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Is it time to say goodbye to culture and sensitivity? The case for culture-independent urology.

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Abstract

Next-Generation sequencing (NGS) has highlighted the limitations of conventional culture methods in the role of urology whilst discovering the intricate details of the role of microbiota in urologic health and disease. This review article explores: the utility and limitations of conventional culture methods; how culture-independent technologies are revolutionising medicine; and how the implementation of these technologies may lead to improved patient outcomes. Finally, this article discusses the barriers to widespread adoption of culture-independent technologies, with suggestions for how these hurdles may be overcome.

Introduction

History of culture methods

Since the inception of conventional culture methods by Dr Robert Koch in the 1880s, traditional microbiology has played an integral role in the identification of bacterial pathogens. With antimicrobial resistance an ever-growing concern, it is important that we determine whether we can rely on culture and sensitivity to accurately diagnose episodes of infection and, if so, when it is appropriate for such reliance on this traditional method.

Before the use of vaccines, the story of infection was relatively simple – planktonic bacteria would seek to penetrate the external defences of the human body, overcome the internal defences of the host, subsequently reaping a potentially ghastly infection. Fortunately for traditional culture methods, these bacteria typically possessed cell walls which exhibited great survival ability in various environments, lacked cell-to-cell connections, and often had adaptations which allowed them to adhere to surfaces[1]. Culture methods, therefore, have successfully aided the development and selection of antibiotics, eradicating many epidemic diseases, saving a great deal of human life.

The present day

However, the efficacy of such methods has ultimately led to their own demise – colonization and survival are now achieved, primarily, through the creation of biofilms[1]. This is the term given to "architecturally complex microbial communities that grow adhered to surfaces and are encased by an extracellular matrix"[2]. Biofilms were recognised due to their persistent nature and their resistance to antibiotic therapies. There is now consensus among the microbiology community that only 1% of microorganisms present in natural and pathogenic ecosystems are planktonic - the remaining 99% grow as a biofilm[3]. The Centers for Disease Control and Prevention has estimated that approximately 65% of all bacterial infections, and an even higher 80% of chronic infections are made up of biofilm infections[1,4]. These statistics suggest that the overwhelming majority of micro-organisms exist in communities with cell-to-cell connections. Importantly, cells within a biofilm exhibit a crucial difference from planktonic bacteria – they fail to create colonies when single

species biofilms have been removed from their microenvironment and set upon traditional petri dishes[5].

Naturally, such a failure to produce colonies will ultimately lead to a ‘negative’ result on culture. It is straightforward to see how this can occur – patients presenting with chronic biofilm infections are often recipients of previous courses of antibiotics; such antimicrobial treatment will have eradicated the planktonic bacteria which would otherwise have been suggestive of an infection, rendering any new culture ‘negative’, despite the presence of a flourishing biofilm. These false negatives are not unimportant; since culture methods are the only widely available diagnostic tools used for the identification of micro-organisms for most institutions in the developed world, many millions of people each year are susceptible to hidden, protracted infections. This is a growing problem and poses a serious dilemma for clinicians tasked with the care of a patient who, by any reasonable clinical criteria, is suffering from an indolent infection, but whose culture results continuously return as ‘negative’. Combining such a fundamental limitation with the inherently slow nature of culture methods, it is not uncommon for a healthcare practitioner to be making what amounts to a ‘best guess’ as to which antibiotic is most appropriate for a given patient, without knowing the identity and characteristics of the culpable microorganism(s). Such clinicians inevitably find themselves in a proverbial ‘catch 22’, expected to be advocates of antibiotic stewardship, with a tool that cannot be relied upon to provide timely, accurate information. Ultimately, this dilemma can have several important ramifications: (1) inefficient use of resources including money, expertise of well-trained microbiologists, and time; (2) contribution to antimicrobial resistance due to the use of inappropriate treatment regimens; (3) microbiota

impairment – e.g. ciprofloxacin and vancomycin have been shown to alter the microbiota of healthy individuals[6].

