in communication with the public.

In Chapter 4, participants considered research through multiple perspectives outlined by Brew (2001). These perspectives could be outcome or process focused and would consider or not the researcher. In this chapter, my findings suggest that the portfolio facilitated opportunities for the participant to see research through each of the four perspectives and understand more about what it is to be a scientist. This occurred through both object and meta level learning. At the object level, the participant increased their knowledge about antibiotics and the process of science. Their specificity and consistency increased as the study progressed. Specific to this theme, the participant experienced meta level learning with regards to the time it takes to undertake scientific research. They realised that rather than being a succession of discoveries, it was instead a process of experimentation which eventually produced outcomes. They also learnt that narratives are built on multiple studies and as such are subject to change as new studies are released. Finally, as they reflected on their own learning experiences, they appreciated the importance of engaging the public whilst critically commenting on the ineffectiveness of one form of public understanding.

In Chapter 4, participants related historical scientific heroes to discoveries and timelines. Research has suggested that in order to mobilise people to act, crises are best explained in story format, including the use of heroes (Shenhav,

2015; Arnold, 2018). In this chapter, my findings suggest that science heroes and famous discoveries are used as familiar ground on which a participant can safely explore unfamiliar topics. Object level learning was seen through increased knowledge about Penicillin, Fleming, Gonorrhoea, classes of antibiotics and their treatment options, the difficulties of the drug discovery pipeline and the threat of antibiotic resistance to human health. This learning was matched with an increased specificity and consistency as the study progressed. With regards to meta level learning, the participant began to appreciate that rather than the heroes of science, discoveries are made by teams of scientists. In further support of the power of stories, the participant self-reported fascination and learning after writing about a Television programme which used storytelling to explain the history of antibiotic discovery.

In Chapter 4, participants admitted that they would make bad decisions in the face of better knowledge in situations where they felt the immediate gain of antibiotic use outweighed the future risk of antibiotic resistance. There is no literary consensus regarding the links between decision making and factual knowledge; better factual knowledge has been linked with both bad decision making (McNulty et al., 2007) and good decision making (European Commission., 2013). In this chapter, the gaining of factual knowledge does seem to facilitate better decision making by the participant. As the participant gains agency, as they gather the fundamental knowledge on which they can make their own evidence-based decisions, they advised their husband to not take medication prescribed by a pharmacist for a throat infection as it contained an antibiotic. This medication is being discontinued as of 2022 and as such, the participant made a positive decision (National Institute for Health and Care Excellence (NICE), 2018). However, they go on to report that as science is an ever-changing field and results can contradict each other, improved knowledge can be a source of confusion.

5.7 Summary

One of the aims of my study was to understand the roles social media and portfolios play in medium- to long-term study of participants discursive transformations. Whilst quantitative analysis of social media metrics provided suggestions on how to best utilise social media, the qualitative analyses of social media comments lacked the richness to highlight discursive transformations. Instead, the short-term interviews and medium-term social media comments were added to the pool of data provided by the longer-term portfolios to present a picture of the evolution of the citizen scientist's learning. Building on the work in Chapter 4 which highlighted eight key themes occurring in participant discourse about antibiotics and antibiotic resistance, I used portfolios to evidence discursive transformations. My analyses indicate that no one theme enabled the mapping of all discursive transformations but instead a combined use of themes was necessary.

My study set out to explore how participants' discourse of antibiotic discovery and antibiotic resistance changed as they participated in self-guided learning. The portfolio focused participant's minds on the topic of antibiotics and

antibiotic resistance. In doing so, the portfolio has encouraged participants to actively seek out new sources of information. In engaging with this information, participants increased their understanding of key discursive objects and utilised learned terminology with increasing specificity and consistency. Finally, the expanding knowledge of discursive objects provided conflict as new objects changed the participant's perception of previously endorsed narratives. In total, participants showed discursive transformation as they engaged with this study.

Chapter 6 Conclusion

6.1 Research Questions and Methodological Reflection

In this final chapter, I highlight the major outcomes of this study, their significance in the wider context of research in this area and their potential societal and educational impact.

6.1.1 Context

In 2019, the US Centers for Disease Control issued a list of antibiotic-resistant pathogenic bacteria which pose an urgent or serious threat to the general public (Centers for Disease Control and Prevention, 2019). The list contains 14 bacteria (Table 1-3) and is not finite.

Bacterial resistance to antibiotics threatens their efficacy (Baym et al., 2016) which in turn threatens modern medicine as we know it. The rate in which bacteria are developing resistance to antibiotics is increasing through human misuse (Bronzwaer et al., 2002; Goossens et al., 2005; O'Neill, 2016b). To combat the development of antibiotic resistance, new antibiotics need to be developed whilst current antibiotics need to be preserved.

Development can be based on (i) designing a new antibiotic derivative of a known antibiotic family with improved properties, (ii) discovering new chemical structures that act on known or a novel bacterial target or (iii) employing alternative therapeutics such as phages or antibodies (Banin et al., 2017). Soil bacteria provide a large reservoir for antibiotic discovery and the actinomycetes in particular have provided one of the richest source of antibiotics to date (Van der Meij et al., 2017). Whilst these bacteria have been well mined (Lewis, 2012), underexploited bacteria and technological advancements have opened a new reservoir for antibiotic discovery (Ling *et al.*, 2015; Hover *et al.*, 2018; Lewis, 2020)

Preservation relies on smarter antibiotic usage, specifically at the point of prescription but also proper use, consumption and adherence to treatment regimes. Reducing antibiotic demand is crucial and is driven by industry and the public. A key component of this is reducing public expectation of receiving a prescription for antibiotics, which has been shown to increase the likelihood of prescriptions being handed out (Cals et al., 2007; Coenen et al., 2013; Coenen et al., 2006; Cole, 2014; Sirota et al., 2017). Science communication in the form of public health campaigns, aims to provide scientific facts to the public in the hope of encouraging desirable behaviour that promote health, following the movement of public understanding of science. These campaigns have suffered from issues such as lack of evaluation to determine success (Bauraind et al., 2004; Huttner et al., 2010), undesirable effects (Price *et al.*, 2004) and absence of cost effectiveness data. The more recent movement of public engagement in science and technology looks beyond providing facts to individuals, but rather engages them in science and research. One form of public engagement, citizen science, is defined as public participation and collaboration in scientific research with the aim to increase awareness of scientific facts as well as of how scientific knowledge is produced (Robinson *et al.*, 2018).

6.1.2 Aims

To pursue both the development of new antibiotics and the preservation of current antibiotics, this PhD used a citizen science approach. Using pop-up stands, interviews, social media interaction and portfolios, I aimed to engage members of the public with the story of antibiotic discovery and the issue of antibiotic resistance. In doing so, I aimed to discover novel antibiotic candidates from soil bacteria. I also aimed to increase understanding of public perceptions on topics surrounding antibiotics (discovery and resistance), as well as perceptions of science and scientists. I aimed to understand how these perceptions changed as participants engaged with the project in a long-term study. I aimed to understand the role social media and portfolios played in evaluating project outcomes for its citizen scientists.

6.1.3 Judging success of this project according to Robinson's ten principles of citizen science

Citizen science projects must include public participation and collaboration in scientific research with the aim to increase awareness of scientific facts as well as how scientific knowledge is produced (Robinson *et al.*, 2018). Whilst citizen science has been an emerging field since 2006, research is criticised as failing to produce new scientific knowledge (Kullenberg and Kasperowski, 2016); instead it is poor science with great communication potential (Heigl & Dörler., 2017). My own literature review suggested that as scientific outputs have increased, research has failed to identify participant outcomes or to conduct proper evaluation (1.5.1). To guide citizen science practice, Robinson *et al* (2018) produced ten key principles which underlie good practice in citizen science (Table 6-1). In this section, I provide a narrative account of the outcomes of this study in relation to these ten principles.

The first principle is that projects actively involve citizens in scientific endeavour that generates new knowledge or understanding. This study has actively involved citizens in scientific endeavour, collecting soil samples, analysing laboratory data and conducting self-guided research. In doing so, it has generated new knowledge and understanding as evidenced in 5.4 and 5.5.

The second principle is that projects have a genuine science outcome. This project has resulted in genuine scientific outcomes. In Chapter 3, I presented a discovery pipeline which collected 454 soil samples, assayed the antagonistic activity of 929 bacterial isolates, leading to the long-term storage of 165 antagonistic isolates ready for future analysis. Further, whole genome sequencing provided 15 genomes, three of which were sequenced a second time to improve read depth and quality. For each of the 15 isolates, phylogenetic trees based on 16S rRNA were constructed. These 15 isolates represented eight species of soil bacteria, none of which were part of the well mined actinomycetes. BGCs were identified in each of the 15 isolates and compared with those present in their nearest characterised relatives. Focusing on five isolates representing underexploited species, BGCs were identified that were not found in my literature search of their nearest relatives. These are Terpene and type III Polyketide Synthases in *Sporosarcina aquimarina.* Further, analysis of BGCs showed that there were some antibiotic and

secondary metabolite clusters which were highly conserved between species, even considering their geographical isolation.

In Chapter 4 I presented the application of a coding schedule compiled from key literature on public discourse of antibiotics and antibiotic resistance. In applying these codes, current themes in the public discourse emerged which contributes to the literature. These themes can be used to direct clinical practice and public health campaigns. In chapter 5, I presented the application of object and meta level learning, as well as the use of consistency and specificity, to identify discursive transformations in participants as they engaged with the study over a 98-200 day period. In doing so, participants were shown to have expanded what they already knew about an existing universe of objects relating to antibiotics and antibiotic resistance, as well as to have explored new objects which contradicted their previously endorsed narratives. They were shown to discuss these discursive objects more consistently, and with greater specificity. The application of these tools is presented as a methodological contribution to the field which can assist with evaluation of other project outcomes, something that was noted as missing from many citizen science projects (1.5).

Combined, these data can benefit science and society, providing a new pool of underexploited soil isolates which show antagonistic activity against medically relevant pathogenic organisms. It also makes a methodological contribution to the field of citizen science in the use of codes, frames and themes to evidence participant learning in future evaluative studies. It offers current public perception data contributing to the knowledge base of what the public understands about antibiotics and offers insight into the power of tracing participant learning as citizens engage with a citizen science project over different time frames including interviews and pop-up stand participation, medium term engagement via Facebook pages, and over several months using portfolios.

The third principle is that a study provides benefits to both science and society. This is one of the more difficult principles to achieve and I present the aspirations of this project to achieve these benefits. This small PhD project, over 5 years, has made a modest contribution to the longstanding issue, debate and effort to make an ecological impact. It has influenced a small number of people; however, it offers the tools to show that, in engaging with scientific research, improvement of scientific literacy can take place in members of the public. Furthermore, this study hopefully provides the foundations to encourage a move in the field of citizen science towards a more evidenced based, qualitative justification of participant outcomes; relying minimally on self-reporting and optimally on change in participant discourse.

The fourth principle is that citizen scientists may participate in various stages of the scientific process. As discussed in the first principle, citizens engaged with data collection, data analysis and exploration of the discursive objects of science.

The fifth principle is that citizen scientists receive feedback from the project. The consistent publication of laboratory data on social media allowed for timely feedback in assisting participants with data analysis. Further, emails and one

phone call were used to offer feedback on portfolio entries when participants were unsure of how they could contribute.

The sixth principle is that the limitations and biases of citizen science should be considered and controlled for. In the literature review, both the limitations and potentials of citizen science were discussed. This understanding guided method selections and participant interactions throughout the study. I discuss the limitations of the project and how these were or could be controlled for in future studies in greater detail in section 6.2.

The seventh principle is that, where suitable, project data and meta-data from citizen science projects are made publicly available and results are published in an open access format. Whilst this is an aspirational achievement of this study, project data and meta-data from the PhD will be open source and a link will be sent to the participants. Further, plans for future publications include open access articles.

The eighth principle is that citizen scientists are suitably acknowledged by projects. Citizen scientists, as a cohort, have featured in the acknowledgements of this PhD and will be acknowledged in any additional publications. Further, a product of this study is a toolkit with which future citizen science projects can better evaluate participant outcomes.

The ninth principle is that citizen science programs offer a range of benefits and outcomes which should be acknowledged and considered in project evaluation. This study has considered and offered a range of benefits in science, participant and socio-ecological and economic dimensions. The three dimensions of citizen science outcomes (Kieslinger *et al.*, 2018) are discussed further in 6.1.4.

The tenth principle is that leaders of citizen science projects take into consideration legal and ethical considerations of the project. Upon choosing a citizen science methodology, the ethics of the study were considered, and ethical approval was sought from and granted by the University of East Anglia ethics committee (Appendix I-1).

When examining the practices of this study in light of the ten principles of good citizen science practice (Robinson *et al.*, 2018), it is clear that this study has achieved many of the ten principles. Whilst Robinson *et al* (2018), did not provide a quantitative approach to understanding how many categories must be achieved to conclude that a project was successful, they did note that whilst some principles are implemented within every citizen science project, others are more challenging to incorporate and require a greater investment of time and resources to fulfil.

Table 6-1. Table showing the ten principles which underlie good citizen science practice (Robinson *et al.,* 2018)**.**

Number	Good Citizen Science Practice
1	Citizen science projects actively involve citizens in scientific endeavour that generates new knowledge or understanding.
2	Citizen science projects have a genuine science outcome.
3	Citizen science provides benefits to both science and society.
4	Citizen scientists may participate in various stages of the scientific process.
5	Citizen scientists receive feedback from the project.
6	Citizen science, as with all forms of scientific inquiry, has limitations and biases that should be considered and controlled for.
7	Where possible and suitable, project data and meta-data from citizen science projects are made publicly available and results are published in an open access format.
8	Citizen scientists are suitably acknowledged by projects.
9	Citizen science programs offer a range of benefits and outcomes which should be acknowledged and considered in project evaluation.
10	The leaders of citizen science projects take into consideration legal and ethical considerations of the project.

6.1.4 Judging success of this project according to Kieslinger's open framework for citizen science evaluation

At the outset of this PhD, there were no commonly established indicators of evaluating citizen science (Kieslinger *et al.*, 2018). Different experts focused on different aspects of evaluation, notably participant learning (Phillips *et al.*, 2014; Masters *et al.*, 2016) or scientific gains and socio-ecological relevance (Jordan, Ballard and Phillips, 2012; Bonney *et al.*, 2014). In 2018, Kieslinger *et al* presented a comprehensive evaluation framework (Kieslinger *et al.*, 2018). This framework is split into three core dimensions: scientific, participant and socio-ecological and economic. For each, criteria were proposed at two levels: process and feasibility as well as outcome and impact. In the following section, I will be focusing on if and to what extent this study has achieved outcomes and impacts in each of the three dimensions.

6.1.4.1 Scientific Dimension

In the scientific dimension, indicators of outcomes and impacts include scientific knowledge and publications, new fields of research and research structures as well as new knowledge resources. These indicators and some of the ways in which the project can evidence its success are presented in Table 6-2. To help identify where these indicators have been met, Kieslinger *et al* (2018) presented supporting questions, which I answer.

To examine scientific knowledge and publications, the framework asks whether the study demonstrates an appropriate publication strategy in scientific and other media outlets. I have, during the PhD, presented preliminary findings through poster presentations at three consecutive Microbiology Society Annual Conferences in Liverpool, Edinburgh and Birmingham. In Birmingham, I also gave a talk and answered questions. I

presented and received an honorary award for a poster of preliminary findings at the American Society of Microbiology conference in New Orleans titled ‘Antibiotic Discovery and Citizen Science’. I have also been an invited speaker at the Open University and South Devon College. There are plans for publication, the writing of which has already began, however this is an area in which progress must be made to ensure this can be considered a successful study. It then asks if citizen scientists are recognised in publications and if so, whether they can participate in dissemination of results. This PhD will be open-source and will be sent to participants who took part in the long-term portfolios. Further, the findings from this PhD will be disseminated in collaboration with the Microbiology Society on their website and social media platforms.

To examine new fields of research and research structures, the framework asks whether the project generated new research questions, projects or proposals. This study highlighted the shortage of citizen science studies which conduct evaluation of participant learning outcomes and utilised portfolios to trace outcomes as participants engaged with the study. The thematic analysis used to trace these discursive transformations and learning experiences is a contribution to the field. Further, this PhD is designed to serve as a new resource in the field of citizen science and act as a lessons-learned guide for future citizen science projects. The isolates generated using the optimised laboratory protocol present a platform for future research projects and already led to collaboration (Cooper Lab – WGS). The framework then asks whether the project contributed to any institutional or structural change. Whilst aspirational, it is hoped that the success of this multidisciplinary PhD encourages other researchers to embark on similarly interdisciplinary studies. This is increasingly important as the role of a researcher begins to incorporate the engagement of citizens in research.

To examine new knowledge resources, the framework asks whether the project eases access to traditional and local knowledge resources. This project did not set out to achieve this goal. The framework then asks whether the study contributed to a better understanding of science in society. It is clear, from analysis in Chapter 5, that participants who engaged with this project have gained an understanding of science both at the object and meta level.

Table 6-2. Examples of evidence from this project which supports the outcome and impact level of the scientific dimension of the citizen science evaluation framework.

Scientific Dimension		
Scientific Knowledge & Publications	**New Research Fields and Structures**	**New Knowledge Resources**
PhD published open source and sent to participants	Thematic Analysis to evidence discursive transformations	Increased scientific literacy of long-term participants
Article in preparation for open source publication with the Microbiology Society titled '*What on Earth? Antibiotics Unearthed*'	Lessons learned guide for future multidisciplinary and citizen science projects	
Presented award winning posters in New Orleans	165 isolates which antagonise medically relevant pathogens	
	Collaboration with Cooper Laboratory at UEA	

6.1.4.2 Participant Dimension

In the participant dimension, indicators of outcomes and impacts include improving knowledge, skills and competencies, improving scientific literacy, encouraging behaviour and ownership and fostering motivation and engagement. These indicators and some of the ways in which the project can evidence its success are presented in Table 6-3.

To examine knowledge, skills and competencies, the framework asks what the learning outcomes are with regards to new knowledge, skills and competencies for the participant. In combination with the portfolios, the tools provided for data analysis on social media facilitated PP1's improvement as a researcher, as they began to compare their own samples to those collected by other people. Participants also showed an increasing diversity of sources they used to obtain their information on science.

To examine science literacy, the framework asks whether the project contributed to a better understanding of science and a better understanding of the scientific topic. In Chapter 5, the expansion of what was already known about scientific objects such as bacteria, viruses, antibiotic discovery and antibiotic resistance highlight increased understanding of this scientific topic especially using medium-term and long-term approaches. The improved specificity and consistency with which these objects were used highlights increased scientific literacy. The use of Brew's conceptions of research (Brew, 2001) highlighted that participants changed their understandings of science, which was captured often by meta-level learning.

To examine behaviour and ownership, the framework asks whether the study fosters ownership amongst participants. The theme which examined 'who is to blame' evidenced ownership, PP1 began to internally attribute responsibility for antibiotic resistance. Further, PP3 admitted a lack of interest in exploring

questions posed in the portfolio handbook and instead took their own course, exploring the relationship between antibiotic resistance and the gut microbiome. The framework next asks if the project contributes to facilitating personal change in behaviour or political citizenship. In Chapter 5, as scientific knowledge increased, PP1's behaviour also changed. The standout example of this was the research they undertook focusing on medication provided by a pharmacist for a sore throat. They concluded that this medication should not be taken due to its containing an antibiotic.

To examine motivation and engagement, the framework asks whether the project raises motivation, self-esteem and empowerment amongst participants. The participant self-reported motivation to learn new things at the start of the study and evidenced continued motivation and engagement through consistent portfolio entries over a 200-day period. Further, all five long-term participants engaged for an extended period; a minimum time of 98-days. Of the 14 participants who received a long-term portfolio, five returned the portfolio. The framework then asks if the project motivated participants to continue the project or involve in similar activities. The actions of the participants following engagement has not been tracked, however would be an avenue for a follow up study.

Table 6-3. Examples of evidence from this project which supports the outcome and impact level of the participant dimension of the citizen science evaluation framework.

Participant Dimension			
Knowledge, Skills & Competencies	**Scientific Literacy**	**Behaviour & Ownership**	**Motivation & Engagement**
Diversifying use of sources of scientific information	Object level learning with regards to *antibiotics*	Not taking antibiotics when prescribed by authority	Participants stayed engaged for between 98 and 200 days
Improved data analysis skills	Meta level learning that science takes teamwork, long efforts and isn't all knowing	Participant taking own course of exploration after saying not interested in mine	Participants self-reported motivation to take part and concluded by expressing enjoyment at having taken part in the portfolios
	Improved consistency and specificity of use of scientific terminology		5/14 participants submitted a portfolio complete with several entries.

6.1.4.3 Socio-ecological and economic dimension

In the socio-ecological and economic dimension, indicators of outcomes and impacts include societal impact, ecological impact and a wider innovation potential. These indicators and some of the ways in which the project can evidence its success are presented in Table 6-4. When it is too early to comment on the impact of this PhD, I cover the aspirations of things that might follow from this study.

Societal impact is split into collective capacity and political participation. Within collective capacity, the framework asks whether the study contributed to the participants collective capacity to achieve common goals. By working directly with the public, the project has shown the potential to improve collective capacity for citizens to work towards the preservation of current antibiotics. For political participation, the framework asks whether the project stimulates political participation and whether the projects impacts on policy processes and decision making. This was not an aim of this project; however, one avenue of future research could examine whether participants have involved themselves with political policies based on their improved understanding of the research topic.