Urine Cultures

Standard practice

Dysuria is a common complaint for women attending an appointment in general practice. The majority of these cases are associated with significant bacteriuria[7]. Usually, empirical antibiotics are started without the need for a urine culture, as classical symptoms such as dysuria, urgency, and frequency, correctly predict a urinary tract infection (UTI) at a high rate[8]. Indeed, Public Health England guidance suggests that a urine culture is normally redundant in females presenting with two or three symptoms of UTI and recommends the prescription of empirical antibiotics without further testing[9].

Thresholds, contamination, and areas of contention

Despite the high-predictive value of such classical symptoms indicating active infection, approximately ¼ of symptomatic women will produce a urine culture eventually labelled as 'negative' according to the cut-off threshold used[7]. There has been considerable debate over what ought to constitute a 'positive' urine culture, with scepticism over the time-honoured threshold of 10^5 CFU/mL in cases of suspected uncomplicated UTI[10]. This debate boils down to concerns over the potential lack of sensitivity in diagnosing a UTI, contrasted with the desire to avoid overdiagnosis and subsequent treatment of unimportant cases of bacteriuria. Even the more liberal threshold of 10^3 CFU/mL is likely to fail in detecting sexually

transmitted infections[11]. Over 30 years ago, it was proposed that an even lower threshold of 10^2 CFU/mL may be suitable for a diagnosis of uncomplicated UTI in women experiencing symptoms[10]. There appears to be very little consensus over an appropriate cut-off for the diagnosis of UTI; indeed, it has been suggested that there is, in fact, no definitive bacterial count which can be considered conclusive for all cases of UTI[12].

A further concern over culture and sensitivity is its high susceptibility to contamination[13]. Cultures are typically viewed as 'spoiled' if more than two isolates of 10^3 CFU/mL are detected[14]. In the United States, the average institution reports a 15% contamination rate of urine cultures[14]. Opposing research has complicated matters, with authors in the 1960s espousing that low bacterial counts can be considered as contamination[15,16], while others, only a few years later, demonstrated that symptomatic women rather frequently had low colony forming units[17,18]. A lack of consensus over what threshold constitutes an infection, combined with seemingly high rates of contamination only serves to complicate matters for a clinician. Indeed, a strongly contested issue has arisen from this inability to provide a clear answer as to whether there is infection, or mere contamination - 'urethral syndrome'[19]. This term refers to those patients who suffer from symptoms typical of a lower urinary tract infection but fail to produce a 'positive' urine culture. Such terminology is still utilised today, commonly described as idiopathic, with bacterial and viral agents not thought to be the cause[20]. Interestingly, 20 years ago, it was demonstrated that, despite being classified as entirely separate entities, with supposedly differing aetiologies, outcomes were equal between those diagnosed with UTI versus those diagnosed with urethral syndrome, after antibacterial treatment had been

administered[21]. This finding suggests that there may be a similar aetiology, one of infection, and it is possible that queries over thresholds have made the issue more complex than it ought to have been.

It is apparent that standard microbiology methods have many downsides: they are unable to detect anaerobic bacteria in cases of UTI due to the difficulty in growing such pathogens via traditional methods; they struggle to detect microbes such as *Aeroccocus urinae*, *Gardnerella vaginalis* etc., as such species can only be caught using particular media types and prolonged incubation periods[22,23]; and certain strains of *E. coli*, for example, exist as intracellular biofilms, rendering the host symptomatic, whilst simultaneously hiding from conventional culture methods[24]. Evidently, therefore, the use of cultures in the detection and identification of complex, diverse, microbial communities existing as biofilms is limited.

Introduction to PCR and NGS

Polymerase chain reaction (PCR) was developed by Kary Mullins in 1983 and its benefits were immediately evident. Compared to culture, PCR is faster, cheaper, and more accurately detects organisms in a sample[25]. PCR works in a three-step process:

1) initial hot denaturation of a double-stranded DNA template
2) annealing of specific primers on the target
3) extending the annealed primers with DNA polymerase

These DNA templates are then amplified to a sufficient degree to identify the pathogens. The high sensitivity and specificity of PCR have enabled the detection of rare microbial targets which has driven its diagnostic clinical applications, specifically in the identification of body fluid infections[26-28]. In the past decade, a multiplex PCR was introduced which enabled direct-from-urine analysis, both identifying more bacteria and discriminating more fastidious bacteria than traditional urine culture in patients with symptoms of UTI[29,30].

Next Generation Sequencing (NGS) is the first cost-effective approach to sequencing human samples for medical genetics and diagnostic purposes[31]. Also known as high-throughput sequencing, NGS can sequence the DNA and RNA of a given sample and is considered revolutionary because it can compute an entire human genome in a day, compared to the previous Sanger sequencing which would take 10 years[32]. NGS can be used to characterise a particular microbiome, such as the urinary microbiome, by comparing these sequences to known motifs specific to certain microorganisms[33]. NGS provides information on which bacteria and fungi are present in the sample, the bacterial load of each microorganism, and antibiotic resistance genes. This diagnostic tool can allow for more targeted clinical therapy by considering the predominant pathogen causing the infection and potential antibiotic resistance.

The utility of culture-independent microbiology

Urine samples

It was not long ago that the medical community held the dogmatic belief that urine is sterile. With the use of DNA-sequencing technologies, this is now known not to be the case, with several studies demonstrating the presence of multiple bacteria in healthy volunteers. One such example was provided by McDonald et al., where 22 healthy volunteers provided urine samples for analysis by NGS. In 5/22, culture results were positive; according to NGS, 21/22 had bacteria detected in their urine, despite being asymptomatic[34]. Although this raises more questions about the normal composition of the urinary microbiome than it does answers, knowledge in this field is increasing at an impressive rate. As a result, interest has begun to turn towards the role of the microbiota in the pathogenesis of numerous conditions[35].

Pearce et al. produced intriguing work on the comparison of the urinary microbiome in women with and without urgency urinary incontinence (UUI)[23]. Previously, Pearce and colleagues had demonstrated that bacteria that had classically been missed by conventional culture methods could be grown by expanded quantitative urine culture (EQUC)[36]. However, EQUC is still not sufficient to detect and identify many bacteria which reside within the urinary tract[23]. As a result, this group of researchers used high-throughput sequencing of the 16S rRNA gene and EQUC to explore urine samples from transurethral catheters in women with UUI and to compare the findings with a control group of women without UUI. Sequencing results revealed bacterial DNA in approximately 65% of women in each group. In comparison to other studies, this overall detection rate seems relatively low, but this could possibly have several explanations e.g. primer errors, low bacterial load etc. This study produced several statistically significant findings[23]:

(1) reduced frequency of *Staphylococcus* within the UUI group

(2) higher frequency of *Gardnerella* and *Aerococcus* within the UUI group

(3) reduced abundance of *Lactobacillus* within the UUI group

(4) increased abundance of *Gardnerella* within the UUI group

(5) *Lactobacillus gasseri* was found more frequently in the UUI group

(6) *Lactobacillus crispatus* was found less frequently in the UUI group

A majority (78.9%) of urine samples grew bacteria when cultivated by EQUC. Of note, 90.1% of these EQUC-positive samples failed to grow on standard culture. The authors discussed that, in this study, the type II error of standard culture was 90.3% in the UUI group, and a similar 90.0% in the control group. This study, however, suggests a degree of superiority of EQUC over sequencing as evidenced by the following results:

- EQUC was capable of detecting bacteria in 14/19 sequence-negative specimens
- DNA sequencing detected bacteria in 3/8 EQUC-negative specimens

With that said, it is worth pointing out that certain genera were detected by DNA sequencing but failed to grow even on EQUC. Evidently, even the most comprehensive culture methods fail to detect particular bacteria. Although EQUC did out-perform NGS in the detection of a total of 9 genera, sequencing successfully detected those same genera in other urine samples, suggesting that this apparent limitation could be overcome by alteration in the amplification of primers[23].

Heytens et al. compared the mid-stream urine samples of 86 asymptomatic women and 220 women with symptoms suggestive of UTI using culture methods and quantitative PCR (qPCR)[37]. Overall, 95.9% of the symptomatic women were qPCR-positive for E. coli while, in the same group, E. coli or another uropathogen was only cultured in 80.9% of the samples. In the asymptomatic group, 11.6% of women had a positive urine sample on qPCR – almost the exact same proportion of those with positive cultures (10.5%). This suggests that the large difference in positive results between culture and qPCR in the symptomatic group cannot be attributed to contamination as a result of improved sensitivity of qPCR. Additionally, 90.5% of the 42 symptomatic women with negative cultures had a positive qPCR result, while only 5.3% of the 76 asymptomatic women with negative cultures had a positive qPCR result ($p < 0.0001$).