Ecological impact focuses on targeted interventions which can be evidenced if the project includes objectives that protect and enhance natural resources and/or foster environmental protection. By engaging the public in the issue of antibiotic resistance and seeing resultant behaviour change, this study has made a modest contribution to highlighting the potential for public engagement to reduce the personal use of antibiotics, which are contributing to sub-therapeutic levels of antibiotics in nature. The framework then asks whether the project contributed to higher awareness, knowledge and responsibility for the natural environment. This topic was not explored in this study, however modification to future studies might include a stronger link between the effect of antibiotic misuse and the natural environment.

Wider innovation potential is split into three categories: New technologies, sustainability and social innovation practice as well as economic potential and market opportunities. To examine new technologies, the framework asks whether the project fosters the use or development of new technologies. As developing new technology was not an objective of this project, this outcome has not been met. With regards to sustainability and social innovation practice, this study did not consider sustainability as part of the project plan. However, the framework also asks whether the project results are transferrable to other contexts or organisations. This study adapted frameworks conceived initially for application in other areas of social science research, mainly mathematics education (Sfard, 2008; Biza *et al*., 2018) and science education (Brew, 2001), to conduct discourse analysis on data collected from the participating citizen scientists. It offers the methodological framework for thematic analysis using eight themes as a flexible tool which can itself be adapted. This study aims to move the field of citizen science towards a more evidenced based, qualitative justification of progress of participants; relying minimally on self-reporting and optimally on their discourse. The framework then asks whether the project contributed to social, technical or political innovation. Whilst aspirational, it is the hope that the potential shown by this study to foster ownership and

behaviours in citizens contributes a method by which science can strengthen civil society to resist not just antibiotic resistance, but other global crises like climate change. To examine economic potential and market opportunities, the framework asks whether the project generated any economic impact or competitive advantages. This study did not achieve this outcome. The framework then asks whether the project fosters co-operation for exploitation. Collaboration with the Cooper Lab highlighted this studies potential to foster co-operation. The 165 antagonistic isolates are to be made use of and the aspiration is that future studies will examine these in greater detail.

Table 6-4. Examples of aspirations of this project which support the outcome and impact level of the socio-ecological and economic dimension of the citizen science evaluation framework.

Socio-ecological and Economic Dimension		
Societal Impact	**Ecological Impact**	**Wider Innovation Potential**
Highlighted the potential of citizen science to improve collective capacity for citizens to work towards the preservation of current antibiotics	Highlighted ownership and behaviour change which has the potential to reduce personal use of antibiotics which are contributing to sub-therapeutic levels of antibiotics in nature	Adaptation of frameworks for application in other fields of research to conduct discourse analysis on citizen scientists
		Methodological framework for thematic analysis as a flexible tool
		Moves away from tick box evaluation exercise
		Fostered opportunities for collaboration

6.1.4.4 Summary

Using Kieslinger et al's (2018) open framework for citizen science evaluation, it is clear that this study has demonstrated outcomes and impacts in each of the three dimensions: scientific, participant and socio-ecological and economic. Whilst not every aspect of each dimension was achieved equally, this was to be expected and concurs with Kieslinger *et al*'s (2018) own thoughts, that criteria need to be prioritised and may receive different weighting depending on project goals. Aspirational goals were discussed where relevant, and further highlight Kieslinger *et al*'s (2018) suggestion that long-term monitoring is necessary to capture a project's far-reaching impact. Ultimately, in conducting this evaluation this study has evidenced its impact on science, participants and the wider field of citizen science.

6.1.5 What citizen scientists have gained from participating in this study

At the outset of this study, citizens were asked whether they would like to take part in a project that is trying to find a new antibiotic. Whilst this overambitious outcome was not achieved in five years, participants have contributed to genuine scientific knowledge with regards to antibiotic discovery in the form of finding environmental isolates which inhibit a range of medically relevant pathogens.

Citizens were asked to contribute to our understanding of the public's perception of antibiotics and antibiotic resistance. Participants have contributed to our understanding of public discourse surrounding these topics. Participants use familiar words and familiar rituals as they attempt to engage with a new discourse. Public health narratives are mimicked to facilitate engagement with the discourse, however, often lead to misunderstandings such as the belief that the human body becomes resistant to antibiotics. Individuals place varying levels of faith in science and scientists. Participants generally consider research to be worthwhile. They derive this worth from different aspects of the scientific process or outcomes. Those who receive the most blame for antibiotic resistance are doctors and people in power.

Participants were asked to follow their soil sample over a longer-time period through social media engagement. In doing so, participants have contributed to understanding of how to best reach users and how to best engage users.

Finally, participants were asked to conduct self-guided research into topics surrounding antibiotics and antibiotic resistance. In doing so, participants experienced discursive transformation. Evidence that this occurred can be seen in Chapter 5, where object level learning surrounding discursive objects like antibiotics, bacteria, viruses and Penicillin was demonstrated. Evidence of meta level learning can also be seen in Chapter 5 where previous narratives were contradicted by knowledge of new objects and as a result, new narratives emerged. This included the increased diversity of sources used to obtain information on science, the evidence-based questioning of authority, an interest in previously unknown topics, development of an objective writing style, defending the motives of doctors, internally attributing responsibility for antibiotic resistance, an appreciation for the need to communicate science to the public, the time it takes to undertake scientific research, the process of experimentation, the importance of scientific consensus in developing narratives and the teams of scientists that contribute to famous discoveries.

6.2 Reflection on strengths and limitations

The strengths of the project have been outlined in the previous sections, and can be summarised as: 1) The engagement of citizens through hands on experimental research which produced genuine scientific outcomes, 2) The contribution of citizens to the current literature surrounding public perception of antibiotics and related topics, 3) The engagement of citizens through self-guided research which resulted in discursive transformations and learning experiences and 4) Evaluation, not often conducted in citizen science projects, suggesting this study as a good, successful citizen science project. Following the strengths of the project, I now discuss the limitations of the project. These provide reflections on opportunities for future studies, which are discussed in this section.

Laboratory study was limited by available resources. Whilst this created opportunity for collaboration, for example WGS with the Cooper Lab, it also meant that experiments of interest such as LC-MS to elucidate which secondary metabolites or antibiotics were being produced by the isolates could not be conducted. This instead provides an area for future study. Further, the

aim of reinvigorating the antibiotic pipeline is a task that was realistically beyond the scope of one PhD. This is made clear in the O'Neill report which suggests that to overhaul the antibiotics pipeline would cost between 16 and 37 million USD over the course of 10 years (O'Neill, 2016b).

Whilst genuine scientific knowledge emerged from the lab work, the balance between scientific data for publication and scientific data to be used as material to be posted specifically to engender social media engagement was difficult to balance. The time demands of the laboratory work took time away from analysing participant engagement. It was only once the social media engagement ended and the laboratory work could be brought to its end (considering the length of time for any further experimentation) that the analysis of participant outcomes could forge ahead. This made it difficult to adapt interviews, social media engagement and portfolios during the study period. This balancing act was further limited by the natural demands placed on this interdisciplinary study by four supervisors. There was an expectation of lab data for the Facebook page which created difficult to meet deadlines. In hindsight, knowing that type of content didn't affect engagement, I would be inclined to release fewer labour intensive media and instead publish media such as easy to digest monthly reviews of scientific literature surrounding antibiotics and resistance, news articles, photos and descriptions of lab methods, rather than lab results. This is not to say that lab results should be neglected entirely. Robinson *et al*'s (2018) fourth principle is that citizen scientists may participate in various stages of the scientific process and this includes analysis of laboratory data.

Analysing the interviews, I feel confident that the questions asked covered a broad range of topics which contribute to the current field of public discourse of antibiotics and antibiotic resistance. However, given more time to analyse interview responses after each pop-up event, I may have tweaked questions to better identify novel areas of interest, such as participant's understanding of the link between antibiotic misuse and the environment, providing evidence for Kieslinger *et al*'s (2018) ecological impact. Further, I may have decided to add explorative questions if I felt underlying reasons behind perceptions or misunderstandings could be uncovered. An example of this would have been understanding why citizens tend to believe the human body becomes resistant to antibiotics rather than infectious agents. When asking if humans could become resistant to bacteria, I might have asked why to see if citizens would refer to the bacteria inside us, which was offered up as an explanation naturally in some instances. In future, studies should consider setting aside time to iterate interview questions after each collection event if themes emerge. Finally, I acknowledge the small sample size for the interviews and as such, the inability to conduct any correlational statistical analysis; for example, to find associations between codes which co-emerge or participants who answer yes/no to all yes/no questions. This lack of statistical power could be resolved through increasing number of participants or length of interviews or number of questions asked in future studies.

The idea of engaging with participants on social media was the driving force behind most of my time spent pursuing certain areas of the PhD. Social media was meant to be medium-term data collection, that is a bridge between the

snapshot interview data and the long-term portfolio data. In hindsight, social-media comments lacked richness and offered opportunity for only descriptive analysis. It was only when the social media data was considered as part of the long-term portfolio analysis, that is as a single part of a participant's long-term journey, that these comments offered a more substantial contribution. Studies may want to consider this if their only form of mid- or long-term data collection is through social media comments.

Furthermore, the social media page was managed by the Microbiology Society. They were the gatekeepers for the information that was posted on their Facebook page set up through their site for this project. This meant that the procedure of publishing data and responses was cumbersome and subject to time restraints. The immediate responses that are inherent in social media interactions were constrained using the procedure for posting that we had in place. One example was a comment from social media participant 24, who posted a question on the 15th December 2017. This was answered on the 20th December 2017. However, whilst this response engaged a further comment on the 23rd December 2017, I was unable to respond until the 15th January 2018; until the team at the Microbiology Society had returned after the Christmas period. In retrospect, I realise that this two-step process limited opportunities to experiment with posting different types of social media content and responses to participant engagements via the Facebook page. This may have impacted on the insights I gained about how to best engage participants. In future, I would have either developed a faster method with which to verify posts with the Microbiology Society Communications Team or created our own Facebook page on which to publish instant responses.

I realise that the limited time I spent considering the social media dimension of this project, because of the constraints of undertaking laboratory analysis of samples, meant that the social media data was not analysed throughout the project. Instead this took place after the laboratory work had been completed. Knowing, for example, that antibiotic awareness week increased reach, I may have arranged an 'Ask Me Anything' type of forum for that week which in turn could have encouraged users to follow the page and provided opportunities for more engagement interactions. The processes I set in place with the Microbiology Society to manage the Facebook page as well as the time constraints I detail above also meant that live forums did not occur. A mix of synchronous (live chats and dedicated times) and asynchronous (people post questions or comments) engagement would have improved the creative aspect of discussion over social media.

The decision to leave portfolios with participants until the end of the study period resulted in the need to analyse, in detail, multiple portfolios at once. This provided little opportunity to act on emerging themes and adapt future portfolios to explore topics of interest more thoroughly. It also meant that it was possible that no portfolios were returned, and this would have had a significant impact on this PhD project. In retrospect, I might have given participants a specific time frame in which to complete the portfolio after the pop-up stand was completed and I would have arranged to have portfolios collected in a timelier and considered manner. Analysing these portfolios during the project might also have allowed me to report back to the participants on questions

they asked, information they wanted to get clarification on or provide feedback on portfolio entries. An example of this would be PP1's mention of the confusion caused by the article in the British Medical Portfolio discussing whether to take the full course.

Further, whilst I had contact details for participants, my expectation was that they would contact me if they had any issues. I realise now that it might have been more sensible to arrange monthly 'check ins' with all portfolio participants to see if any questions had arisen that I could answer, establishing a conversation and demonstrating two-way public engagement. For example, I did arrange a phone call with one participant who was struggling with what to write (PP3) which resulted in them pursuing their own interests in this area, rather than the questions I had highlighted and provided to the participants in the portfolio handbook (Appendix G-1). I would also have liked to hand out more portfolios to participants. Participants were not always keen in contributing a significant portion of their time to engaging with the project, but due to the power of this tool to highlight impact and engender participant learning, it should be a focus of future studies. I note that in this study, transcribing and analysing portfolios took significant amounts of time, making iterative analysis during the study period more difficult. However, modern technology is making these techniques faster. Autograph software is beginning to make this method scalable in that much of the transcription is done automatically. Further, if a research team undertook a similar study, analysis of portfolios could be split amongst team members to increase throughput.

6.3 My concluding remarks

The work presented in this thesis has generated 165 antagonistic isolates which kill or inhibit a variety of medically relevant pathogens, 15 of which were analysed with WGS (Chapter 3). It has contributed to the current understanding of the public's perception of antibiotics and related topics (Chapter 4), has examined social media as a tool for public engagement (Chapter 5) and has shown that long-term engagement with a citizen science study engenders participant learning (Chapter 5).

The scientific isolates require analysis by future researchers to assess their value as candidates for the antibiotic pipeline. This research will include a bioinformatic approach to improve WGS read lengths and as such confidence in phylogenetic analysis and identification of BGCs. It will also include a culture-dependent approach to conduct further bioassays, purification of secondary metabolites (LC-MS) and knock-out mutation experiments.

The interview data gathered in this project was analysed using a coding schedule (Table 2-5) based on key topics emerging from public perception literature, as well as frameworks for understanding individuals concepts of research (1.3). The interview questions and coding schedule provide tools with which to build a detailed understanding of how the public perceive antibiotics and antibiotic resistance. This also provides a time point prior to the arrival of the Covid19 pandemic, from which the change in science optimism of the public could be measured.

The social media data collected through Facebook metrics suggests that user engagement is not affected by the types of content released. However, the number of people who see any given content is driven by external events such as antibiotic awareness week.

The use of portfolios to track participant outcomes is my contribution to the field of citizen science. Further, the portfolio data shows that engaging citizens in a citizen science study results in a multitude of participant outcomes whilst simultaneously contributing general science outcomes. This should encourage future studies to deploy the citizen science design and data analysis methods presented in this thesis.

In total, this study has achieved most of the principles which underlie good citizen science practice (6.1.3) and has evidenced outcomes and impacts across the scientific, participant and social-ecological and economic dimensions (6.1.4).

Whilst this study has focused upon the learning experienced by the participant, it has also facilitated learning for its researcher. I have learnt that a multidisciplinary PhD offers many unique opportunities. These include the opportunity to consult with a variety of experts, to learn how to balance multiple avenues of research and to develop expertise on several fronts in order to put the results into context in several fields. It also offers a unique set of challenges: managing the demands of two or more fields of research, supervisors and interested parties. Whilst not easy, it is rewarding to be able to evidence that participants have gained as much from this study as I have. Many citizen science projects use participants as tools for data collection. Being able to say that as well as the science outcomes, the participants have experienced outcomes too is a wonderful feeling. Most of all, this project has taught me that perfection is aspirational, elusive, and pursuing it can be an enemy of productivity. In working in several fields, I constantly find experts who are more familiar with the discourses of science and social science than myself and who have a deeper grasp of the fundamentals than myself. However, I feel that the amount of data I collected, how I analysed it and the impact I had on participants has been productive. Whilst it may take finer tuning of analysis to tease the most out of each element of this study, I feel optimistic that I have laid an important foundation for future citizen science projects and future work.

Chapter 7 References

Abd El-Rahman, H. A. *et al.* (2002) 'Two novel psychrotolerant species, Bacillus psychrotolerans sp. nov. and Bacillus psychrodurans sp. nov., which contain ornithine in their cell walls', *International Journal of Systematic and Evolutionary Microbiology*, 52(6), pp. 2127–2133. doi: 10.1099/ijs.0.01665-0.

Adger, W. N. and Anger, W. N. (2003) 'Social capital, collective action, and adaptation to climate change', *Economic geography*, 79(4), pp. 387–404. doi: 10.1126/science.11.277.620.

Agate, L. *et al.* (2016) 'Scientific Citizenship The Search for Violacein-Producing Microbes to Combat Batrachochytrium dendrobatidis : A Collaborative Research Project between Secondary School and College Research Students †', *J Microbiol Biol Educ*, 17(1), pp. 70–73. doi: 10.1128/jmbe.v17i1.1002.

Alexander, H. (2020) 'Donald Trump accuses the FDA of holding up Pfizer vaccine until after the election', *Daily Mail*, 11 November. Available at: https://www.dailymail.co.uk/news/article-8931911/Donald-Trump-accuses-FDA-holding-Pfizer-vaccine-election.html (Accessed: 13 November 2020).

Alikhan, N. F. *et al.* (2011) 'BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons', *BMC Genomics*, 12(1), p. 402. doi: 10.1186/1471-2164-12-402.

Altschul, S. F. *et al.* (1990) 'Basic local alignment search tool', *Journal of Molecular Biology*. 1990/10/05, 215(3), pp. 403–410. doi: 10.1016/S0022-2836(05)80360-2.

Aminov, R. I. (2010) 'A brief history of the antibiotic era: Lessons learned and challenges for the future', *Frontiers in Microbiology*, 1(DEC). doi: 10.3389/fmicb.2010.00134.

Anagnostopoulos, C. and Spizizen, J. (1961) 'Requirements for Transformation in Bacillus Subtilis', *Journal of Bacteriology*, 81(5), pp. 741–746. doi: 10.1128/jb.81.5.741-746.1961.

Anderson-Lee, J. *et al.* (2016) 'Principles for Predicting RNA Secondary Structure Design Difficulty', *Journal of Molecular Biology*, 428(5), pp. 748–757. doi: 10.1016/j.jmb.2015.11.013.

Arnold, A. (2018) 'Climate Change and Storytelling', *Climate Change and Storytelling*. doi: 10.1007/978-3-319-69383-5.

Ash, C., Priest, F. G. and Collins, M. D. (1993) 'Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test - Proposal for the creation of a new genus Paenibacillus', *Antonie van Leeuwenhoek*, 64(3–4), pp. 253–260. doi: 10.1007/BF00873085.

Baindara, P., Nayudu, N. and Korpole, S. (2020) 'Whole genome mining reveals a diverse repertoire of lanthionine synthetases and lanthipeptides among the genus Paenibacillus', *Journal of Applied Microbiology*, 128(2), pp. 473–490. doi: 10.1111/jam.14495.

Baltz, R. H. (2007) 'Antimicrobials from actinomycetes: Back to the future', *Microbe*, 2(3), pp. 125–131.

Banin, E., Hughes, D. and Kuipers, O. P. (2017) 'Editorial: Bacterial pathogens, antibiotics and antibiotic resistance', *FEMS Microbiology Reviews*.

Oxford University Press, pp. 450–452. doi: 10.1093/femsre/fux016.

Baquero, F., Martínez-Beltrán, J. and Loza, E. (1991) 'A review of antibiotic resistance patterns of Streptococcus pneumoniae in Europe.', *The Journal of antimicrobial chemotherapy*, 28, pp. 31–38. doi: 10.1093/jac/28.suppl_C.31.

Barbara, S. (2011) *Famine Early Warning Systems NETwork*. Famine Early Warning System. Available at: http://www.fews.net.

Bardaji, R. *et al.* (2016) 'Estimating the underwater diffuse attenuation coefficient with a low-cost instrument: The KdUINO DIY buoy', *Sensors (Switzerland)*, 16(3), p. 373. doi: 10.3390/s16030373.

Barrett, H. (2005) *White Paper: Researching Electronic Portfolios and Learner Engagement*. Available at: http://electronicportfolios.org/ (Accessed: 16 November 2020).

Battaglia, M. (2008) 'Encyclopedia of Survey Research Methods', in Lavrakas, P. J. (ed.) *Encyclopedia of Survey Research Methods*. Sage Publications, Inc., pp. 449–450. doi: 10.4135/9781412963947.

Bauraind, I. *et al.* (2004) 'Association between antibiotic sales and public campaigns for their appropriate use.', *Jama*, pp. 2468–70. doi: 10.1001/jama.292.20.2468-b.

Baym, M., Stone, L. K. and Kishony, R. (2016) 'Multidrug evolutionary strategies to reverse antibiotic resistance', *Science*, 351(6268). doi: 10.1126/science.aad3292.

BBC TWO (2017) 'Britain's Greatest Invention'. United Kingdom: BBC.

Benson, D. A. *et al.* (2013) 'GenBank', *Nucleic Acids Research*, 41(D1). doi: 10.1093/nar/gks1195.

BERTANI, G. (1951) 'Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli.', *Journal of bacteriology*, 62(3), pp. 293–300. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14888646 (Accessed: 27 September 2019).

Bignell, C. J. (2001) 'European guideline for the management of gonorrhoea', *International Journal of STD and AIDS*, 12(SUPPL. 3), pp. 27–29. doi: 10.1258/0956462011924074.

Bignell, C. J. (2004) 'BASHH guideline for gonorrhoea', *Sexually Transmitted Infections*, 80(5), pp. 330–331. doi: 10.1136/sti.2004.012781.

Biza, I., Nardi, E. and Zachariades, T. (2018) 'Competences of Mathematics Teachers in Diagnosing Teaching Situations and Offering Feedback to Students: Specificity, Consistency and Reification of Pedagogical and Mathematical Discourses', in *Diagnostic Competence of Mathematics Teachers*. Cham: Springer International Publishing, pp. 55–78. doi: 10.1007/978-3-319-66327-2_3.

Bodmer, W. F. (1985) *The public understanding of science.*, *Royal Society*. The Royal Society. doi: 10.1038/340011a0.

Bonney, R. *et al.* (2009) *Public Participation in Scientific Research: Defining the Field and Assessing Its Potential for Informal Science Education. A CAISE Inquiry Group Report*, *A CAISE Inquiry Group Report*. Center for Advancement

of Informal Science Education (CAISE), Cornell Lab of Ornithology, University of California, Davis, Rutgers University, Carnegie Museum of Natural History, Dickinson College. Available at: http://files.eric.ed.gov/fulltext/ED519688.pdf.

Bonney, R. *et al.* (2014) 'Next steps for citizen science', *Science*, 343(6178), pp. 1436–1437. doi: 10.1126/science.1251554.

Boucher, H. W. *et al.* (2009) 'Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America', *Clinical Infectious Diseases*, 48(1), pp. 1–12. doi: 10.1086/595011.

Bowater, L. (2017) *The Microbes Fight Back: Antibiotic Resistance.*, *The Microbes Fight Back: Antibiotic Resistance.* Cambridge: Royal Society of Chemistry.

Brew, A. (2001) 'Conceptions of Research: A phenomenographic study', *Studies in Higher Education*, 26(3), pp. 271–285. doi: 10.1080/03075070120076255.

Brew, A. *et al.* (2016) 'Research productivity and academics' conceptions of research', *Higher Education*, 71(5), pp. 681–697. doi: 10.1007/s10734-015-9930-6.

Brew, A. and Lucas, L. (2009) *Academic Research and Researchers*. Edited by A. Brew and L. Lucas. Open University Press. Available at: https://books.google.co.uk/books?id=QuSa_spTW1oC&printsec=frontcover#v=onepage&q&f=false (Accessed: 27 March 2020).

Bronzwaer, S. L. A. M. *et al.* (2002) 'The relationship between antimicrobial use and antimicrobial resistance in Europe', *Emerging infectious diseases*, 8(3), pp. 278–282. doi: 10.3201/eid0803.010192.

Brookes-Howell, L., Hood, K., *et al.* (2012) 'Clinical influences on antibiotic prescribing decisions for lower respiratory tract infection: A nine country qualitative study of variation in care', *BMJ Open*, 2(3). doi: 10.1136/bmjopen-2011-000795.

Brookes-Howell, L., Elwyn, G., *et al.* (2012) '"The body gets used to them": Patients' interpretations of antibiotic resistance and the implications for containment strategies', *Journal of General Internal Medicine*, 27(7), pp. 766–772. doi: 10.1007/s11606-011-1916-1.

Brooks, L. *et al.* (2008) 'Towards a better understanding of patients' perspectives of antibiotic resistance and MRSA: a qualitative study', *Fam Pract*, 25(5), pp. 341–348. doi: 10.1093/fampra/cmn037.

Brossard, D. (2013) 'New media landscapes and the science information consumer', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, pp. 14096–14101. doi: 10.1073/pnas.1212744110.

Budiharjo, A. *et al.* (2017) 'Complete genome sequence of Bacillus altitudinis P-10, a potential bioprotectant against Xanthomonas oryzae pv. oryzae, isolated from rice rhizosphere in Java, Indonesia', *Genome Announcements*, 5(48). doi: 10.1128/genomeA.01388-17.

Bush, K. *et al.* (2011) 'Tackling antibiotic resistance', *Nature Reviews Microbiology*, 9(12), pp. 894–896. doi: 10.1038/nrmicro2693.

Butler, C. C. *et al.* (1998) 'Understanding the culture of prescribing: qualitative study of general practitioners' and patients' perceptions of antibiotics for sore throats', *Bmj*, 317(7159), pp. 637–642. doi: 10.1136/bmj.317.7159.637.

Cals, J. W. L. *et al.* (2007) 'Public beliefs on antibiotics and respiratory tract infections: An internet-based questionnaire study', *British Journal of General Practice*, 57(545), pp. 942–947. doi: 10.3399/096016407782605027.

Cardamone, C., Lobel, L. and Cardamone, C. (2016) 'Using Citizen Science to Engage Introductory Students: From Streams to the Solar System †', *Journal of Microbiology & Biology Education*, 17(1), pp. 117–119. doi: 10.1128/jmbe.v17i1.1082.

Caruso, J. P. *et al.* (2016) 'Citizen Science: The Small World Initiative Improved Lecture Grades and California Critical Thinking Skills Test Scores of Nonscience Major Students at Florida Atlantic University', *Journal of Microbiology & Biology Education*, 17(1), pp. 156–162. doi: 10.1128/jmbe.v17i1.1011.

Carver, C. S., Scheier, M. F. and Segerstrom, S. C. (2010) 'Optimism', *Clinical Psychology Review*. Pergamon, pp. 879–889. doi: 10.1016/j.cpr.2010.01.006.

Cassini, A. *et al.* (2019) 'Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis', *The Lancet Infectious Diseases*, 19(1), pp. 56–66. doi: 10.1016/S1473-3099(18)30605-4.

Caulier, S. *et al.* (2019) 'Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group', *Frontiers in Microbiology*. Frontiers Media S.A., p. 302. doi: 10.3389/fmicb.2019.00302.

Centers for Disease Control and Prevention (1999a) *Achievements in Public Health, 1900-1999: Control of Infectious Diseases.* Available at: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm4829a1.htm.

Centers for Disease Control and Prevention (1999b) 'Interagency Task Force on Antimicrobial Resistance', *Task Force on Antimicrobial Resistance*, p. 46. Available at: https://www.cdc.gov/drugresistance/actionplan/aractionplan.pdf (Accessed: 1 March 2018).

Centers for Disease Control and Prevention (2000) *Leading Causes of Death, 1900-1998.* Available at: https://www.cdc.gov/nchs/data/dvs/lead1900_98.pdf.

Centers for Disease Control and Prevention (2006) 'Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs.', *The Annals of pharmacotherapy.*, 40(5), pp. 1007–1008. doi: 10.1345/aph.1N108.

Centers for Disease Control and Prevention (2013a) *Antibiotic resistance threats in the United States, 2013.*, *U.S. Department of Health and Human Services*. Available at: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf (Accessed: 23 August 2019).

Centers for Disease Control and Prevention (2013b) *Antibiotic Resistance Threats in the United States*.

Centers for Disease Control and Prevention (2019) *Antibiotic*

ResistanceThreats in the United States. doi: 10.15620/cdc:82532.

Centers for Disease Control and Prevention (2020) *Scientific Brief: Community Use of Cloth Masks to Control the Spread of SARS-CoV-2.* Available at: https://www.cdc.gov/coronavirus/2019-ncov/more/masking-science-sars-cov2.html (Accessed: 12 November 2020).

Chaisson, M., Pevzner, P. and Tang, H. (2004) 'Fragment assembly with short reads', *Bioinformatics*, 20(13), pp. 2067–2074. doi: 10.1093/bioinformatics/bth205.

Chaudhary, D. K., Khulan, A. and Kim, J. (2019) 'Development of a novel cultivation technique for uncultured soil bacteria', *Scientific Reports*, 9(1). doi: 10.1038/s41598-019-43182-x.

Clarke, T. A., Yousafzai, F. K. and Eady, R. R. (1999) 'Klebsiella pneumoniae nitrogenase: Formation and stability of putative beryllium fluoride-ADP transition state complexes', *Biochemistry*, 38(31), pp. 9906–9913. doi: 10.1021/bi9904353.

Cleary, G. P. *et al.* (2016) 'Avian assemblages at bird baths: A comparison of urban and rural bird baths in Australia', *PLoS ONE*, 11(3), p. e0150899. doi: 10.1371/journal.pone.0150899.

Clift, C. (2019) *Review of Progress on Antimicrobial Resistance: Background and Analysis*, *Centre on Global Health Security*. Available at: https://www.chathamhouse.org/publication/review-progress-antimicrobial-resistance (Accessed: 24 November 2020).

Coenen, S. *et al.* (2006) 'Antibiotic prescribing for acute cough: The effect of perceived patient demand', *British Journal of General Practice*, 56(524), pp. 183–190. Available at: /pmc/articles/PMC1828261/?report=abstract (Accessed: 22 September 2020).

Coenen, S. *et al.* (2013) 'Are Patient Views about Antibiotics Related to Clinician Perceptions, Management and Outcome? A Multi-Country Study in Outpatients with Acute Cough', *PLoS ONE*, 8(10). doi: 10.1371/journal.pone.0076691.

Cohen, L., Manion, L. and Morrison, K. (Keith R. B. . (2007) *Research methods in education*. Routledge Falmer.

Cole, A. (2014) 'GPs feel pressurised to prescribe unnecessary antibiotics, survey finds.', *BMJ (Clinical research ed.)*. BMJ. doi: 10.1136/bmj.g5238.

Cooke, J. *et al.* (2015) 'Longitudinal trends and cross-sectional analysis of English national hospital antibacterial use over 5 years (2008-13): Working towards hospital prescribing quality measures', *Journal of Antimicrobial Chemotherapy*, 70(1), pp. 279–285. doi: 10.1093/jac/dku328.

Cornell University. Laboratory of Ornithology. and Bonney, R. (1991) *Living bird.*, *Living bird*. Cornell Laboratory of Ornithology. Available at: http://sfx-44uea.hosted.exlibrisgroup.com/44uea?sid=google&auinit=R&aulast=Bonney&atitle=Citizen+science:+A+lab+tradition&title=Living+bird&volume=15&issue=4&date=1996&spage=7&issn=1059-521X (Accessed: 4 September 2019).

Costelloe, C. *et al.* (2010) 'Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-

analysis', *Bmj*, 340(may18 2), pp. c2096–c2096. doi: 10.1136/bmj.c2096.

Cowan, M. M. (1999) *Plant Products as Antimicrobial Agents*, *CLINICAL MICROBIOLOGY REVIEWS*. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC88925/pdf/cm000564.pdf (Accessed: 18 January 2019).

Creswell, J. W. (2012) *Planning, Conducting, and Evaluating Quantitative and Qualitative Research*. 4th edn. Pearson. Available at: http://basu.nahad.ir/uploads/creswell.pdf (Accessed: 10 June 2019).

Crits-Christoph, A. *et al.* (2018) 'Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis', *Nature*, 558(7710), pp. 440–444. doi: 10.1038/s41586-018-0207-y.

D'Costa, V. M. *et al.* (2006) 'Sampling the antibiotic resistome', *Science*, 311(5759), pp. 374–377. doi: 10.1126/science.1120800.

D'Onofrio, A. *et al.* (2010) 'Siderophores from Neighboring Organisms Promote the Growth of Uncultured Bacteria', *Chemistry and Biology*, 17(3), pp. 254–264. doi: 10.1016/j.chembiol.2010.02.010.

Dale, G. E. *et al.* (1995) 'Characterization of the gene for the chromosomal dihydrofolate reductase (DHFR) of Staphylococcus epidermidis ATCC 14990: The origin of the trimethoprim-resistant S1 DHFR from Staphylococcus aureus?', *Journal of Bacteriology*, 177(11), pp. 2965–2970. doi: 10.1128/jb.177.11.2965-2970.1995.

Danielsen, F. *et al.* (2007) 'Increasing conservation management action by involving local people in natural resource monitoring.', *A Journal of the Human Environment*. 2007/12/14, 36(7), pp. 566–570. doi: 10.1579/0044-7447(2007)36{[}566:ICMABI]2.0.CO;2.

Daud, N. S. *et al.* (2019) 'Paenibacillus polymyxa bioactive compounds for agricultural and biotechnological applications', *Biocatalysis and Agricultural Biotechnology*. Elsevier Ltd, p. 101092. doi: 10.1016/j.bcab.2019.101092.

Daume, S. and Galaz, V. (2016) '"Anyone know what species this is?" - Twitter conversations as embryonic citizen science communities', *PLoS ONE*, 11(3), p. e0151387. doi: 10.1371/journal.pone.0151387.

Davies, J. and Davies, D. (2010) 'Origins and Evolution of Antibiotic Resistance', *Microbiology and Molecular Biology Reviews*, 74(3), pp. 417–433. doi: 10.1128/MMBR.00016-10.

Davies, S. C. (2011) *Infections and the Rise of Antimicrobial Resistance*, *Annual Report of the Chief Medical Officer - Infections and the rise of antimicrobial resistance*. Edited by D. O. Health. England: Department Of Health. doi: 10.1016/S0140-6736(13)60604-2.

Davies, S. C. *et al.* (2013) 'Annual report of the chief medical officer: Infection and the rise of antimicrobial resistance', *The Lancet*, 381(9878), pp. 1606–1609. doi: 10.1016/S0140-6736(13)60604-2.

Davis, M. E. *et al.* (2017) 'Exploring patient awareness and perceptions of the appropriate use of antibiotics: A mixed-methods study', *Antibiotics*, 6(4). doi: 10.3390/antibiotics6040023.

Deguines, N. *et al.* (2016) 'Functional homogenization of flower visitor

communities with urbanization', *Ecol Evol*, 6(7), pp. 1967–1976. doi: 10.1002/ece3.2009.

Delcher, A. L. *et al.* (2007) 'Identifying bacterial genes and endosymbiont DNA with Glimmer', *Bioinformatics*, 23(6), pp. 673–679. doi: 10.1093/bioinformatics/btm009.

Delgado, S. and Nichols, W. J. (2005) 'Saving Sea Turtles from Ground Up: Awakening Sea Turtle Conservation in Northwestern Mexico', *Marine Studies*, 3(2), pp. 89–104. Available at: http://www.wallacejnichols.org/234/419/mast-saving-sea-turtles-from-ground-up.html.

Department of Health and Social Care (2020) *New campaign to prevent spread of coronavirus indoors this winter -*, *Gov.Uk*. Available at: https://www.gov.uk/government/news/new-campaign-to-prevent-spread-of-coronavirus-indoors-this-winter (Accessed: 12 November 2020).

Dickinson, J., Zuckerberg, B. and Bonter, D. (2010) 'Citizen Science as an Ecological Research Tool: Challenges and Benefits', *Annual Review of Ecology, Evolution, and Systematics*, 41(1), pp. 149–172. doi: 10.1146/annurev-ecolsys-102209-144636.

Dijksterhuis, J. *et al.* (1999) 'Antibiosis plays a role in the context of direct interaction during antagonism of Paenibacillus polymyxa towards Fusarium oxysporum', *Journal of Applied Microbiology*, 86(1), pp. 13–21. doi: 10.1046/j.1365-2672.1999.t01-1-00600.x.

van Dijl, J. M. and Hecker, M. (2013) 'Bacillus subtilis: From soil bacterium to super-secreting cell factory', *Microbial Cell Factories*, 12(1), p. 3. doi: 10.1186/1475-2859-12-3.

Do, T. C. M. V. *et al.* (2020) 'Development and validation of a LC-MS/MS method for determination of multi-class antibiotic residues in aquaculture and river waters, and photocatalytic degradation of antibiotics by TiO2 nanomaterials', *Catalysts*, 10(3), p. 356. doi: 10.3390/catal10030356.

Dodd, P. J., Sismanidis, C. and Seddon, J. A. (2016) 'Global burden of drug-resistant tuberculosis in children: a mathematical modelling study', *The Lancet Infectious Diseases*, 16(10), pp. 1193–1201. doi: 10.1016/S1473-3099(16)30132-3.

Donnelly, L. (2017) 'Britain could face "post-antibiotic apocalypse" warns top doctor ', *The Telegraph*, 13 October. Available at: https://www.telegraph.co.uk/news/2017/10/13/britain-could-face-post-antibiotic-apocalypse-warns-top-doctor/ (Accessed: 18 November 2020).

Donoghue, E. M. and Sturtevant, V. E. (2007) 'Social Science Constructs in Ecosystem Assessments: Revisiting Community Capacity and Community Resiliency', *Society & Natural Resources*, 20(10), pp. 899–912. doi: 10.1080/08941920701561114.

Dowell, E. (2017) *Pop Up Science: Transforming empty shops into creative spaces for science engagement*. Available at: https://www.imperial.ac.uk/media/imperial-college/medicine/nhli/public-engagement/Pop_Up_Science_eBook.pdf (Accessed: 11 November 2020).

Van Driel, M. L. *et al.* (2006) 'Are sore throat patients who hope for antibiotics actually asking for pain relief?', *Annals of Family Medicine*, 4(6), pp. 494–499.

doi: 10.1370/afm.609.

Dunn, R. R., Urban, J., *et al.* (2016) ‘Symbiosis in the Soil: Citizen Microbiology in Middle and High School Classrooms †’, *Journal of Microbiology & Biology Education*, 17(1), pp. 60–62. doi: 10.1128/jmbe.v17i1.1016.

Dunn, R. R., Cooper, C. B., *et al.* (2016) ‘The Tragedy of the Unexamined Cat: Why K-12 and University Education Are Still in the Dark Ages and How Citizen Science Allows for a Renaissance’, *J Microbiol Biol Educ*, 17(1), pp. 4–6. doi: 10.1128/jmbe.v17i1.1049.

Dyar, O. *et al.* (2018) ‘Assessing the Knowledge, Attitudes and Behaviors of Human and Animal Health Students towards Antibiotic Use and Resistance: A Pilot Cross-Sectional Study in the UK’, *Antibiotics*, 7(1), p. 10. doi: 10.3390/antibiotics7010010.

Dye, C. (2014) ‘After 2015: Infectious diseases in a new era of health and development’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1645). doi: 10.1098/rstb.2013.0426.

Eastman, A. W., Heinrichs, D. E. and Yuan, Z. C. (2014) ‘Comparative and genetic analysis of the four sequenced Paenibacillus polymyxa genomes reveals a diverse metabolism and conservation of genes relevant to plant-growth promotion and competitiveness’, *BMC Genomics*, 15(1), pp. 1–22. doi: 10.1186/1471-2164-15-851.

Edgar, R. C. (2004) ‘MUSCLE: Multiple sequence alignment with high accuracy and high throughput’, *Nucleic Acids Research*, 32(5), pp. 1792–1797. doi: 10.1093/nar/gkh340.

Ehrenberg CG (1835) ‘Physikalische Abhandlungen der Koeniglichen Akademie der Wissenschaften zu Berlin aus den Jahren 1833–1835’, pp. 145–336.

Eitinger, T., Fuchs, G. and Schlegel, H. G. (2014) *Allgemeine Mikrobiologie*. Thieme.

El-Rahman, T. M. A. *et al.* (2020) ‘Antimicrobial Potential of Paenibacillus Polymyxa AALI Endophyte Isolated from Calotropis Procera’, *International Journal of Progressive Sciences and Technologies*, 20(2), pp. 418–422. Available at: http://ijpsat.es/index.php/ijpsat/article/view/1833 (Accessed: 5 November 2020).

Ensor, J. (2020) ‘Donald Trump accuses Pfizer of delaying coronavirus vaccine announcement until after election’, *The Telegraph*, 10 November. Available at: https://www.telegraph.co.uk/news/2020/11/09/donald-trumps-son-questions-timing-pfizers-coronavirus-vaccine/ (Accessed: 13 November 2020).

European Center for Disease Prevention and Control (2012) *ECDC Antimicrobial resistance - Factsheet for experts*, *Factsheet for experts*. Available at: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/basic_facts/Pages/factsheet_experts.aspx (Accessed: 21 March 2018).

European Commission (2010) *Special Eurobarometer 338 - Antimicrobial Resistance*. Survey commissioned by the Directorate-General for Health and Consumers and coordinated by the Directorate-General Communication

("Research and Political Analysis" Unit). doi: 10.1097/CCM.0b013e3181de0c26.

European Commission (2013) *Special Eurobarometer 407 -Antimicrobial Resistance*. TNS Opinion & Social, at the request of the European Commission, Directorate-General for Health and Consumers. Available at: http://ec.europa.eu/public_opinion/archives/ebs/ebs_407_en.pdf.

European Commission (2016) *Special Eurobarometer 445 - Antimicrobial Resistance*. doi: 10.2875/760366.

Fang, F. C. *et al.* (2016) 'Crowdsourced Data Indicate Widespread Multidrug Resistance in Skin Flora of Healthy Young Adults †', *Journal of Microbiology & Biology Education*, 17(1), pp. 172–182. doi: 10.1128/jmbe.v17i1.1008.

Felsenstein, J. (1985) 'Confidence Limits on Phylogenies: An Approach Using the Bootstrap', *Evolution*, 39(4), p. 783. doi: 10.2307/2408678.

Fenton, K. A. *et al.* (2003) 'Ciprofloxacin resistance in Neisseria gonorrhoeae in England and Wales in 2002', *Lancet*, 361(9372), pp. 1867–1869. doi: 10.1016/S0140-6736(03)13489-7.

Fernandez-Gimenez, M. E., Ballard, H. L. and Sturtevant, V. E. (2008) 'Adaptive management and social learning in collaborative and community-based monitoring: a study of five community-based forestry organizations in the western USA', *Ecol Soc*, 13(2).

Filipetto, F. A. *et al.* (2008) 'Patient knowledge and perception of upper respiratory infections, antibiotic indications and resistance', *Patient Preference and Adherence*, 2(2), pp. 35–39. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2770410/pdf/ppa-2-35.pdf (Accessed: 6 June 2018).

Finch, R. *et al.* (2004) 'Educational interventions to improve antibiotic use in the community: Report from the International Forum on Antibiotic Resistance (IFAR) colloquium, 2002', *Lancet Infectious Diseases*, 4(1), pp. 44–53. doi: 10.1016/S1473-3099(03)00860-0.

Fleming, A. (1929) 'On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae.', *British journal of experimental pathology*, 10(3), p. 226. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2048009/ (Accessed: 26 November 2020).