McDonald et al. compared conventional culture methods and NGS in the treatment of 44 patients with symptoms of acute cystitis[34]. Patients were randomised into two arms. Arm A was treated based on results of culture, while Arm B was treated based on results of NGS. If cultures were negative in Arm A, patients were treated according to results of NGS. Overall, 29.5% (13/44) of urine samples were positive on culture, compared to 100% of samples on NGS. Interestingly, NGS demonstrated that 77% of samples were polymicrobial, compared to only 15% of positive cultures. Symptom scores (based on a UTI self-assessed questionnaire) were significantly better in Arm B (NGS-based group). Of clinical importance was the ability of NGS to detect anaerobic bacteria; such species were present in 45.5% of samples and, in 50% of those cases, anaerobic bacteria constituted the main infectious component of the urine.

At the European Association of Urology Congress in March 2019, Dixon et al. presented work assessing the comparative value of standard culture and sensitivity versus NGS in chronic UTI[38]. A total of 69 patients were included, all of whom received NGS on their mid-stream catch urine samples. 49/69 patients also had culture and sensitivity performed, allowing a comparison between the two methods of diagnosis in 49 patients. Overall, NGS detected a microbial presence in 98.6% (68/69) of patients, while culture and sensitivity only produced a positive result in 61% (30/49) of patients. In the single case of an NGS-negative result, culture methods did detect the presence of an organism. In the 19 culture-negative cases, NGS detected microbes in 18. A single patient produced a negative result on both tests.

Lewis et al. assessed MSU samples from healthy males (n =6) and healthy females (n=10)[39]. Their work showed a trend towards a heterogenous mix of bacterial genera within females, with a larger range of genera, and a greater diversity of genera on average, at a level that was statistically significant (p = 0.042). Of significant importance was the ability of NGS to detect and identify organisms that would not be grown by conventional culture methods; the authors explained that NGS results revealed 94 bacterial genera, 63 of which would either not usually be cultivated by National Health Service laboratories or would not be reported individually. Their work also produced the first report of the presence of the genus *Soehngenia* in humans. NGS detected this in 4 individuals, 3 of whom were female. Once again, such a finding would not have been observed if dependent on conventional culture methods, as bacteria within this genus are anaerobic.

Liss et al. explored the role of NGS in the selection of prophylactic antibiotics for ureteroscopy[40]. 20 patients provided urine samples for culture and NGS prior to surgery. Cultures returned positive for just 2/20 samples, while NGS provided positive results in 12/20. In the 18 culture-negative cases, NGS revealed the presence of microbes in 10. In the 2 culture-positive samples, NGS concurred and produced a positive result.

Shrestha et al. sought to explore any association between the urinary microbiome and the presence of prostate cancer, grading of said cancer, as well as the type of prostatic inflammation in men undergoing prostate biopsy[41]. Their work revealed that men with prostate cancer more commonly exhibited bacteria associated with infections of the urogenital tract such as prostatitis. Some bacteria were also noted to be more prevalent in those with higher grade prostate cancer e.g. *Streptococcus.* For an overview of the studies incorporating culture-independent methods, see **Table 1.**

Rectal swabs

Culture-independent methods have recently been used to examine the microbial composition of rectal samples, showing that men receiving anti-androgen therapy for prostate cancer had different microbiota to those being treated with only GnRH therapies or not being treated at all[42]. There has been some evidence, also produced by NGS, that there is no difference in the rectal bacterial profiles of men

with prostate cancer and those without, with the exception of an elevated frequency of *Bacteroides* and *Streptococcus* in men with prostate cancer[43].

Preliminary work has also been carried out using NGS to create individualised antimicrobial prophylaxis for men undergoing TRUS-guided prostate biopsy[44]. Although only performed with a small sample of 68 participants, this method of precision-prophylaxis resulted in a complete avoidance of severe infectious complications (0/68) at 30 days post-procedure. This is in contrast to the usual rate of complications which can be as high as 17%[45].