Fleming, A. (1945) *Nobel Lecture*. Available at: https://www.nobelprize.org/prizes/medicine/1945/fleming/lecture/%3E (Accessed: 30 August 2019).

Follett, R. and Strezov, V. (2015) 'An analysis of citizen science based research: Usage and publication patterns', *PLoS ONE*, 10(11), p. e0143687. doi: 10.1371/journal.pone.0143687.

Foster, W. and Raoult, A. (1974) 'Early descriptions of antibiosis.', *The Journal of the Royal College of General Practitioners*, 24(149), pp. 889–94. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2157443&tool=pmcentrez&rendertype=abstract (Accessed: 21 March 2018).

Furusawa, C., Horinouchi, T. and Maeda, T. (2018) 'Toward prediction and control of antibiotic-resistance evolution', *Current Opinion in Biotechnology*, 54, pp. 45–49. doi: 10.1016/j.copbio.2018.01.026.

Gaarslev, C. *et al.* (2016) 'A mixed methods study to understand patient expectations for antibiotics for an upper respiratory tract infection', *Antimicrobial Resistance and Infection Control*, 5(1), p. 39. doi: 10.1186/s13756-016-0134-3.

Gallagher, J. (2017) 'Bug resistant to all antibiotics kills woman', *BBC News Website*, 13 January. Available at: http://www.bbc.co.uk/news/health-38609553 (Accessed: 17 November 2020).

Gans, J., Wolinsky, M. and Dunbar, J. (2005) 'Microbiology: Computational improvements reveal great bacterial diversity and high toxicity in soil', *Science*, 309(5739), pp. 1387–1390. doi: 10.1126/science.1112665.

Gao, X. *et al.* (2017) 'A Bayesian taxonomic classification method for 16S rRNA gene sequences with improved species-level accuracy', *BMC Bioinformatics*, 18(1), pp. 1–10. doi: 10.1186/s12859-017-1670-4.

Gonzales, R. *et al.* (2008) '"Get smart Colorado": Impact of a mass media campaign to improve community antibiotic use', *Medical Care*, 46(6), pp. 597–605. doi: 10.1097/MLR.0b013e3181653d2e.

Goossens, H. *et al.* (2005) 'Outpatient antibiotic use in Europe and association with resistance: A cross-national database study', *Lancet*, 365(9459), pp. 579–587. doi: 10.1016/S0140-6736(05)17907-0.

Gordon, R. *et al.* (1973) *The Genus Bacillus*. Edited by United States Department of Agriculture. Washington, DC: United States Department of Agriculture. Available at: https://books.google.co.uk/books?hl=en&lr=&id=F4kwAAAAYAAJ&oi=fnd&pg=PA1&dq=Bacillus+genus+spores&ots=P627Ff08-a&sig=uFFShOaEyaFpylz6weTWYQlePLg#v=onepage&q&f=true (Accessed: 3 November 2020).

Gosling, L. *et al.* (2016) 'Citizen science identifies the effects of nitrogen dioxide and other environmental drivers on tar spot of sycamore', *Environ Pollut*, 214, pp. 549–555. doi: 10.1016/j.envpol.2016.04.066.

Grant, J. R., Arantes, A. S. and Stothard, P. (2012) 'Comparing thousands of circular genomes using the CGView Comparison Tool', *BMC Genomics*, 13(1), p. 202. doi: 10.1186/1471-2164-13-202.

Gregory, J. and Lock, S. J. (2008) 'The Evolution of "Public Understanding of Science": Public Engagement as a Tool of Science Policy in the UK', *Sociology Compass*, 2(4), pp. 1252–1265. doi: 10.1111/j.1751-9020.2008.00137.x.

Grijalva, C. G., Nuorti, J. P. and Griffin, M. R. (2009) 'Antibiotic prescription rates for acute respiratory tract infections in US ambulatory settings', *JAMA - Journal of the American Medical Association*, 302(7), pp. 758–766. doi: 10.1001/jama.2009.1163.

Gross, H. and Loper, J. E. (2009) 'Genomics of secondary metabolite production by Pseudomonas spp.', *Natural Product Reports*. Royal Society of Chemistry, pp. 1408–1446. doi: 10.1039/b817075b.

Gudnadottir, U. *et al.* (2013) 'Reducing health care-associated infections: Patients want to be engaged and learn about infection prevention', *American Journal of Infection Control*, 41(11), pp. 955–958. doi: 10.1016/j.ajic.2013.03.310.

Hamaki, T. *et al.* (2005) 'Isolation of novel bacteria and actinomycetes using soil-extract agar medium', *Journal of Bioscience and Bioengineering*, 99(5), pp. 485–492. doi: 10.1263/jbb.99.485.

Harshey, R. M. (1994) 'Bees aren't the only ones: swarming in Gram-negative bacteria', *Molecular Microbiology*, 13(3), pp. 389–394. doi: 10.1111/j.1365-2958.1994.tb00433.x.

Hasman, H. *et al.* (2014) 'Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples', *Journal of Clinical Microbiology*, 52(1), pp. 139–146. doi: 10.1128/JCM.02452-13.

Hawker, J. I. *et al.* (2014) 'Trends in antibiotic prescribing in primary care for clinical syndromes subject to national recommendations to reduce antibiotic resistance, UK 1995-2011: Analysis of a large database of primary care consultations', *Journal of Antimicrobial Chemotherapy*, 69(12), pp. 3423–3430. doi: 10.1093/jac/dku291.

Hawkey, P. M. (1998) 'The origins and molecular basis of antibiotic resistance', *British Medical Journal*, 317(7159), pp. 657–660.

Hawking, M. K. D. *et al.* (2017) 'Attitudes and behaviours of adolescents towards antibiotics and self-care for respiratory tract infections: A qualitative study', *BMJ Open*, 7(5), p. e015308. doi: 10.1136/bmjopen-2016-015308.

Hawkings, N. J., Butler, C. C. and Wood, F. (2008) 'Antibiotics in the community: A typology of user behaviours', *Patient Education and Counseling*, 73(1), pp. 146–152. doi: 10.1016/j.pec.2008.05.025.

Hawkings, N. J., Wood, F. and Butler, C. C. (2007) 'Public attitudes towards bacterial resistance: A qualitative study', *Journal of Antimicrobial Chemotherapy*, 59(6), pp. 1155–1160. doi: 10.1093/jac/dkm103.

Health Protection Agency (2012) *English National Point Prevalence Survey on Healthcare Associated Infections and Antimicrobial Use, 2011*, *Healthcare associated infections (HCAI): guidance, data and analysis*. Edited by D. of Health. Available at: https://www.gov.uk/government/publications/healthcare-associated-infections-hcai-point-prevalence-survey-england.

Heigl, F. *et al.* (2018) 'Quality Criteria for Citizen Science Projects on Österreich forscht | Version 1.1'. doi: 10.31219/osf.io/48j27.

Heigl, F. and Dörler, D. (2017) 'Public participation: Time for a definition of citizen science', *Nature*, 551(7679), pp. 168–168. doi: 10.1038/d41586-017-05745-8.

Herman, J. and Winters, L. (1994) 'Portfolio Research: A Slim Collection.', *Educational Leadership*.

Hernandez, S., Tsang, T. and Handelsman, J. (2015) *SMALL WORLD INITIATIVE: Research Protocols*. Available at: https://bblearn.callutheran.edu/bbcswebdav/pid-797987-dt-content-rid-3544052_2/courses/62944/2016.8.1%2BSWI%2BStudent%2BResearch%2B

Protocols%2B3rd%2BEdition%2B%28w-%2Binserted%2Bpages%29.pdf (Accessed: 12 September 2019).

Hoiseth, S. K. and Stocker, B. A. D. (1981) 'Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines', *Nature*, 291(5812), pp. 238–239. doi: 10.1038/291238a0.

Holmes, A. H. *et al.* (2016) 'Understanding the mechanisms and drivers of antimicrobial resistance', *The Lancet*. Lancet Publishing Group, pp. 176–187. doi: 10.1016/S0140-6736(15)00473-0.

Hover, B. M. *et al.* (2018) 'Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens', *Nature Microbiology*, 3(4), pp. 415–422. doi: 10.1038/s41564-018-0110-1.

Huber, B. *et al.* (2019) 'Fostering public trust in science: The role of social media', *Public Understanding of Science*, 28(7), pp. 759–777. doi: 10.1177/0963662519869097.

Huttner, B. *et al.* (2010) 'Characteristics and outcomes of public campaigns aimed at improving the use of antibiotics in outpatients in high-income countries', *The Lancet Infectious Diseases*, 10(1), pp. 17–31. doi: 10.1016/S1473-3099(09)70305-6.

IFRC (2000) *World Disasters Report.*, *Disasters*. Geneva: International Federation of the Red Cross and Red Crescent Societies. doi: 10.1037/e569662006-003.

Irwin, A. (1995) *Citizen Science*. Routledge. doi: 10.4324/9780203202395.

Ison, C. *et al.* (2013) 'Decreased susceptibility to cephalosporins among gonococci: Data from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) in England and Wales, 2007-2011', *The Lancet Infectious Diseases*, 13(9), pp. 762–768. doi: 10.1016/S1473-3099(13)70143-9.

Jeong, H. *et al.* (2019) 'Chronicle of a soil bacterium: Paenibacillus polymyxa E681 as a Tiny Guardian of Plant and Human Health', *Frontiers in Microbiology*. Frontiers Media S.A., p. 467. doi: 10.3389/fmicb.2019.00467.

Jiang, N. J. *et al.* (2016) 'Ureolytic activities of a urease-producing bacterium and purified urease enzyme in the anoxic condition: Implication for subseafloor sand production control by microbially induced carbonate precipitation (MICP)', *Ecological Engineering*, 90, pp. 96–104. doi: 10.1016/j.ecoleng.2016.01.073.

Jin, X.-B. *et al.* (2012) 'Isolation and Identification of Bacillus altitudinis ZJ 186 from Marine Soil Samples and its Antifungal Activity Against Magnaporthe oryzae', *Current Research in Bacteriology*, 5(1), pp. 13–23. doi: 10.3923/crb.2012.13.23.

Johnson, K. A. (2016) 'Real Life Science with Dandelions and Project BudBurst', *Journal of Microbiology & Biology Education*, 17(1), pp. 115–116. doi: 10.1128/jmbe.v17i1.1064.

Jordan, R. C., Ballard, H. L. and Phillips, T. B. (2012) 'Key issues and new approaches for evaluating citizen-science learning outcomes', *Frontiers in*

Ecology and the Environment, 10(6), pp. 307–309. doi: 10.1890/110280.

Kearns, D. B. (2010) 'A field guide to bacterial swarming motility', *Nature Reviews Microbiology*. NIH Public Access, pp. 634–644. doi: 10.1038/nrmicro2405.

Kieser, T. *et al.* (2000) *Practical Streptomyces Genetics. 2nd ed.*, *International microbiology: official journal of the Spanish Society for Microbiology*. Norwich: John Innes Foundation.

Kieslinger, B. *et al.* (2018) *Evaluating Citizen Science: Towards an open framework*, *Citizen Science - Innovation in Open Science, Society and Policy*. Available at: https://www.researchgate.net/publication/328334525_Evaluating_citizen_science_-_Towards_an_open_framework (Accessed: 26 September 2020).

Klein, E. Y. *et al.* (2018) 'Global increase and geographic convergence in antibiotic consumption between 2000 and 2015', *Proceedings of the National Academy of Sciences of the United States of America*, 115(15), pp. E3463–E3470. doi: 10.1073/pnas.1717295115.

Kluyver, A. J. and van Niel., C. B. (1936) 'Prospects for a natural system of classification of bacteria. Zentralblatt für Bakteriologie.', *Parasitenkunde and Infektionskrankheiten*, 2(94), pp. 369–403. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2679376 (Accessed: 5 November 2020).

Kong, Q. *et al.* (2016) 'MyShake: A smartphone seismic network for earthquake early warning and beyond', *Sci Adv*, 2(2), p. e1501055. doi: 10.1126/sciadv.1501055.

Kridelbaugh, D. M. (2016) 'The Use of Online Citizen-Science Projects to Provide Experiential Learning Opportunities for Nonmajor Science Students †', *Journal of Microbiology & Biology Education*, 17(1), pp. 105–106. doi: 10.1128/jmbe.v17i1.1022.

Krieg, N. (1984) *Bergey's Manual of Systematic Bacteriology*. Edited by Williams & Wilkins. Baltimore.

Kullenberg, C. and Kasperowski, D. (2016) 'What is citizen science? - A scientometric meta-analysis', *PLoS ONE*, 11(1), p. e0147152. doi: 10.1371/journal.pone.0147152.

Kumar, S., Stecher, G. and Tamura, K. (2016) 'MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets', *Molecular biology and evolution*, 33(7), pp. 1870–1874. doi: 10.1093/molbev/msw054.

Lawana, V., Korrapati, M. C. and Mehendale, H. M. (2014) 'Cycloheximide', in *Encyclopedia of Toxicology: Third Edition*. Elsevier, pp. 1103–1105. doi: 10.1016/B978-0-12-386454-3.00298-0.

Lazarus, R. J. (2009) *Super wicked problems and climate change: Restraining the present to liberate the future*, *Cornell Law Review*. Available at: https://www.researchgate.net/publication/47505469_Super_Wicked_Problems_and_Climate_Change_Restraining_the_Present_to_Liberate_the_Future (Accessed: 13 November 2020).

Lee, I. *et al.* (2016) 'OrthoANI: An improved algorithm and software for calculating average nucleotide identity', *International Journal of Systematic*

and Evolutionary Microbiology, 66(2), pp. 1100–1103. doi: 10.1099/ijsem.0.000760.

Lertcanawanichakul, M. and Sawangnop, S. (2008) 'A Comparison of Two Methods Used for Measuring the Antagonistic Activity of Bacillus Species', *Walailak Journal of Sciences Technology*, pp. 161–171. Available at: http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.503.4715 (Accessed: 16 September 2019).

Lewis, D. A. (2010) 'The Gonococcus fights back: Is this time a knock out?', *Sexually Transmitted Infections*, 86(6), pp. 415–421. doi: 10.1136/sti.2010.042648.

Lewis, K. *et al.* (2010) 'Uncultured microorganisms as a source of secondary metabolites', *Journal of Antibiotics*. J Antibiot (Tokyo), pp. 468–476. doi: 10.1038/ja.2010.87.

Lewis, K. (2012) 'Antibiotics: Recover the lost art of drug discovery', *Nature*, 485(7399), pp. 439–440. doi: 10.1038/485439a.

Lewis, K. (2013) 'Platforms for antibiotic discovery', *Nature Reviews Drug Discovery*. Nature Publishing Group, pp. 371–387. doi: 10.1038/nrd3975.

Lewis, K. (2020) 'The Science of Antibiotic Discovery', *Cell*. Cell Press, pp. 29–45. doi: 10.1016/j.cell.2020.02.056.

Liao, C. H. and Shollenberger, L. M. (2003) 'Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline', *Letters in Applied Microbiology*, 37(1), pp. 45–50. doi: 10.1046/j.1472-765X.2003.01345.x.

Liebeke, M. *et al.* (2009) 'Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria', *Applied Microbiology and Biotechnology*, 83(1), pp. 161–173. doi: 10.1007/s00253-009-1965-0.

Lieberman, P. B. and Wootan, M. G. (1998) *Protecting the Crown Jewels of Medicine: A Strategic Plan to Preserve the Effectiveness of Antibioitcs*. Center for Science in the Public Interest: Center for Science in the Public Interest.

Ling, L. L. *et al.* (2015) 'A new antibiotic kills pathogens without detectable resistance', *Nature*, 517(7535), pp. 455–459. doi: 10.1038/nature14098.

Lintott, P. R. *et al.* (2016) 'Differential responses of cryptic bat species to the urban landscape', *Ecology and Evolution*, 6(7), pp. 2044–2052. doi: 10.1002/ece3.1996.

Little, P. *et al.* (2004) 'Importance of patient pressure and perceived pressure and perceived medical need for investigations, referral, and prescribing in primary care: Nested observational study', *British Medical Journal*, 328(7437), pp. 444–446. doi: 10.1136/bmj.38013.644086.7c.

Livermore, D. M. *et al.* (2013) 'Declining cephalosporin and fluoroquinolone non-susceptibility among bloodstream Enterobacteriaceae from the UK: Links to prescribing change?', *Journal of Antimicrobial Chemotherapy*, 68(11), pp. 2667–2674. doi: 10.1093/jac/dkt212.

Lloyd, K. G. *et al.* (2018) 'Phylogenetically Novel Uncultured Microbial Cells Dominate Earth Microbiomes', *mSystems*, 3(5). doi: 10.1128/msystems.00055-18.

Loudon, I. (2006) ‘A brief history of homeopathy’, *Journal of the Royal Society of Medicine*, 99, pp. 607–610.

Macfarlane, J. *et al.* (1997) ‘Influence of patients’ expectations on antibiotic management of acute lower respiratory tract illness in general practice: Questionnaire study’, *British Medical Journal*, 315(7117), pp. 1211–1214. doi: 10.1136/bmj.315.7117.1211.

Macfarlane, J. T., Holmes, W. F. and Macfarlane, R. M. (1997) ‘Reducing reconsultations for acute lower respiratory tract illness with an information leaflet: a randomized controlled study of patients in primary care.’, *The British journal of general practice : the journal of the Royal College of General Practitioners*, 47(424), pp. 719–22. Available at: http://bjgp.org/content/47/424/719.abstract (Accessed: 6 June 2018).

Magiorakos, A. P. *et al.* (2012) ‘Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance’, *Clinical Microbiology and Infection*, 18(3), pp. 268–281. doi: 10.1111/j.1469-0691.2011.03570.x.

Mair, L. and Ruete, A. (2016) ‘Explaining spatial variation in the recording effort of citizen science data across multiple taxa’, *PLoS ONE*, 11(1), p. e0147796. doi: 10.1371/journal.pone.0147796.

Marconi, P. *et al.* (1976) ‘Utilizzazione di soluzione salina ipertonica di cloruro di potassio (3M KC1) per l’estrazione di antigeni solubili da Candia albicans’, *Annali Sclavo; rivista di microbiologia e di immunologia*, 18(1), pp. 61–66.

Martin, J. E. *et al.* (1970) ‘Comparative study of gonococcal susceptibility to penicillin in the United States, 1955–1969’, *Journal of Infectious Diseases*, 122(5), pp. 459–461. doi: 10.1093/infdis/122.5.459.

Masters, K. *et al.* (2016) ‘Science learning via participation in online citizen science’, *Journal of Science Communication*, 15(3). doi: 10.22323/2.15030207.

McClelland, M. *et al.* (2001) ‘Complete genome sequence of Salmonella enterica serovar Typhimurium LT2’, *Nature*, 413(6858), pp. 852–856. doi: 10.1038/35101614.

McNulty, C. *et al.* (2007a) ‘Don’t wear me out - The public’s knowledge of and attitudes to antibiotic use’, *Journal of Antimicrobial Chemotherapy*, 59(4), pp. 727–738. doi: 10.1093/jac/dkl558.

McNulty, C. *et al.* (2007b) ‘The public’s attitudes to and compliance with antibiotics.’, *The Journal of antimicrobial chemotherapy*. 2007/09/14, 60 Suppl 1(suppl_1), pp. i63-8. doi: 10.1093/jac/dkm161.

McNulty, C. *et al.* (2012) ‘Have the public’s expectations for antibiotics for acute uncomplicated respiratory tract infections changed since the H1N1 influenza pandemic? A qualitative interview and quantitative questionnaire study’, *BMJ Open*, 2(2), p. e000674. doi: 10.1136/bmjopen-2011-000674.

McNulty, C. *et al.* (2016) ‘How much information about antibiotics do people recall after consulting in primary care?’, *Family Practice*, 33(4), pp. 395–400. doi: 10.1093/fampra/cmw022.

Medema, M. H. *et al.* (2011) ‘AntiSMASH: Rapid identification, annotation and

analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences', *Nucleic Acids Research*, 39(SUPPL. 2), pp. W339–W346. doi: 10.1093/nar/gkr466.

Meek, R. W., Vyas, H. and Piddock, L. J. V. (2015) 'Nonmedical Uses of Antibiotics: Time to Restrict Their Use?', *PLoS Biology*, 13(10), pp. 1–11. doi: 10.1371/journal.pbio.1002266.

Meibohm, B. and Derendorf, H. (1997) 'Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling.', *International journal of clinical pharmacology and therapeutics*, 35(10), pp. 401–13. doi: 10.1155/2014/506287.

van der Meij, A. *et al.* (2017) 'Chemical ecology of antibiotic production by actinomycetes', *FEMS Microbiology Reviews*. Oxford University Press, pp. 392–416. doi: 10.1093/femsre/fux005.

Mendelson, M. *et al.* (2017) 'Antibiotic resistance has a language problem', *Nature*, 545(7652), pp. 23–25. doi: 10.1038/545023a.

Microbiology Society (2017a) *Schoolzone: Antibiotics Unearthed: teacher experiences*. Available at: https://microbiologysociety.org/publication/past-issues/microbiology-in-popular-culture/article/schoolzone-antibiotics-unearthed-teacher-experiences.html# (Accessed: 4 December 2020).

Microbiology Society (2017b) *Schoolzone: Teaching antimicrobial resistance in schools*. Available at: https://microbiologysociety.org/publication/past-issues/halting-epidemics/article/schoolzone-teaching-antimicrobial-resistance-in-schools.html (Accessed: 4 December 2020).

Microbiology Society (2017c) *The history of antibiotics*, *Microbiology Society*. Available at: https://microbiologysociety.org/education-outreach/antibiotics-unearthed/antibiotics-and-antibiotic-resistance/the-history-of-antibiotics.html (Accessed: 27 August 2019).

Microbiology Society (2018) *What are antibiotics and how do they work?* | *Microbiology Society*. Available at: https://microbiologysociety.org/education-outreach/antibiotics-unearthed/antibiotics-and-antibiotic-resistance/what-are-antibiotics-and-how-do-they-work.html (Accessed: 21 March 2018).

Microbiology Society (2019a) *Antibiotics Unearthed*, *Microbiology Society*. Available at: https://microbiologysociety.org/education-outreach/antibiotics-unearthed.html (Accessed: 24 November 2020).

Microbiology Society (2019b) *Antimicrobial Resistance*, *Microbiology Society*. Available at: https://microbiologysociety.org/our-work/antimicrobial-resistance.html (Accessed: 30 August 2019).

Migula, N. (1894) *Arbeiten aus dem Bakteriologischen*, *Inst. Technischen Hochschule Karlsruhe*. Available at: https://books.google.co.uk/books/about/Arbeiten_aus_dem_bakteriologischen_Insti.html?id=M05CngEACAAJ&redir_esc=y (Accessed: 5 November 2020).

Miyazaki, Y., Teramura, A. and Senou, H. (2016) 'Biodiversity data mining from Argus-eyed citizens: the first illegal introduction record of Lepomis macrochirus macrochirus Rafinesque, 1819 in Japan based on Twitter information', *ZooKeys*, 2016(569), pp. 123–133. doi: 10.3897/zookeys.569.7577.

Montefusco, A., Nakamura, L. K. and Labeda, D. P. (1993) 'Bacillus peoriae sp. nov.', *International Journal of Systematic Bacteriology*. Microbiology Society, pp. 388–390. doi: 10.1099/00207713-43-2-388.

Moore, D. (2016) *Antibiotic Classification & Mechanism - Basic Science*, *Orthobulllets*. Available at: https://www.orthobullets.com/basic-science/9059/antibiotic-classification-and-mechanism (Accessed: 1 December 2020).

Moradali, M. F., Ghods, S. and Rehm, B. H. A. (2017) 'Pseudomonas aeruginosa lifestyle: A paradigm for adaptation, survival, and persistence', *Frontiers in Cellular and Infection Microbiology*. Frontiers Research Foundation. doi: 10.3389/fcimb.2017.00039.

Morgan, E. (2020) 'Those who won't wear masks put us all at risk, but confrontation is not the answer.', *The Guardian*. Available at: https://www.theguardian.com/commentisfree/2020/sep/17/masks-risk-confrontation-covid-19-guidelines-empathy (Accessed: 12 November 2020).

Moss, E. L., Maghini, D. G. and Bhatt, A. S. (2020) 'Complete, closed bacterial genomes from microbiomes using nanopore sequencing', *Nature Biotechnology*, 38(6), pp. 701–707. doi: 10.1038/s41587-020-0422-6.

National Institute for Health and Care Excellence (NICE) (2018) *Sore throat (acute): antimicrobial prescribing*, *NICE Guideline*. Available at: https://www.nice.org.uk/guidance/ng84 (Accessed: 17 November 2020).

Nesta (2017) *Longitude prize*. Available at: https://longitudeprize.org/ (Accessed: 18 January 2019).

Nguyen, T. M. *et al.* (2018) 'Effective soil extraction method for cultivating previously uncultured soil bacteria', *Applied and Environmental Microbiology*, 84(24), pp. 1145–1163. doi: 10.1128/AEM.01145-18.

NHS.UK (2016) *Antibiotics*. Available at: https://www.nhs.uk/conditions/antibiotics/ (Accessed: 21 March 2018).

Nichols, D. *et al.* (2010) 'Use of ichip for high-throughput in situ cultivation of "uncultivable microbial species▽', *Applied and Environmental Microbiology*, 76(8), pp. 2445–2450. doi: 10.1128/AEM.01754-09.

Norris, P. *et al.* (2013) 'Public Beliefs about Antibiotics, Infection and Resistance: A Qualitative Study', *Antibiotics*, 2(4), pp. 465–476. doi: 10.3390/antibiotics2040465.

Nyquist, A. C. *et al.* (1998) 'Antibiotic prescribing for children with colds, upper respiratory tract infections, and bronchitis', *Journal of the American Medical Association*, 279(11), pp. 875–877. doi: 10.1001/jama.279.11.875.

O'Neill, J. *et al.* (2015) *Securing New Drugs for Future Generations: The Pipeline of Antibiotics*, *The Review on Antimicrobial Resistance*. Available at: http://amr-review.org/sites/default/files/SECURING NEW DRUGS FOR FUTURE GENERATIONS FINAL WEB_0.pdf%5Cnhttp://amr-review.org/Publications (Accessed: 16 September 2020).

O'Neill, J. (2016a) *Infection prevention, control and surveillance: limiting the development and spread of drug resistance*, *The Review on Antimicrobial Resistance*. Review on Antimicrobial Resistance. Available at: http://amr-

review.org/sites/default/files/Health infrastructure and surveillance final version_LR_NO CROPS.pdf.

O'Neill, J. (2016b) *TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS*, *The Review on Antimicrobial Resistance*.

O'Neill, J. (2018) 'Two Years On: An update on achievement towards the recommendations of the Antimicrobial Resistance Report', *Journal of Applied Microbiology*. doi: 10.1111/jam.13933.

Obama, B. and The Task Force for Combating Antibitic-Resistant Bacteria (2015) *National Action Plan For Combating Antibiotic-Resistant Bacteria*. Office of the Press Secretary: The White House, Washington. Available at: https://www.whitehouse.gov/sites/default/files/docs/national_action_plan_for_combating_antibotic-resistant_bacteria.pdf.

Onghena, S. (2011) 'The survival of 19th-century scientific optimism: The public discourse on science in Belgium in the aftermath of the Great War (ca. 1919-1930)', *Centaurus*, 53(4), pp. 280–305. doi: 10.1111/j.1600-0498.2011.00239.x.

Oxford English Dictionary (2018) *'Antibiotic, Adj. and N.'.* Available at: http://www.oed.com/view/Entry/8513?redirectedFrom=Antibiotics#eid (Accessed: 21 March 2018).

Padda, K. P., Puri, A. and Chanway, C. P. (2017) 'Paenibacillus polymyxa: A prominent biofertilizer and biocontrol agent for sustainable agriculture', in *Agriculturally Important Microbes for Sustainable Agriculture*. Singapore: Springer Singapore, pp. 165–191. doi: 10.1007/978-981-10-5343-6_6.

Pallen, M. J., Loman, N. J. and Penn, C. W. (2010) 'High-throughput sequencing and clinical microbiology: Progress, opportunities and challenges', *Current Opinion in Microbiology*. Elsevier Current Trends, pp. 625–631. doi: 10.1016/j.mib.2010.08.003.

Park, M., Satta, G. and Kon, O. M. (2019) 'An update on multidrug-resistant tuberculosis', *Clinical Medicine, Journal of the Royal College of Physicians of London*. Royal College of Physicians, pp. 135–139. doi: 10.7861/CLINMEDICINE.19-2-135.

Parkinson, J. E. *et al.* (2016) 'A citizen science approach to monitoring bleaching in the zoantharian Palythoa tuberculosa', *PeerJ*, 4, p. e1815. doi: 10.7717/peerj.1815.

Patuxent Wildlife Research Center (1970) *North American Bird Phenology Program*, *Patuxent Wildlife Research Center, USGS*. Available at: http://www.birds.cornell.edu/citscitoolkit/projects/pwrc/nabirdphenologyprogram/ (Accessed: 4 September 2019).

Payne, D. J. *et al.* (2007) 'Drugs for bad bugs: Confronting the challenges of antibacterial discovery', *Nature Reviews Drug Discovery*. Nature Publishing Group, pp. 29–40. doi: 10.1038/nrd2201.

Perez, T. *et al.* (2016) 'Collective intelligence: Aggregation of information from neighbors in a guessing game', *PLoS ONE*, 11(4), p. e0153586. doi: 10.1371/journal.pone.0153586.

Peters, N. K. *et al.* (2008) 'The research agenda of the National Institute of Allergy and Infectious Diseases for antimicrobial resistance', *J.Infect.Dis.*, 197(0022-1899 (Print)), pp. 1087–1093. doi: 10.1086/533451.

Pham, V. H. T. and Kim, J. (2012) 'Cultivation of unculturable soil bacteria', *Trends in Biotechnology*, pp. 475–484. doi: 10.1016/j.tibtech.2012.05.007.

Phillips, T. *et al.* (2014) *Introduction to the User ' s Guide for Evaluating Learning Outcomes from Citizen Science*, *Public Underst. Sci.* Ithaca, NY: Cornell Lab of Ornithology.

Picard, A. (2017) 'Misuse of antibiotics is leading to dangerous resistance', *The Globe and Mail*, 20 June. Available at: https://www.theglobeandmail.com/opinion/misuse-of-antibiotics-is-leading-to-dangerous-resistance/article35362410/ (Accessed: 1 December 2020).

Piuri, M., Sanchez-Rivas, C. and Ruzal, S. M. (1998) 'A novel antimicrobial activity of a Paenibacillus polymyxa strain isolated from regional fermented sausages', *Letters in Applied Microbiology*, 27(1), pp. 9–13. doi: 10.1046/j.1472-765X.1998.00374.x.

Poppe, C. *et al.* (1998) 'Salmonella typhimurium DT104: A virulent and drug-resistant pathogen', *Canadian Veterinary Journal*, 39(9), pp. 559–565. Available at: /pmc/articles/PMC1539434/?report=abstract (Accessed: 25 November 2020).

Pray, L. (2008) 'Antibiotic Resistance , Mutation Rates and MRSA', *Nature Education*, 1(1), p. 30. Available at: http://www.nature.com/scitable/topicpage/antibiotic-resistance-mutation-rates-and-mrsa-28360.

Price, D. B. *et al.* (2004) 'Community-acquired pneumonia mortality: A potential link to antibiotic prescribing trends in general practice', *Respiratory Medicine*, 98(1), pp. 17–24. doi: 10.1016/j.rmed.2003.08.011.

Priyodip, P. and Balaji, S. (2019) 'A Preliminary Study on Probiotic Characteristics of Sporosarcina spp. for Poultry Applications', *Current Microbiology*, 76(4), pp. 448–461. doi: 10.1007/s00284-019-01647-2.

Public Health England (2014) *Voluntary surveillance of Clostridium difficile, England, Wales and Northern Ireland: 2013*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/346324/Voluntary_reporting_S._aureus_bacteraemia_England_Wales_Northern_Ireland_2013.pdf (Accessed: 2 September 2019).

Public Health England (2017) *Keep Antibiotics Working*. Available at: https://campaignresources.phe.gov.uk/resources/campaigns/58-keep-antibiotics-working/Overview (Accessed: 4 September 2019).

Public Health England (2019) *English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR)*. Available at: www.facebook.com/PublicHealthEngland (Accessed: 16 September 2020).

Qin, Y. *et al.* (2017) 'Antimicrobial resistance and molecular characteristics of methicillin-resistant Staphylococcus aureus isolates from child patients of high-risk wards in Shenzhen, China', *Japanese Journal of Infectious Diseases*, 70(5), pp. 479–484. doi: 10.7883/yoken.JJID.2016.328.

Qin, Z. *et al.* (2017) 'Formicamycins, antibacterial polyketides produced by Streptomyces formicae isolated from African Tetraponera plant-ants', *Chemical Science*, 8(4), pp. 3218–3227. doi: 10.1039/c6sc04265a.

Quinn, R. A. *et al.* (2017) 'Molecular Networking As a Drug Discovery, Drug Metabolism, and Precision Medicine Strategy', *Trends in Pharmacological Sciences*, 38(2), pp. 143–154. doi: 10.1016/j.tips.2016.10.011.

Rabbee, M. F. *et al.* (2019) 'Bacillus velezensis: A valuable member of bioactive molecules within plant microbiomes', *Molecules*, 24(6). doi: 10.3390/molecules24061046.

Rajbhandary, S. S., Marks, S. M. and Bock, N. N. (2004) 'Costs of patients hospitalized for multidrug-resistant tuberculosis', *Int J Tuberc Lung Dis*, 8(8), pp. 1012–1016. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15305486 (Accessed: 16 February 2018).

Ramaswamy, S. and Musser, J. M. (1998) 'Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update', *Tubercle and Lung Disease*, 79(1), pp. 3–29. doi: 10.1054/tuld.1998.0002.

Raoult, V. *et al.* (2016) 'GoPros™ as an underwater photogrammetry tool for citizen science', *PeerJ*, 4, p. e1960. doi: 10.7717/peerj.1960.

REEF (2011) *Reef Environmental Education Foundation Volunteer Survey Project Database*, *World Wide Web electronic Publication, www.reef.org*. doi: reef.

Rice, L. (2008a) 'Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE', *The Journal of Infectious Diseases*, 197(8), pp. 1079–1081. doi: 10.1086/533452.

Rice, L. (2008b) 'The Maxwell Finland Lecture: For the Duration-- Rational Antibiotic Administration in an Era of Antimicrobial Resistance and Clostridium difficile', *Clinical Infectious Diseases*, 46(4), pp. 491–496. doi: 10.1086/526535.

Robinson, L. D. *et al.* (2018) 'Ten principles of citizen science', in Hecker, S. et al. (eds) *Citizen Science: Innovation in Open Science, Society and Policy*. London: UCL Press, pp. 27–40. doi: 10.14324/111.9781787352339.

Rogers, Y. H. and Venter, J. C. (2005) 'Genomics: Massively parallel sequencing', *Nature*. Nature Publishing Group, pp. 326–327. doi: 10.1038/437326a.

Rosado, A. S. and Seldin, L. (1993) 'Production of a potentially novel anti-microbial substance by Bacillus polymyxa', *World Journal of Microbiology & Biotechnology*, 9(5), pp. 521–528. doi: 10.1007/BF00386287.

Roy, H. E. *et al.* (2016) 'Focal plant observations as a standardised method for pollinator monitoring: Opportunities and limitations for mass participation citizen science', *PLoS ONE*, 11(3), p. e0150794. doi: 10.1371/journal.pone.0150794.

Rutledge, P. J. and Challis, G. L. (2015) 'Discovery of microbial natural products by activation of silent biosynthetic gene clusters', *Nature Reviews Microbiology*. Nature Publishing Group, pp. 509–523. doi: 10.1038/nrmicro3496.

Sagarra, O. *et al.* (2016) 'Citizen Science Practices for Computational Social Science Research: The Conceptualization of Pop-Up Experiments', *Frontiers in Physics*, 3, p. 93. doi: 10.3389/fphy.2015.00093.

Saitou, N. and Nei, M. (1987) 'The neighbor-joining method: a new method for reconstructing phylogenetic trees.', *Molecular Biology and Evolution*, 4(4), pp. 406–425. doi: 10.1093/oxfordjournals.molbev.a040454.

Sample, I. (2015) 'New class of antibiotic could turn the tables in battle against superbugs', *The Guardian*, 8 January. Available at: https://www.theguardian.com/science/2015/jan/07/antibiotic-drug-resistance-teixobactin.

Sarkar, S., Hermes DeSantis, E. R. and Kuper, J. (2007) 'Resurgence of colistin use', *American Journal of Health-System Pharmacy*, 64(23), pp. 2462–2466. doi: 10.2146/ajhp060501.

Schäberle, T. F. (2016) 'Biosynthesis of α-pyrones', *Beilstein Journal of Organic Chemistry*. Beilstein-Institut Zur Forderung der Chemischen Wissenschaften, pp. 571–588. doi: 10.3762/bjoc.12.56.

Schäfer, M. S. (2009) 'From public understanding to public engagement: An empirical assessment of changes in science coverage', *Science Communication*, 30(4), pp. 475–505. doi: 10.1177/1075547008326943.

Schatz, A., Bugle, E. and Waksman, S. A. (1944) 'Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria', *Proceedings of the Society for Experimental Biology and Medicine*, 55(1), pp. 66–69. doi: 10.3181/00379727-55-14461.

Schnetzer, J. *et al.* (2016) 'Scientific Citizenship MyOSD 2014 : Evaluating Oceanographic Measurements Contributed by Citizen Scientists in Support of Ocean Sampling Day', *Journal of Microbiology and Biology Education*, 17(1), pp. 163–171. doi: 10.1128/jmbe.v17i1.1001.

Schöner, T. A. *et al.* (2016) 'Aryl Polyenes, a Highly Abundant Class of Bacterial Natural Products, Are Functionally Related to Antioxidative Carotenoids', *ChemBioChem*, 17(3), pp. 247–253. doi: 10.1002/cbic.201500474.

Schultz, J. A., Cloutier, R. N. and Côté, I. M. (2016) 'Evidence for a trophic cascade on rocky reefs following sea star mass mortality in British Columbia', *PeerJ*, 4, p. e1980. doi: 10.7717/peerj.1980.

Schwartz, A. *et al.* (2013) 'Bacillus simplex—A Little Known PGPB with Anti-Fungal Activity—Alters Pea Legume Root Architecture and Nodule Morphology When Coinoculated with Rhizobium leguminosarum bv. viciae', *Agronomy*, 3(4), pp. 595–620. doi: 10.3390/agronomy3040595.

Seeley, H. M. and Vandemark, P. J. (1962) *Microbes in Action*. San Francisco: W. H. Freeman and Company. Available at: https://www.abebooks.co.uk/servlet/BookDetailsPL?bi=22802206461&searchurl=sortby%3D20%26tn%3Dmicrobes%2Bin%2Baction&cm_sp=snippet-_-srp1-_-title1 (Accessed: 16 November 2020).

Seemann, T. (2014) 'Prokka: Rapid prokaryotic genome annotation', *Bioinformatics*, 30(14), pp. 2068–2069. doi: 10.1093/bioinformatics/btu153.

Segata, N. *et al.* (2012) ‘Metagenomic microbial community profiling using unique clade-specific marker genes’, *Nature Methods*, 9(8), pp. 811–814. doi: 10.1038/nmeth.2066.

Seifert, V. A. *et al.* (2016) ‘Community Partnership Designed to Promote Lyme Disease Prevention and Engagement in Citizen Science.’, *Journal of microbiology & biology education*, 17(1), pp. 63–9. doi: 10.1128/jmbe.v17i1.1014.

Seldin, L. *et al.* (1999) ‘Inhibitory activity of Paenibacillus polymyxa SCE2 against human pathogenic micro-organisms’, *Letters in Applied Microbiology*, 28(6), pp. 423–427. doi: 10.1046/j.1365-2672.1999.00563.x.

Seppälä, H. *et al.* (1997) ‘The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group a streptococci in Finland’, *New England Journal of Medicine*, 337(7), pp. 441–446. doi: 10.1056/NEJM199708143370701.

Sfard, A. (1998) *On Two Metaphors for Learning and the Dangers of Choosing Just One*, *Educational Researcher*. Available at: http://er.aera.net (Accessed: 24 May 2019).

Sfard, A. (2007) ‘When the rules of discourse change, but nobody tells you: Making sense of mathematics learning from a commognitive standpoint’, *Journal of the Learning Sciences*, 16(4), pp. 565–613. doi: 10.1080/10508400701525253.

Sfard, A. (2008) *Thinking as Communicating: Human Development, the Growth of Discourses, and Mathematizing (Learning in Doing: Social, Cognitive and Computational Perspectives)*. Cambridge University Press. Available at: citeulike-article-id:8146415%5Cnhttp://www.amazon.ca/exec/obidos/redirect?tag=citeulike09-20&amp%5Cnpath=ASIN/0521867371 (Accessed: 12 October 2018).

Sfard, A. (2015) ‘Learning, commognition and mathematics.’, in D. Scott & E. Hargreaves (ed.) *The Sage Handbook of Learning, Chapter: Learning, commognition and mathematics*. London: Sage, pp. 129–138. Available at: https://www.researchgate.net/publication/288839755_Sfard_A_2015_Learning_commognition_and_mathematics_In_D_Scott_E_Hargreaves_Eds_The_Sage_Handbook_of_Learning_pp_129_-_138_London_Sage (Accessed: 19 March 2019).

Shah, N. S. *et al.* (2007) ‘Worldwide emergence of extensively drug-resistant tuberculosis’, *Emerging Infectious Diseases*, 13(3), pp. 380–387. doi: 10.3201/eid1303.061400.

Shehab, N. *et al.* (2008) ‘Emergency Department Visits for Antibiotic-Associated Adverse Events’, *Clinical Infectious Diseases*, 47(6), pp. 735–743. doi: 10.1086/591126.

Shenhav, S. R. (2015) *Analyzing social narratives*, *Analyzing Social Narratives*. Taylor and Francis Inc. doi: 10.4324/9780203109083.

Shirk, J. L. *et al.* (2012) ‘Public participation in scientific research: A framework for deliberate design’, *Ecology and Society*, 17(2), p. 29. doi: 10.5751/ES-04705-170229.

Shivaji, S. *et al.* (2006) ‘Bacillus aerius sp. nov., Bacillus aerophilus sp. nov.,

Bacillus stratosphericus sp. nov. and Bacillus altitudinis sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes', *International Journal of Systematic and Evolutionary Microbiology*, 56(7), pp. 1465–1473. doi: 10.1099/ijs.0.64029-0.

Silver, L. L. (2011) 'Challenges of antibacterial discovery', *Clinical Microbiology Reviews*, 24(1), pp. 71–109. doi: 10.1128/CMR.00030-10.

Sirota, M. *et al.* (2017) 'Expectations for antibiotics increase their prescribing: Causal evidence about localized impact', *Health Psychology*, 36(4), pp. 402–409. doi: 10.1037/hea0000456.

Slezak, M. (2017) 'Australian babies given antibiotics at some of the highest rates in the world', *The Guardian*, 27 July. Available at: https://www.theguardian.com/society/2017/jul/28/australian-babies-given-antibiotics-at-some-of-the-highest-rates-in-the-world (Accessed: 18 November 2020).

Small World Initiative, I. (2019) *Our Approach — Small World Initiative*. Available at: http://www.smallworldinitiative.org/about (Accessed: 27 September 2020).

Snow, C. P. (1959) 'The Two Cultures And The Scientific Revolution', *Nature*, 184(4684), pp. 411–412. doi: 10.1038/184411a0.

Spellberg, B. and Shlaes, D. (2014) 'Prioritized current unmet needs for antibacterial therapies', *Clinical Pharmacology and Therapeutics*, 96(2), pp. 151–153. doi: 10.1038/clpt.2014.106.

Spellberg, B. and Taylor-Blake, B. (2013) *On the exoneration of Dr. William H. Stewart: Debunking an urban legend*, *Infectious Diseases of Poverty*. doi: 10.1186/2049-9957-2-3.

Srivastava, Alok Kumar *et al.* (2020) 'Pan-genome analysis of Exiguobacterium reveals species delineation and genomic similarity with Exiguobacterium profundum PHM 11', *Environmental Microbiology Reports*, 12(6), pp. 639–650. doi: 10.1111/1758-2229.12890.

Stein, T. (2005) 'Bacillus subtilis antibiotics: Structures, syntheses and specific functions', *Molecular Microbiology*, 56(4), pp. 845–857. doi: 10.1111/j.1365-2958.2005.04587.x.

Steiner, K. K. *et al.* (2020) 'Isolation and whole-genome sequencing of Pseudomonas sp. RIT 623, a slow-growing bacterium endowed with antibiotic properties', *BMC Research Notes*, 13(1), p. 370. doi: 10.1186/s13104-020-05216-w.

Stewart, E. J. (2012) 'Growing unculturable bacteria', *Journal of Bacteriology*. American Society for Microbiology (ASM), pp. 4151–4160. doi: 10.1128/JB.00345-12.

Stiggins, R. J. (1994) *Student-centered classroom assessment*. New York: Merrill.

Sundquist, A. *et al.* (2007) 'Whole-genome sequencing and assembly with high-throughput, short-read technologies', *PLoS ONE*. Edited by A. Christoffels, 2(5), p. e484. doi: 10.1371/journal.pone.0000484.

Taylor, C. B. (1951) 'Nature of the factor in soil-extract responsible for bacterial

growth-stimulation [6]', *Nature*, pp. 115–116. doi: 10.1038/168115a0.

The PEW Charitable Trusts (2020) *Tracking the Global Pipeline of Antibiotics in Development, April 2020*, *Antibiotic Resistance Project*. Available at: https://www.pewtrusts.org/en/research-and-analysis/issue-briefs/2020/04/tracking-the-global-pipeline-of-antibiotics-in-development (Accessed: 24 November 2020).

Thelwall, S. *et al.* (2018) *Annual epidemiological commentary: Gram-negative bacteraemia, MRSA bacteraemia, MSSA bacteraemia and C. difficile infections, up to and including financial year April 2017 to March 2018*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/724030/Annual_epidemiological_commentary_2018.pdf (Accessed: 2 September 2019).

Tommasi, R. *et al.* (2015) 'ESKAPEing the labyrinth of antibacterial discovery', *Nature Reviews Drug Discovery*. Nature Publishing Group, pp. 529–542. doi: 10.1038/nrd4572.

Torsvik, V., Goksoyr, J. and Daae, F. L. (1990) 'High diversity in DNA of soil bacteria', *Applied and Environmental Microbiology*, 56(3), pp. 782–787. doi: 10.1128/aem.56.3.782-787.1990.

Trantas, E. A. *et al.* (2015) 'Comparative genomic analysis of multiple strains of two unusual plant pathogens: Pseudomonas corrugata and Pseudomonas mediterranea', *Frontiers in Microbiology*, 6(AUG), p. 811. doi: 10.3389/fmicb.2015.00811.

Trautman, P., Eric, M. and Crawford, J. M. (2015) 'Linking Biosynthetic Gene Clusters to their Metabolites via Pathway- Targeted Molecular Networking', *Current Topics in Medicinal Chemistry*, 16(15), pp. 1705–1716. doi: 10.2174/1568026616666151012111046.

Trivella, D. B. B. and de Felicio, R. (2018) 'The Tripod for Bacterial Natural Product Discovery: Genome Mining, Silent Pathway Induction, and Mass Spectrometry-Based Molecular Networking', *mSystems*, 3(2). doi: 10.1128/msystems.00160-17.

Tvrzová, L. *et al.* (2006) 'Pseudomonas moraviensis sp. nov. and Pseudomonas vranovensis sp. nov., soil bacteria isolated on nitroaromatic compounds, and emended description of Pseudomonas asplenii', *International Journal of Systematic and Evolutionary Microbiology*, 56(11), pp. 2657–2663. doi: 10.1099/ijs.0.63988-0.

UK Cabinet Office (2015) *National Risk Register of Civil Emergencies: 2015 edition*, *National Risk Register of Civil Emergencies*.

Vallejos, M. A. V. *et al.* (2016) 'Human-Induced Landscape Changes Homogenize Atlantic Forest Bird Assemblages through Nested Species Loss', *PLoS ONE*, 11(2), p. e0147058. doi: 10.1371/journal.pone.0147058.

Vikeli, E. *et al.* (2020) 'In situ activation and heterologous production of a cryptic lantibiotic from an african plant ant-derived saccharopolyspora species', *Applied and Environmental Microbiology*, 86(3). doi: 10.1128/AEM.01876-19.

Wakefield, M. A., Loken, B. and Hornik, R. (2010) 'Use of mass media

campaigns to change health behaviour', *The Lancet*, 376(9748), pp. 1261–1271. doi: 10.1016/S0140-6736(10)60809-4.

Waksman, S. A. (1947) 'What Is an Antibiotic or an Antibiotic Substance?', *Mycologia*, 39(5), p. 565. doi: 10.2307/3755196.

Walker, T. M. *et al.* (2013) 'Contact investigations for outbreaks of Mycobacterium tuberculosis: Advances through whole genome sequencing', *Clinical Microbiology and Infection*. Blackwell Publishing Ltd, pp. 796–802. doi: 10.1111/1469-0691.12183.

Wang, M. Q. *et al.* (2020) 'Pseudomonas laoshanensis sp. nov., isolated from peanut field soil', *Archives of Microbiology*, 1, p. 3. doi: 10.1007/s00203-020-02067-8.

Wang, Q. *et al.* (2007) 'Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy', *Applied and Environmental Microbiology*, 73(16), pp. 5261–5267. doi: 10.1128/AEM.00062-07.

Watkins, L. K. F. *et al.* (2015) 'Knowledge and Attitudes Regarding Antibiotic Use Among Adult Consumers, Adult Hispanic Consumers, and Health Care Providers — United States, 2012–2013', *MMWR. Morbidity and Mortality Weekly Report*, 64(28), pp. 767–770. doi: 10.15585/mmwr.mm6428a5.

Weber, T. *et al.* (2015) 'AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters', *Nucleic Acids Research*, 43(W1), pp. W237–W243. doi: 10.1093/nar/gkv437.

Von Der Weid, I. *et al.* (2003) 'Antimicrobial activity of Paenibacillus peoriae strain NRRL BD-62 against a broad spectrum of phytopathogenic bacteria and fungi', *Journal of Applied Microbiology*, 95(5), pp. 1143–1151. doi: 10.1046/j.1365-2672.2003.02097.x.

Wellcome Trust (2014) *Internationally focused commission on antimicrobial resistance announced by PM*. Available at: https://wellcome.ac.uk/press-release/internationally-focused-commission-antimicrobial-resistance-announced-pm (Accessed: 23 August 2019).

Welschen, I. *et al.* (2004) 'Antibiotics for acute respiratory tract symptoms: Patients' expectations, GPs' management and patient satisfaction', *Family Practice*, 21(3), pp. 234–237. doi: 10.1093/fampra/cmh303.

Wilcox, M. H. *et al.* (2004) 'Long-term surveillance of cefotaxime and piperacillin-tazobactam prescribing and incidence of Clostridium difficile diarrhoea', *Journal of Antimicrobial Chemotherapy*, 54(1), pp. 168–172. doi: 10.1093/jac/dkh285.

Winchester, C. C. *et al.* (2009) 'Antibiotic prescribing and outcomes of lower respiratory tract infection in UK primary care', *Chest*, 135(5), pp. 1163–1172. doi: 10.1378/chest.07-2940.

Winterberg, H. (1898) 'Zur Methodik der Bakterienzählung', *Zeitschrift für Hygiene und Infectionskrankheiten*, 29(1), pp. 75–93. doi: 10.1007/BF02217377.

Wood, A. J. J. and Iseman, M. D. (1993) 'Treatment of Multidrug-Resistant Tuberculosis', *New England Journal of Medicine*, 329(11), pp. 784–791. doi: 10.1056/NEJM199309093291108.

Wood, D. E. and Salzberg, S. L. (2014) 'Kraken: Ultrafast metagenomic sequence classification using exact alignments', *Genome Biology*, 15(3), p. R46. doi: 10.1186/gb-2014-15-3-r46.

Woodside, A. G. (2010) 'Brand-consumer storytelling theory and research: Introduction to a Psychology & Marketing special issue', *Psychology and Marketing*, 27(6), pp. 531–540. doi: 10.1002/mar.20342.

World Health Organisation (2014) 'Antimicrobial resistance. Global report on surveillance', *World Health Organization*, 61(3), pp. 383–394. doi: 10.1007/s13312-014-0374-3.

World Health Organisation (2017) 'Antibiotic resistance', *World Health Organization*. Available at: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/ (Accessed: 21 March 2018).

World Health Organisation (2019) *Infectious Diseases.* Available at: https://www.who.int/topics/infectious_diseases/en/.

World Health Organisation (2020) *Tuberculosis*, *World Health Organization*. Available at: https://www.who.int/news-room/fact-sheets/detail/tuberculosis (Accessed: 27 March 2020).

Wright, G. D. (2014) 'Something old, something new: revisiting natural products in antibiotic drug discovery', *Canadian Journal of Microbiology*, 60(3), pp. 147–154. doi: 10.1139/cjm-2014-0063.

Wright, H. D. (1934) 'The preparation of nutrient agar with special reference to pneumococci, streptococci and other Gram-positive organisms', *The Journal of Pathology and Bacteriology*, 39(2), pp. 359–373. doi: 10.1002/path.1700390210.

Yamamotot, S. and Harayama, S. (2017) *P h y I og enet ic re la t ions h ips of Pseudornonas putida strains deduced from the nucleotide sequences of gyrB, rpoD and 16s rRNA genes*, *International Journal of Systematic Bacteriology*.

Yoon, J. H. *et al.* (2001) 'Sporosarcina aquimarina sp. nov., a bacterium isolated from seawater in Korea, and transfer of Bacillus globisporus (larkin and stokes 1967), Bacillus psychrophilus (Nakamura 1984) and Bacillus pasteurii (Chester 1898) to the genus Sporosarcina as Sporosa', *International Journal of Systematic and Evolutionary Microbiology*, 51(3), pp. 1079–1086. doi: 10.1099/00207713-51-3-1079.

Ziemert, N., Alanjary, M. and Weber, T. (2016) 'The evolution of genome mining in microbes-a review', *Natural Product Reports*. Royal Society of Chemistry, pp. 988–1005. doi: 10.1039/c6np00025h.

Zignol, M. *et al.* (2006) 'Global Incidence of Multidrug-Resistant Tuberculosis', *The Journal of Infectious Diseases*, 194(4), pp. 479–485. doi: 10.1086/505877.

Chapter 8 Appendices

Appendix A

Figure A-1. Search String for PubMed to identify Citizen Science publications in the field of Microbiology which produce scientific output. Based on search string presented by Kullenberg & Kasperowski (2016).

(TS=("Antibio*") NOT TS=("lantibiotic")) OR (TS=("Antifung*")) OR (TS=("Antimicro*")) OR (TS=("Archae*") NOT TS=("Archaeology")) OR (TS=("Bacteri*") NOT TS=("Bacteria" OR "pathogenic bacteria")) OR (TS=("Disease*") NOT TS=("Diseases" OR "disease management" OR "disease mapping" OR "disease risk" OR "disease spread" OR "cardiovascular disease" OR "Digital disease detection" OR "Emerging infectious disease" OR "infectious-disease" OR "infectious-diseases" OR "rare-disease diagnostics")) OR (TS=("Dust") NOT TS=("dust" OR "Heritage industry" OR "industrial biotechnology" OR "zodiacal dust")) OR (TS=("Fung*") NOT TS=("fungal" OR "fungal diversity" OR "fungal fruiting" OR "fungi" OR "fungicide" OR "ectomycorrhizal fungus" OR "amphibian chytrid fungus")) OR (TS=("Genetic*") NOT TS=("genetic algorithm" OR "genetic and reproductive technologies" OR "Genetic counselling" OR "genetic discoveries" OR "genetic diversity" OR "genetic privacy" OR "genetic research" OR "genetic science" OR "genetic species" OR "genetically modified organisms" OR "Genetically-modified" OR "genetically-modified insects" OR "genetically-modified mosquitos" OR "genetic-analysis" OR "geneticists" OR "genetic-modification" OR "genetics" OR "genetics and ethics" OR "human genetic research" OR "human-genetics" OR "phylogenetic diversity" OR "Plant genetic resources" OR "population genetics" OR "population genetic-structure" OR "psychiatric genetics" OR "public health genetics")) OR (TS=("Genom*") NOT TS=("genome" OR "genome interpretation" OR "genome survey sequencing" OR "genomic" OR "genomics" OR "human genome project" OR "human-genome-project" OR "Personal Genome Project" OR "personal genomics" OR "plant genomics" OR "whole-genome sequencing" OR "direct-to-consumer genomics")) OR (TS=("Infect*") NOT TS=("infectious-disease" OR "infectious-diseases" OR "zoonotic infections" OR "Emerging infectious disease")) OR (TS=("Microbi*") NOT TS=("microbial diversity" OR "microbiome" OR "Biotechnology & Applied Microbiology" OR "human microbiome" OR "Indoor microbiology")) OR (TS=("Microorganism*")) OR (TS=("Microsco*") NOT TS=("microscopy" OR "automated electron microscopy")) OR (TS=("Mycobacteri*")) OR (TS=("Pathogen*") NOT TS=("pathogen" OR "pathogen transmission" OR "pathogenic bacteria" OR "pathogens")) OR (TS=("Phage*")) OR (TS=("Pharmac*") NOT TS=("pharmaceuticals" OR "pharmacogenomics" OR "Pharmacology & Pharmacy")) OR (TS=("Phytopathology")) OR (TS=("Protist*")) OR (TS=("Protozo*")) OR (TS=("Resistan*") NOT TS=("resistance habitus" OR "resistant staphylococcus-aureus")) OR (TS=("Tuberculo*") NOT TS=("Tuberculosis" OR "tuberculosis complex")) OR (TS=("Vira")) OR (TS=("Viro*") NOT TS=("Built environment" OR "deliberative environmental democracy" OR "environment" OR "environmental" OR "Environmental communication" OR "environmental exposure" OR "environmental health" OR "environmental intervention" OR "environmental justice" OR "environmental ngos" OR "environmental radioactivity" OR "environmental risk analysis" OR "environmental-health" OR "environmental-policy" OR "Public, Environmental & Occupational Health")) OR (TS=("Viru*") NOT TS=("virulence" OR "Virunga Massif" OR "virus transmission" OR "west-nile-virus")) OR (TS=("Mycoplasma gallisepticum")) OR (TS=("capodacus-mexicanus")) OR (TS=("Veterinary Sciences"))

Appendix B

TableB-1. Logic model. Maps inputs (what is put into project), activities (things somebody will do), outputs (what comes out of the project) and outcomes (measurable outcome/impact) over the course of the project. These were considered for citizen scientists and the research team. I mapped my expected outcomes for the citizen science project to the framework for evaluating citizen science learning outcomes (Phillips *et al.*, 2014).

		Inputs	Activities	Outputs	Short-Term Outcomes	Medium-Term Outcomes	Long-Term Impacts
Audience	**Participants (Citizen Scientists)**	• Time • Access to internet • Private landowner access or access to a forest • Baseline Interest/Motivation and confidence • Camera/phone	• Understand background material for project • Collect Data (soil sample) • Engage in training • Learn and understand project protocol • Make/log/detail observations of collection site • View/explore data	• Amount of volunteer collected data • Publicly accessible data • Data visualisation tools • Number of individuals engaged with program • Number of individual hours engaged. • Number if individual hours or total effort? • Personal data accounts	• Increase public access to scientific enterprise • Increased confidence to engage with science and the environment • Increased interest in science and the environment	• Improved knowledge of science content and process • Improved data collection an interpretation skills • Increased appreciation for science and the environment • Serve as project	• Increased public support of science • Improved science-society relationship • Better informed citizenry • Enhanced scientific and environmental literacy • Increased conservation of natural resources • Healthier communities

		• Communicate with other citizen scientists • Provide feedback to project staff • Communicate project findings to wide audience		• Increased motivation to do and learn science	ambassadors to promote the project • Increased environmental activism • Scientific recognition of value of volunteer collected observation data • Increased social capital/community capacity

Program (Me Supervisory Team, MicSoc)	• Funding sources • Expertise • Lab Resources • Designing protocol, • Research topic, • Questions we will ask • Protocol • Evaluation form for participants • Equipment to collect sample • Website with data uploaded • Twitter/forum for discussion • Tools or logging information/development (diary). • Leaflets/information on background of subject	• Develop project design, protocol, educational materials • Recruit participants • Provide training and support to volunteers • Implement and operate project • Analyse volunteer collected data • Communicate project findings to wide audience • Network with partner organisations	• Interactive website • Data quality/data assurance filters • Reference data set for scientific community • Number of training and workshops offered • Number of sites or acreage monitored • Data tools for managing data rewards • Increased exposure of project to wider audience • Peer review publications/reports/meetings	• Increased knowledge of natural systems • Collection and central organisation of scientific data • Increased utility and accessibility of data • Peer-reviewed publications, reports, meetings	• Enable and inform conservation actions • Program sustainability • Increased knowledge of best practices for operating citizen science projects • Improved relationships between project and **local** community	• Policy initiatives that address environmental issues

Appendix C

Table C-1. Media composition.

Media	Description
Luria-Bertani (LB) Broth (g L^{-1}) 10 Sodium chloride 10 Tryptone 5 Yeast extract	(BERTANI, 1951) Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving.
Luria-Bertani (LB) Agar (g L^{-1}) 10 Sodium chloride 10 Tryptone 5 Yeast extract 1.5% (w/v) Agar	(BERTANI, 1951) Prepare LB broth as described. Take 200 mL of LB and add 3 g of agar. Sterilise by autoclaving. NB. For indicator strain plates, increase agar to 4%, 12 g per 200 mL.
Nutrient Agar (NA) (g L^{-1}`) 6 Peptone 5 Sodium chloride 2 Yeast extract 1 Beef extract 1.5% (w/v) agar	(Wright, 1934) Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving.
Tryptic Soy Agar (TSA) (g L^{-1}) 15 Casein peptone (pancreatic) 5 Sodium chloride 5 Soya peptone (papainic) 1.5% (w/v) agar	(TSB; Sigma Aldrich) Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving.
Soy Flour Mannitol (SFM) Agar (g L^{-1}) 20 Soya flour 20 Mannitol 2% (w/v) agar	(Kieser *et al.*, 2000) Dissolve mannitol in ~800 mL dH_2O, add Soya flour and Agar. Make up to 1 L with dH_2O and sterilise by autoclaving.
Technical agar with Calcium chloride (TA + $CaCl_2$) (g L^{-1}) 1.5% (w/v) Oxoid™ agar technical 8.82 Calcium chloride dihydrate	(TSB; Sigma Aldrich) Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving. To add the Calcium chloride, add 1.47 g of $CaCl_2$ dihydrate to 10 mL dH_2O, sterile filter through a 0.22 nm filter disc. Add 60 µL of this to 1 L.
All Culture (AC) Broth (g L^{-1})	(TSB; Sigma Aldrich)

20 Protease peptone 3 Beef extract 3 Yeast extract 3 Malt extract 5 Dextrose 0.2 Ascorbic acid	Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving.
All Culture (AC) Agar (g L^{-1}) 20 Protease peptone 3 Beef extract 3 Yeast extract 3 Malt extract 5 Dextrose 0.2 Ascorbic acid 1.5% (w/v) Agar	(TSB; Sigma Aldrich) Prepare AC broth as described. Take 200 mL of AC and add 3 g of agar. Sterilise by autoclaving. NB. For indicator strain plates, increase agar to 4%, 12 g per 200 mL.
Brain Heart Infusion (BHI) Broth (g L^{-1}) 12.5 Calf brain 10 Peptone 5 Beef heart 5 Sodium chloride 2.5 Disodium hydrogen phosphate 2 Dextrose	(TSB; Sigma Aldrich) Dissolve in ~800 mL dH_2O. Make up to 1 L with dH_2O and sterilise by autoclaving.
Brain Heart Infusion (BHI) Broth (g L^{-1}) 12.5 Calf brain 10 Peptone 5 Beef heart 5 Sodium chloride 2.5 Disodium hydrogen phosphate 2 Dextrose 1.5% (w/v) Agar	(TSB; Sigma Aldrich) Prepare BHI broth as described. Take 200 mL of BHI and add 3 g of agar. Sterilise by autoclaving. NB. For indicator strain plates, increase agar to 4%, 12 g per 200 mL.
Soil Extract Media 100g topsoil 300 mL MOPS buffer 1.5% (w/v) Agar	(Adapted from Liebeke et al., 2009) 100 g of air-dried topsoil suspended in 300 mL of sterile MOPS buffer (10 mM, pH 7.0) at 37 °C with shaking at 200 rpm for 1 h. Extract filtered sequentially through filter paper (Whatman) and filters with a pore size of 5 and 0.45 µM in order to remove particulate matter. Directly after preparation, the extract was filtered to sterility using a 0.22-µm pore size and stored at 4 °C until use.
dH_2O SEM 100g topsoil	(Adapted from Liebeke et al., 2009)

300 mL dH_2O 1.5% (w/v) Agar	300 mL MOPS replaced with 300 mL dH_2O as homogenising diluent.
Autoclaved dH_2O SEM 100g topsoil 300 mL dH_2O 1.5% (w/v) Agar	(Adapted from Liebeke et al., 2009) 300 mL MOPS replaced with 300 mL dH_2O. Once particles removed via sequential filtering, autoclave rather than use sterile filter.
Concentrated dH_2O SEM 100g topsoil 100 mL dH_2O 1.5% (w/v) Agar	(Adapted from Liebeke et al., 2009) MOPS replaced with dH_2O. 1:3 soil to water ratio changed to 1:1 before filtration. Once filtered, add 200 mL dH_2O to reach equivalent media volume.

Appendix D

Table D-1. Antifungals. All antifungals were aliquoted in 1 mL fractions and stored at -20°C. Nystatin was made in DMSO, rather than dH_2O.

Antifungal	Stock Concentration ($mg\ mL^{-1}$)	Final Concentration ($\mu g\ mL^{-1}$)
Cycloheximide	100	50
Nystatin	5	20

Appendix E

Figure E-1. Factual Summary for Interview 32, interviewees 32a and 32b. After reading the transcript of the interview twice, I compiled a factual summary of the parts of the interview which I considered key. This was done for all interviews.

The child (32b) knew that there were bacteria in the soil from which antibiotics can be found and knew antibiotics were made by bacteria and fungi. They pointed out that humans can use bacteria and fungi to make antibiotics and answered yes to plants incorrectly. They did say no to viruses. They knew bacterial infections were treatable by antibiotics, and only bacterial. They thought that discovery was recent and had an optimistic guess about how many we had taken to market. They knew that resistance is a property of an antibiotic losing effectiveness treating bacteria and attributed this to overuse. The parent (32a) knew that we would go back to pre-world war standards of medicine and mentioned the idea of being pricked by a rose. Interestingly they pointed out the idea that this issue is known but might be at the back of people's minds. The child focused mainly on environmental spread of resistant bacteria between humans but did touch on the idea that bacteria multiply. The parent used a specific story from the radio, where flies would carry resistant bacteria from faeces of contaminated animals. The child targeted non-serious diseases that would become more serious as the biggest problem. They focused on taking the full course and unnecessary use where the immune system could do the job as ways of making a difference. In terms of use, the child went for prophylactic use in chickens. Regarding misuse, they focused on this prophylactic use in animals also, but were unsure whether this is unnecessary. After some discussion, they decided this was misuse as it leads to resistance. They also then mentioned over-prescription in hospitals.

In the post-interview discussion, the child wanted to know whether we can treat MRSA, and the parent wanted to know what leads to bacteria becoming untreatable in the first place and how long this takes. An expression was how something as tiny as a bacterium could be so sophisticated. The parent used an interesting 'carrots won't cook in warm water' metaphor for 'bacteria not dying in suboptimal antibiotic doses'. The parent also wanted to know whether the bacteria we use to make antibiotics could mutate and become harmful. Discussion about irresponsible others lead to a discussion about other countries like China and India, but not Australia having less controls over antibiotic dosing. The problem of antibiotic resistance was then called 'analogous to climate change' in that we know there is a problem, but it is always someone else's. The participant believed making the switch to accepting personal responsibility and understanding the personal inconvenience that could be caused might help. A special quote from the parent was 'I would say I was a fairly sort of conscious person around the issue, until it really bites I suppose' (on giving son medicine if sick, even if worse for overall community). The parent had also seen a documentary about doctors feeling pressure to prescribe. The parent also mentioned the irresponsibility of the "vaccines cause autism" paper and the long-lasting effect/irresponsibility that has. Parent says that science works to dispute the consensus and to challenge it (disprove), based on 'evidentiary'.

Appendix F

Table F-1. Chronological account of types of content released to Antibiotics Unearthed Facebook Page. Table shows the number of each of the 47 posts released as part of Antibiotics Unearthed social media engagement. The date of release of each post is shown, as well as the type of media that the post represented. Web links would take users to a page, such as a news article. Event links specifically advertised upcoming Antibiotics Unearthed pop-up stand events. Images provided visual aids to discussion. Videos linked to relevant media from YouTube. Photo refers to a change of cover photo. Sample Images were images of data emerging from the laboratory, labelled so users could identify their own soil samples.

Post Number	Date of Post	Media Type	Media Type Code
1	05/07/2016	Web Link to the Antibiotics Unearthed Webpages	A
2	18/07/2016	Web Link to news event about the pop up stands to take place at Garwnant Visitor Centre in the Brecon Beacons, and Kielder Castle	A
3	25/07/2016	Event Link to the Pop Up Event taking place at Garwnant Forest	B
4	10/08/2016	Photograph of a participants attending the Pop Up Event at Garwnant Forest	C
5	16/08/2016	Link to a BBC News article that discus finding a Colicin Resistant Bacteria found in Scotland https://www.bbc.co.uk/news/uk-scotland-36962781?fbclid=IwAR2BPBLEwWCSZdpVCJFhVZVMPhAEgfxzT2Jobm6L07CGo9gC91_lV05XiMY	A
6	18/08/2016	Video about Phage created by the Microbiology Society Link to Microbiology Today society magazine	D
7/8	23/08/2016	Web Link and Photo New Image to represent Antibiotics Unearthed project and link to website	A/E
9/10	24/08/2016	Image and Sample Images Photo images of agar plates streaked from soil samples brought in by participants who attended the Pop up event at Garwnant Forest and Kielder Forest during summer 2016 In addition, Colony Morphology Image	C/F
11	22/09/2016	Web Link Link to a News Article about United Nations signing a landmark declaration to fight the global challenge of antibiotic resistance	A
12	23/09/2016	Sample Images Photo images of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Pop up event at Garwnant Forest and Kielder Forest during summer 2016	F
13	04/10/2016	Web Link Link to News Story about The Microbiology Society trip to the Ministerial Side-Event on AMR held in New York to hear about the global commitment in tackling the issue https://microbepost.org/2016/10/04/nations-and-leaders-commit-to-tackle-antimicrobial-resistance/?fbclid=IwAR2b-hbVFv_rqg_sWLD6wH9ySghJfFqFBhGwZErIv4gEVcNwy9EhEHgIXrk	A
14	03/11/2016	Web Link Link to an interactive microscope created by the Microbiology Society https://microbiologyonline.org/students/microbe-passports-1?fbclid=IwAR3aSjgXqIPRXvI2UqoVM7k6g3OhjY01zcnFNDvWqptmwLIVhgI4HGOpmLI	A
15	14/11/2016	Web Link Microbiology Society Fact sheet about Antibiotic Resistance released as part of Antibiotic Awareness Week https://microbiologyonline.org/file/e610e1e6da2ebcfe7ae85d59057ec91f.pdf?fbclid=IwAR372heac4VzIK9UK8dgIFne9QVQzebPcfxi1wmnze-D5AHRp0l7RMcogRQ	A
16	16/11/2016	Video Link to You Tube animation about Antibiotic Resistance https://www.facebook.com/MicrobiologySociety/videos/10153929663711078/UzpfSTEyNzY1NzQ0MDkwOTcyMjozNzg2NjQ5NDkxNDIzMDI/	D
17	23/11/2016	Sample Images Photo images of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop up event at Garwnant Forest and Kielder Forest during summer 2016	F

18/19	21/12/2016	Sample Images and Web Link Advert for a talk at Nottingham University about the recently launched O'Neil Review on Antimicrobial Resistance.	A/F
20	09/01/2017	Sample Images Two photographs of agar plates from Samples taken the 2016 pop-up events at Garwnant Forest and Kielder Forest	F
21	12/01/2017	Web Link News article about spider silk being used to augment antibiotic-releasing bandages https://microbepost.org/2017/01/12/antibiotic-spider-silk-that-can-heal-wounds/?fbclid=IwAR3XlKairJKIGF8xhMXtPjU5TPRxIykeGXaTwAoJF12Kb73woa62EVIwVB4	A
22	23/01/2017	Web Link News article about a moth gut bacterium the defends its host by making antibiotics https://www.sciencedaily.com/releases/2017/01/170119134628.htm?fbclid=IwAR0q7If_HvpJjA-WI7cWclhPlAzMc_BEUAEGRJCzkViW6oaWAOeRnF133OI	A
23	26/01/2017	Web Link Link to BBSRC highlighting a research call that address AMR in agriculture Link is no longer active	A
24	09/02/2017	Web Link News article about scientists showing that currently available antibiotics can still stop resistant bacteria – by exerting enough force. https://www.ucl.ac.uk/news/2017/feb/brute-force-can-overcome-antibiotic-resistance?fbclid=IwAR3vKCPhDmQ2AhFVH0ck5wK4axNk2dQAhPdwplQDdK676KiUUG2td0rHJ1k	A
25	22/02/2017	Web Link News article from UKRI about uncovering the molecular mechanisms that make multidrug-resistant *Klebsiella pneumoniae* so resistant to antibiotics Link is no longer active.	A
26	27/02/2017	Web Link NICE article:- Children and young people should be taught simple hygiene measures to curb the spread of infections. http://indepth.nice.org.uk/children-and-young-people-should-be-taught-simple-hygiene-measures-to-curb-the-spread-of-infections-says-nice/index.html?fbclid=IwAR3ISLOJndbE9pi-Vxx8jZmqFWWHOsnQVL5Nv3vnoR_TakGcIMzohY-5Fmk	A
27	03/03/2017	Web Link Announcement of The BBC Radio 4 drama called Resistance a three-part drama series about an outbreak of antibiotic resistance. https://microbepost.org/2017/03/03/review-resistance-on-bbc-radio-4/?fbclid=IwAR2owKC0KdlISHUKnW9KqUIGvhlfVSQSY50PxyPKeZjxi5a4LplE9NrqDVA	A
28	24/03/2017	Web Link School Pupil Winners of Antibiotic Unearthed Schools programme. http://www.utcoxfordshire.org.uk/winners-of-antibiotics-unearthed-announced/?fbclid=IwAR0yUkcydVg2hlp3DuSeR4upDBFNi-eOmGp8a2V5jSoi0oahTLghncvFVOg	A
29	05/04/2017	Image Agar plate demonstrating the microbial handprint of an eight and a half year old boy after he'd been playing outside	C
30/31	16/05/2017	Sample Images and Event Link Photo images of agar plates streaked from soil samples brought in by participants who attended the Glasgow Botanic Garden Pop up event that took place in May	F
32	30/05/2017	Sample Images Photo images of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Thetford Forest pop up event in May	F
33	27/06/2017	Sample Images Photo images of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop up event at Thetford Forest in May.	F
34	31/07/2017	Web Link	A

		Lespar comment on the BMJ article that discusses how long people should take a course of antibiotics for. https://microbiologysociety.org/news/society-news/lespar-comment-on-the-bmj-article-on-antibiotic-course-duration.html?fbclid=IwAR2lCFLFmThfyfWn5cCCO7P5Yvqm1ZTuQazoByYthXNESRzDwsgUZzQJ9Lo	
35	10/08/2017	Sample Images Photo images of species identified by Ethan from soil samples brought to the pop up event at Thetford Forest that took place in July. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected.	F
36	19/09/2017	Web Link Article about the up and coming launch of the Microbiology Society new microbiome-themed colouring book, that became available to pre-order	A
37	02/10/2017	Sample Images Photo images of agar plates streaked from soil samples brought in by participants who attended the Glasgow Botanic Garden Pop up event that took place in September	F
38/39	10/10/2017	Web Link and Sample Images Photo images of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples found by participants that attended the Glasgow Botanic Garden pop up event. Article about the launch of the Microbiology Society new microbiome-themed colouring book, that became available to pre-order	A/F
40	06/11/2017	Sample Images Photo images of agar plates streaked from soil samples brought in by participants for the Norwich Science Festival event that took place in October 2017.	F
41	09/11/2017	Sample Images Photo images of zones of inhibition gathered from bacterial species purified from soil samples brought to the Pop up event at Glasgow Botanic Gardens.	F
42	15/11/2017	Image Infographic from the WHO that explains the antibiotic resistance cycle. Published in Antibiotic Awareness week.	C
43	20/11/2017	Sample Images Photo images of agar plates, lab-grown by Ethan at the University of East Anglia, using the brain-heart infusion media using samples brought in by participants to the Norwich Science Festival pop-up event in October 2017.	F
44	05/12/2017	Sample Images Photo images of species identified by Ethan from soil samples brought to the pop up event at Glasgow Botanic Garden that took place in September. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected.	F
45	06/12/2017	Sample Images Photo images of zones of inhibition gathered from bacterial species purified from soil samples brought to the Norwich Science Festival.	F
46	15/12/2017	Sample Images Photo images of species identified by Ethan from soil samples brought to the Norwich Science Festival. Post provided information gathered from sequencing to identify the groups of bacteria that were found in the various soils collected.	F
47	21/02/2018	Web Link BBC news article: New Family of Antibiotics found in Dirt. https://www.bbc.co.uk/news/health-43032602?fbclid=IwAR1a_uWpj7ZHgzVIO3qowO77RkUPUIDTds7LS81_xVncbpgxA_e1rDekOy8	A

Appendix G

Figure G-1. Participant portfolio handbook. Handbook was created in collaboration with the supervisor team to explain the purpose of the study and provide examples of ways participants could contributed. This also laid out the suggested template for making entries, including adding dates which assisted with later analysis. It also provided instructions for returning the portfolio and contact details in case questions arose.

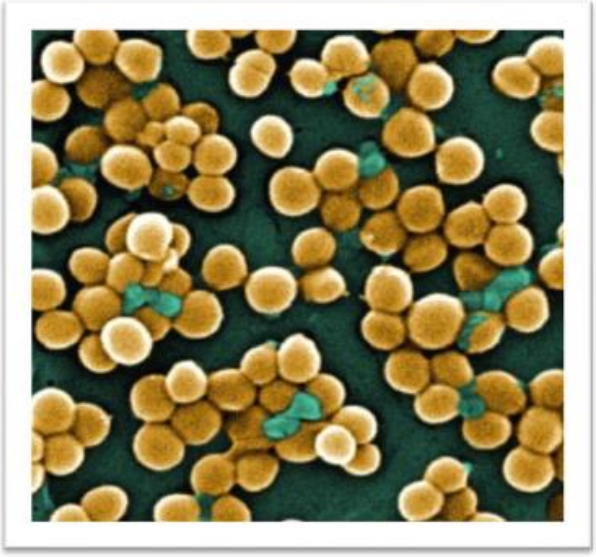
Antibiotics and Antibiotic Resistance Portfolio.

This portfolio is designed to capture your experiences of antibiotics and antibiotic resistance. We want to understand your views on antibiotics and antibiotic resistance and how you come in to contact with these themes on a day to day basis. By contributing your time to this portfolio, you will be potentially shaping future campaigns based on the preservation of current antibiotics in the hope of tackling the global problem of antibiotic resistance.

There are many ways you can contribute and you are welcome to do so in any way you feel able to demonstrate your experiences. We offer a few suggestions below in case you need help to get started.

To begin with, there is a place for you to fill in your details, attached to the inside cover. We have also included a **survey**; filling this in as best you can will help us when it comes to understanding and appreciating your views.

IMPORTANT: Please write the date each time you make an entry.



This is a photograph of a bacteria that you may have heard of? It is MRSA, a bacterium that can cause nasty infections when it gets into wounds. This bacterium is resistant to several antibiotics including Penicillin and Methicillin (Methicillin Resistant *Staphylococcus Aureus*). *Figure permission from the centre of disease control.*

Types of experience you may want to tell us about

To start, you may want to reflect on your time in the forest. We would love to hear your thoughts.

What are your thoughts on our Forest Event?

1. Did you learn anything new during your time at the stand?

2. Did you have any questions you didn't get to ask or that have come up since?

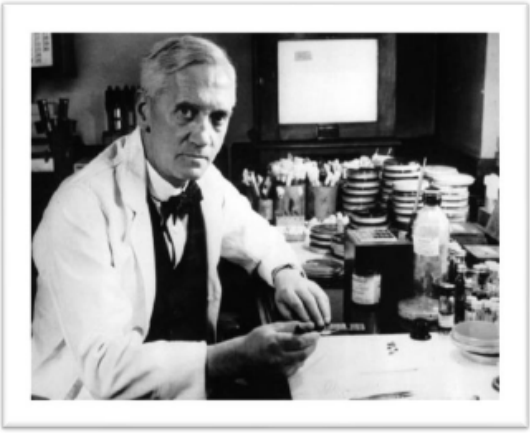
3. Is there anything that particularly sticks in your mind that you have learned?

You may wish to use a few pages to research some questions that we often ask. For those of you who took part in the interviews, these may sound familiar. These include:

Your Research:

- What is in your medicine cabinet?
- What are most antibiotics made by?
- What kind of infections do you think antibiotics combat?
- What is the difference between a bacterial, a fungal and a viral infection?
- What is Teixobactin?
- What is antibiotic resistance?
- Is antibiotic resistance a problem in bacteria, viruses, fungi, humans or plants?
- What type of infection is cold and flu?
- How does antibiotic resistance spread?
- What is the cause of antibiotic resistance?
- What is the risk to humans if antibiotic resistance spreads?
- Can you make a difference to the spread of antibiotic resistance?
- How do we use/misuse antibiotics?

Personal experiences and views:



You may wish to include your own personal experiences instead of - or as well as - responding to these questions. This may include thoughts about:

- What does a bacterium look like/is?
- Yours or other people's use of antibiotics: have they worked?
- Are you on a course of antibiotics? If yes, describe.

Alexander Fleming

Over the next few months, up until the end of September, you may notice bits and pieces that are related to antibiotics and antibiotic resistance. We would love you to tell us when this happens. This maybe in the form of:

Media, Propaganda and Advertising:

- Online news and reports
- Newspapers
- Radio
- Television
- Doctors leaflets

Thoughts on Facebook posts from us:

We post all our experiments on Facebook and you can track your own sample there using the sample number you received in the forest. If you do, you could let us know your thoughts:

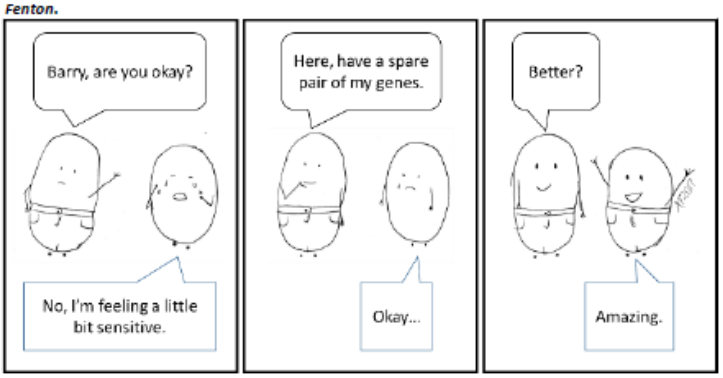
- Did reading the Facebook posts, seeing the images or receiving the explanatory emails raise any questions, teach you something new or reinforce something you already understood?
- Perhaps you want to print and annotate what you are seeing or redraw what you see with annotations/questions.
- Do you want to know more?
- https://www.facebook.com/antibioticsunearthed

Don't forget, you can put anything you like in this portfolio. We hope that below we provide a few ideas!

Ways in which you can tell us about your experiences

Visual means and artwork: Drawings/Paintings/Photographs/Songs/Poems/Graphic novel/ Animation

Barry the Bacterium Combats Low levels of Antibiotics by Borrowing Resistance Genes *by Ashley Fenton*.



Written: Blog/Diary/Reflection/Brainstorm

We encourage you to capture your own thoughts or experiences relating to antibiotics and antibiotic resistance in any way that you deem convenient or natural to you. There is no right or wrong way to do it! The example here shows how you may want to draw a comic style conversation to depict how bacteria share resistance genes to allow them to survive against the use of antibiotics.

Social Media:

If you want to show us what you are doing as you fill things in, please feel free to take pictures and use the #AntibioticsUnearthed on twitter or post on the Facebook page, https://www.facebook.com/antibioticsunearthed

How to return the Portfolio so that we can make best use of your efforts:

Once you have started filling in the portfolio, we will send you an email requesting your address so that we can send you a stamped address envelope which you can put the portfolio inside. Once we receive the portfolio back from you, we can capture your experiences and use it to influence campaigns going forward. Anything you include in this portfolio will be completely anonymous and confidential. Following this process, we will send the portfolio straight back to you with a gift, as a thank you for all the time and effort you have put in to helping us and helping shape one part of the future of antimicrobial resistance.

Questions or Concerns:

If you have any questions or concerns that emerge as you begin to fill in the portfolio that you want to ask, please do not hesitate to get in touch.

You can email me:

E.drury@uea.ac.uk

Message me on twitter:

@EthanMMB with the optional #AntibioticsUnearthed

Contact me and the rest of the team on the AntibioticsUnearthed Facebook Page:

https://www.facebook.com/antibioticsunearthed

Thank you:

I hope that the valuable time you are giving to help with my doctoral project is a worthy and enjoyable learning experience. I thank you warmly for your support in the global fight against antimicrobial resistance!

Ethan Drury

Ethan Drury

Appendix H

Figure H-1. Factual Summary for Portfolio Participant 3 (NSF01). After reading the transcript of the portfolio twice, I compiled a factual summary of the parts of the portfolio which I considered key. This was done for all portfolios.

PP3 NSF 01 (1,087 words, 9 pages)

'NSF01' began by noting they had seen nothing in the news about antibiotics since our meeting. They went on to describe what they thought they knew about antibiotics and what they didn't know (nothing that they didn't really care to know). They discussed their upcoming hip replacement op and the advice regarding antibacterial soap use prior to this. They repeated information given at the event, that antibiotics are found in the soil. They also described the contents of their medicine cabinet. They mentioned a Guardian article discussing the microbiome and wondered whether a prescribed diet post-antibiotic treatment could help re-establish the gut microbiome. They created a spider diagram which discussed the benefits of organic food production, something they go on to mention several more times in the portfolio. They discussed the lack of antibiotics prescribed after the hip operation was finished. They wondered whether poorer people would struggle more with resistance as they can't afford to eat as well. They also wondered whether lack of sleep contributes to resistance based on a link with the microbiome. They mentioned that they originally thought antibiotics were grown on cheese and bread. Following on from soil antibiotics, they wondered whether their chickens could become resistant to antibiotics and pass this on to them through their eggs. A discussion with a retired GP friend bought up topics such as tightening on antibiotic prescription and focus away from completing course. The GP also mentioned the habitual use of antibiotics in food production being more substantial that onus on patients. They discussed a report on the link between a sugar food additive and C diff. They then took the stance that we should not eat processed food, repeating their fondness for organic food. They wondered whether their diet or their genes were responsible for not needing antibiotics in over 10 years. They watched a play about a future with one antibiotic and no resistance left which scared them, whilst also discussing how the term incentivisation is patronising as they already listen to and follow advice. They wondered whether this was aimed at poorer and less educated individuals. They attempted to listen to the infinite monkey cage on radio 4, heard about resistant Gonorrhoea and Phages, but gave up as this was too difficult to follow. They noticed on the BBC website the discovery of malacidins in dirt. They also noticed the discovery of 'blackberry antibiotics' by BT Young Scientist. They were notably pleased about these discoveries. They were similarly excited about finding a new supplier of raw milk organic kefir, which they attributed to looking after your gut bacteria. They wondered if it is possible to have resistance to a disease they've not had before or to an antibiotic that they've never taken. They thanked me for allowing them to take part as it heightened their awareness for antibiotic items, and they learned a lot. They also hoped I would find something interesting amongst their scribbles.

Appendix I

Figure I-1. Ethics Approval Form. Ethics application form from East Anglia's Faculty of Education's Research Ethics Committee approving the collection of participant interview, social media and portfolio data as of the 11th July 2016.

EDU ETHICS APPLICATION FEEDBACK 2016-2017

APPLICANT DETAILS	
Name:	Ethan Drury
School:	MED (Jointly supervised with BIO and EDU)
Current Status:	**PGR Student**
UEA Email address:	**Jcj15dzu@uea.ac.uk**

EDU Recommendation	
Approved, data collection can begin	✓
Minor revisions/further details required (see feedback below)	
Not Approved, resubmission required (see feedback below)	

EDU REC feedback to applicant: Chair review date 11.7.16……..

Comments: thank you for sending your response to my query regarding the use (or not) of video material. As you have made the decision to no longer include that as a data source full approval can now be given. Good luck with the project

Action required by applicant:

Ethical approval has now been given:	

Signed: Kate Russell EDU Chair, Research Ethics Committee

Appendix J

Figure J-1. Participant Information Statement. A participant information statement was provided to each participant prior to interview data collection at the pop-up stands. It explained details of the study, how long it would take and rights to withdrawal.

University of East Anglia

Using Citizen Science to explore antibiotic discovery

PARTICIPANT INFORMATION STATEMENT – Ethan Drury

(1) What is this study about?

You are invited to take part in a research study about your views on the use of antibiotics. This Participant Information Statement is designed to tell you what is involved and will help you decide if you want to take part in the study. This Participant Information Statement tells you about the research study. We will be happy to answer any questions you may have after reading this sheet. Participation in this project is voluntary. By giving consent to take part in this study you are telling us that you:

- ✓ Understand what you have read.
- ✓ Agree to take part in the research study as outlined below.
- ✓ Agree to the use of your personal information as described.

(2) Who is running the study?

The study is being carried out by the following researchers: Ethan Drury, PhD Student with supervision from Dr Laura Bowater and Prof Elena Nardi, School of Education and Lifelong Learning, University of East Anglia.

(3) What will the study involve for me?

You will be asked to submit a soil sample collected from the Forest, complete an anonymous survey that will ask you questions about your views on antibiotics and their use. Video recording will be taking place to use as observational data.

(4) How much of my time will the study take?

It is expected that the survey will take less than 10 minutes to complete.

(5) Do I have to be in the study? Can I withdraw from the study once I've started?

Being in this study is completely voluntary and you do not have to take part. Of course, if you decide to take part in the study (donate a soil sample) and then change your mind, you are free to withdraw your information at any time. Once you have submitted your questionnaire data however, and if you agree to being audio-recorded at the stand, your responses cannot be withdrawn because they will be anonymous and therefore we will not be able to tell which one is yours.

(6) Are there any risks or costs associated with being in the study?

Aside from giving up your time, we do not expect that there will be any risks or costs associated with taking part in this study.

(7) Are there any benefits associated with being in the study?

Your responses are likely to provide details about the way that antibiotics are used in our society and your views on their usefulness. It may also help to identify any potential issues or barriers to your use of these resources. Observational data will be useful to qualify our survey data and provide information to the forests.

(8) What will happen to information about me that is collected during the study?

By providing your consent, you are agreeing to us collecting personal information about you for the purposes of this research study. Your information will only be used for the purposes outlined in this Participant Information Statement, unless you consent otherwise. Data management will follow the 1998 Data Protection Act and the University of East Anglia Research Data Management Policy (2013). Your information will be stored securely and your identity/information will be kept strictly confidential, except as required by law. Study findings may be published, but

you will not be identified in these publications if you decide to participate in this study. In this instance, data will be stored for a period of 10 years and then destroyed.

(9) What if I would like further information about the study?
When you have read this information, Laura will be available to discuss it with you further and answer any questions you may have. You can contact her on Laura.bowater@uea.ac.uk.

(10) Will I be told the results of the study?
We would be delighted to share the overall results of this study with you. As this is an anonymous questionnaire we do not want you to provide us with your contact details but we will be posting our findings on this website www.???.co.uk [TBD] following the end of the project (September 2018). Of course, you can tell us that you wish to receive more personalised feedback by ticking the relevant box on the consent form and providing an email address.

(11) What if I have a complaint or any concerns about the study?
The ethical aspects of this study have been approved under the regulations of the University of East Anglia's School of Education and Lifelong Learning Research Ethics Committee. If there is a problem please let me know. You can contact me via the University at the following address:
Dr Laura Bowater
Norwich Medical School
University of East Anglia
NORWICH NR4 7TJ
Laura.bowater@uea.ac.uk

If you are concerned about the way this study is being conducted or you wish to make a complaint to someone independent from the study, please contact please contact the Head of the Norwich Medical School, Prof M. Frenneaux, at M.Frenneaux@uea.ac.uk

(12) OK, I want to take part – what do I do next?
Please keep the letter, information sheet and the 2nd copy of the consent form for your information.

This information sheet is for you to keep

Appendix K

Figure K-1. Participant Interview, Soil Sample and Social Media Consent Form. Developed with the Microbiology Society, the consent form notes Sample Number and Name, as well as Email Address as a signature. The participant could agree to any or all parts.

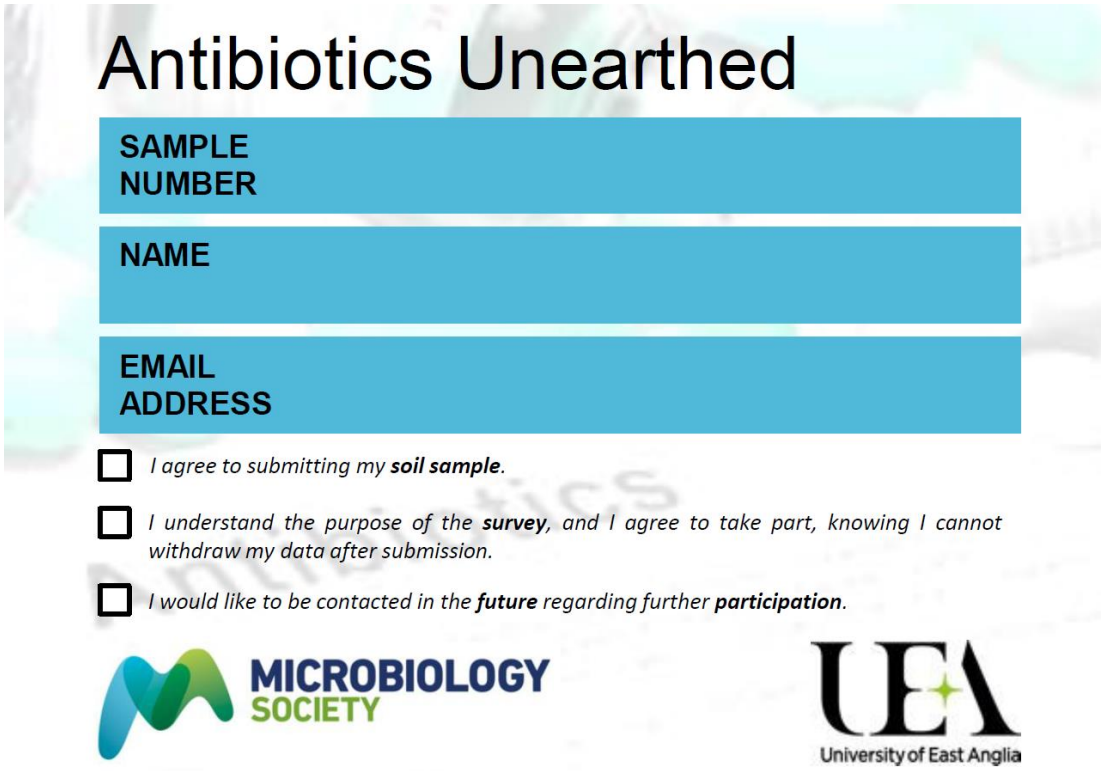
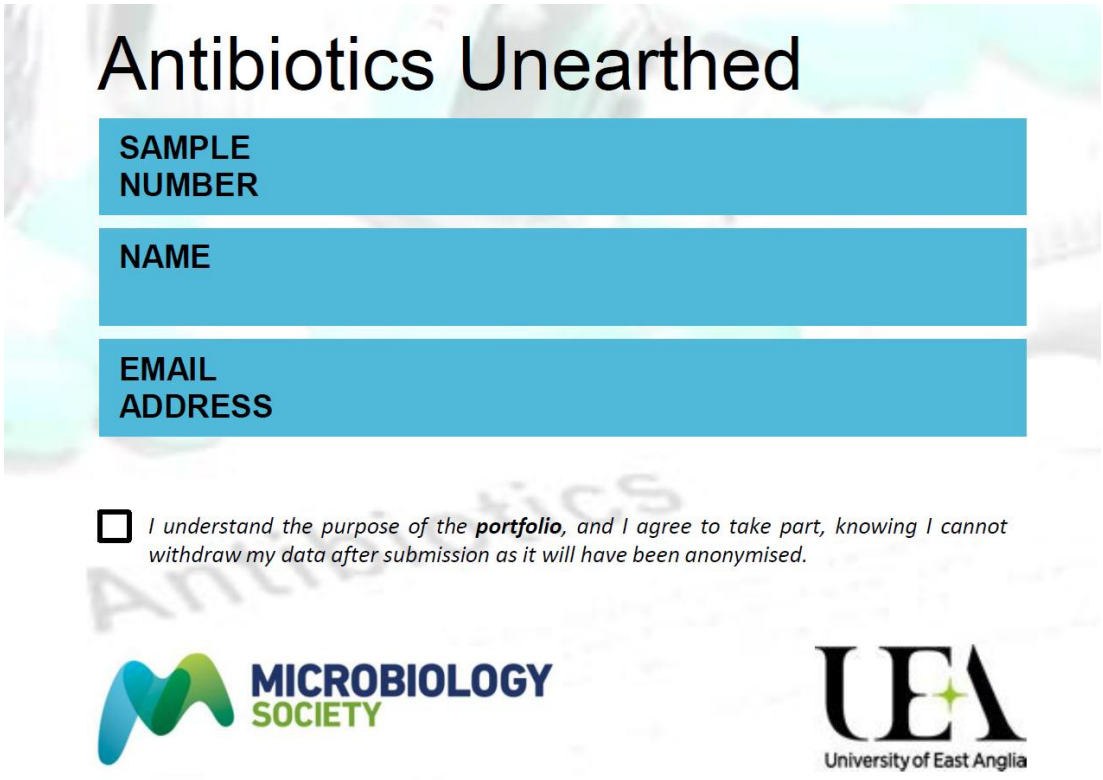


Figure K-2. Participant Portfolio Consent Form. Developed with the Microbiology Society, the consent form notes Sample Number and Name, as well as Email Address as a signature.



Appendix L

Table L-1. Norwich Science Festival antagonistic isolates stored at -80°C. Colonies are divided into which growth media they were grown on (AC, BHI or LB). They are presented as the plate number they were picked from and their sequential colony number. The results of the pre-event (1st) and post-WGS (2nd) antagonistic assays are shown.

		1st Test						2nd Test					
Participant Plate Number	Colony Number	*Bacillus subtilis*	*Staphylococcus epidermis*	*Salmonella* Typhimurium 1344	*Klebsiella pneumoniae*	*Salmonella* Typhimurium DT104	*Candida albicans*	*Bacillus subtilis*	Methicillin-Resistant *Staphylococcus aureus*	Vancomycin-Resistant *Enterococcus faecium*	*Salmonella* Typhimurium 1344	*Salmonella* Typhimurium DT104	*Candida albicans*
All Culture Media													
1	1						Y						
2	4			Y		Y	Y						Y
3	8					Y	Y						Y
3	9			Y		Y	Y						
4	13			Y		Y							
4	15						Y		Y	Y			Y
4	17	Y				Y				Y			
4	17	Y				Y				Y			
6	24						Y		Y			Y	Y
7	28			Y		Y			Y				
8	29			Y		Y							
10	34			Y		Y			Y				Y

12	37			Y		Y							Y
16	41						Y				Y	Y	Y
16	42			Y		Y			Y		Y		Y
17	43					Y	Y		Y		Y		Y
17	44						Y						Y
21	49			Y		Y	Y			Y		Y	
23	50					Y	Y						Y
23	51			Y		Y	Y						Y
24	52					Y							Y
26	56			Y		Y	Y	Y	Y	Y			Y
31	59						Y				Y		Y
31	61	Y		Y		Y	Y			Y			Y
32	62	Y		Y		Y	Y						Y
34	65						Y						Y
40	73						Y	Y					Y
43	76	Y		Y		Y	Y		Y	Y			Y
44	77			Y		Y	Y						
45	79			Y		Y	Y						Y
48	80			Y		Y	Y						Y
48	81			Y		Y	Y						Y
52	84			Y		Y	Y						Y

52	85		[redacted]		[redacted]		Y	Y		Y			Y
55	89		[redacted]		[redacted]		Y						
Brain Heart Infusion Media													
5	14		[redacted]	Y	[redacted]	Y			Y	Y			
6	17		[redacted]		[redacted]		Y		Y	Y		Y	
11	22		[redacted]	Y	[redacted]	Y			Y		Y	Y	
14	25												
14	26												
18	34		[redacted]	Y	[redacted]	Y							Y
19	38												
20	40												
34	51		[redacted]		[redacted]		Y		Y	Y		Y	Y
34	51		[redacted]		[redacted]		Y		Y	Y		Y	Y
42	56		[redacted]		[redacted]		Y			Y			
52	62		[redacted]		[redacted]	Y	Y		Y		Y	Y	
52	63	Y	[redacted]	Y	[redacted]	Y				Y			Y

Table L-2. Glasgow Botanical Gardens antagonistic isolates stored at -80°C. Colonies are divided into which growth media they were grown on (AC, BHI or LB). They are presented as the plate number they were picked from and their sequential colony number. The results of the pre-event (1st) and post-WGS (2nd) antagonistic assays are shown.

		1st Test						2nd Test					
Participant Plate Number	Colony Number	*Bacillus subtilis*	*Staphylococcus epidermis*	*Salmonella* Typhimurium 1344	*Klebsiella pneumoniae*	*Salmonella* Typhimurium DT104	*Candida albicans*	*Bacillus subtilis*	Methicillin-Resistant *Staphylococcus aureus*	Vancomycin-Resistant *Enterococcus faecium*	*Salmonella* Typhimurium 1344	*Salmonella* Typhimurium DT104	*Candida albicans*
All Culture Media													
3	3												
6	5	Y		Y		Y							Y
6	5	Y		Y		Y							Y
11	7	Y		Y		Y	Y					Y	
11	8	Y		Y		Y	Y					Y	
12	13	Y		Y		Y	Y				Y	Y	
12	14	Y		Y		Y	Y	Y	Y	Y			
12	15	Y		Y		Y	Y					Y	
21	34	Y		Y		Y						Y	
15	20			Y		Y						Y	
23	38			Y		Y		Y		Y		Y	Y
27	44	Y		Y		Y						Y	
28	46						Y						
39	58	Y		Y		Y	Y					Y	

41	62					Y						Y	
42	66			Y		Y						Y	
58	79					Y							
59	84						Y					Y	
Brain Heart Infusion Media													
2	1	Y		Y		Y					Y	Y	
3	7	Y		Y		Y					Y	Y	
4	10			Y		Y			Y		Y	Y	
6	21					Y			Y				Y
13	49	Y		Y		Y			Y		Y		
15	54			Y		Y			Y		Y	Y	
18	62			Y		Y					Y		
20	68	Y		Y		Y			Y		Y	Y	
21	71												
21	73												
28	84						Y			Y	Y	Y	Y
40	96	Y		Y		Y	Y		Y		Y	Y	
48	105			Y		Y	Y						
LB Media													
35	21						Y	Y		Y			
48	26			Y		Y					Y		Y

Table L-3. Thetford Forest antagonistic isolates stored at -80°C. Colonies are divided into which growth media they were grown on (AC, BHI or LB). They are presented as the plate number they were picked from and their sequential colony number. The results of the pre-event (1st) and post-WGS (2nd) antagonistic assays are shown.

		1st Test						2nd Test					
Participant Plate Number	Colony Number	*Bacillus subtilis*	*Staphylococcus epidermis*	*Salmonella* Typhimurium 1344	*Klebsiella pneumoniae*	*Salmonella* Typhimurium DT104	*Candida albicans*	*Bacillus subtilis*	Methicillin-Resistant *Staphylococcus aureus*	Vancomycin-Resistant *Enterococcus faecium*	*Salmonella* Typhimurium 1344	*Salmonella* Typhimurium DT104	*Candida albicans*
All Culture Media													
11	29	Y		Y			Y	Y	Y		Y	Y	Y
11	30	Y		Y			Y						
40	78	Y		Y			Y		Y				Y
40	79			Y									Y
40	80	Y		Y			Y	Y	Y				Y
41	81	Y		Y									
44	92	Y		Y					Y				
45	94	Y		Y			Y					Y	
45	97	Y		Y			Y						
46	98	Y		Y			Y						Y
46	99	Y		Y									
46	100	Y		Y									Y

Brain Heart Infusion Media													
15	37	Y		Y			Y					Y	
7	42						Y						
8	45			Y									
9	46			Y									
9	47	Y		Y			Y						
10	52			Y									
11	53			Y							Y		
12	58			Y							Y	Y	
12	59			Y							Y		
19	76			Y									
22	77			Y									
30	86						Y		Y				
32	87	Y		Y			Y		Y		Y	Y	
34	88						Y		Y		Y	Y	
36	91						Y						
40	92						Y						
	100	Y		Y									Y
LB Media													
34	72	Y		Y			Y	Y	Y				Y
34	76						Y						

35	78	Y	[redacted]	Y	[redacted]		Y						
38	81	Y		Y			Y				Y		Y
40	83	Y		Y			Y						
40	84	Y		Y			Y				Y		
40	86						Y	Y		Y			
41	90	Y		Y			Y						Y