Limitations of PCR

Although PCR enables amplification of bacterial species, it can still be challenging to diagnose a bacterial infection that has multiple aetiologies. In the diagnosis of UTI, PCR is limited to the detection of a single pathogen or the gram-status[46]. PCR does not allow determination of a causal agent for an infection and necessitates multiple individual amplifications and analyses. Because of this, drug susceptibility and genetic typing still need to be performed; thus, PCR testing alone may be limited as a diagnostic tool. This issue has been addressed with the advent of multiplex real-time PCR, which has sensitivity to 25 common bloodstream pathogens[46].

Another limitation of PCR is the likelihood of false positive and negative results. When urine is collected using the most common “clean catch” method, bacteria within the sample could be contaminated by commensal urethral flora[30]. PCR reports the presence of bacteria but does not quantify this; therefore, it can be difficult to discern physiologic bacteria contaminating the sample from a potential causative

agent of infection. Another false positive result can involve the detection of a pathogen by PCR in the absence of clinical UTI symptoms. It is unclear if this would represent a subclinical or passed infection and how clinically relevant this would be[46]. One of the most important limitations of PCR, however, is that it relies upon a 'targeted detection methodology', whereby prior knowledge is used to put forward a hypothesis as to which aetiological agent is likely to be found, with subsequent primer development and utilisation in the effort to identify the aetiological microbe. High-throughput DNA sequencing does not assume this methodology and so is capable of circumventing this problem entirely, providing a thorough description of all the genomic content of a sample.

Limitations of NGS

Despite the revolutionary diagnostic relevance NGS provides, there are limitations of NGS of which providers should be aware. NGS has a high sensitivity for bacterial and fungal species, making it difficult to interpret the results and discerning their clinical relevance. The NGS panel states the bacteria and its dominance (proportion) in the sample. The issue with this structure is the potential inference that the dominant bacteria are the causative bacteria; however, the dominance of a microorganism does not mean it is pathophysiological, especially in the case of UTIs which have multiple different aetiologies. This issue encourages NGS companies to provide certain thresholds and physiologic percentages that allow clinicians to have a reference point for diagnosis.

Another limitation of NGS is its inability to test for phenotypic antibiotic sensitivity. Although NGS can determine a genetic link between antibiotic resistance and the sample DNA, the genotypic-phenotypic link is not clear[47]. There are some studies that have shown a correlation between genetic and phenotypic antibiotic resistance, but virulence determinants of resistance are still being developed[33, 48]. From these studies, it is clear that NGS can provide information on genetic susceptibility to antibiotic resistance, but the genetic and environmental factors responsible for an antibiotic resistant phenotype are not well understood.

Finally, one of the most crucial impediments to widespread implementation of NGS is the quality of the data available for the genomic reference library. Publicly available databases are, unfortunately, impaired by incorrect annotations of data – the importance of this must not be understated, as the accuracy of the databases upon which healthcare providers must rely is of the highest priority due to its effects on the ability to correctly identify the organisms culpable for infection[49]. In order for NGS platforms to be used in everyday practice, this problem must be overcome by comprehensive quality control, and it is likely that regulatory bodies would need to be included in this[49].

Conclusion

Conventional culture methods have proven invaluable since their discovery in the late 1800s. Today, still, they are used as the gold-standard diagnostic tool in even the most developed regions of the world. However, with the continuous improvement of culture-independent technologies such as NGS, it is evident that there are several areas in which culture methods are substantially inferior: an inability to detect the

entire spectrum of microbes present within a sample; high rates of false-negatives; difficulty detecting anaerobic microbes without extended techniques; high rates of contamination; slow to produce results; and biofilms, which now comprise the majority of infections, are capable of avoiding detection. Furthermore, there is now a wealth of information in the public domain which serves to demonstrate the utility of DNA-sequencing methods in the exploration of the urinary and rectal microbiomes, their complex interactions, and their potential role in the pathophysiological processes of a great number of urological diseases. Although not without their own limitations such as inaccuracies within reference databases, difficulty determining the culpable organism(s) from the spectrum of identified microbes, and the start-up costs for each medical centre, forthcoming advances will likely minimise these pitfalls. Such advances will likely be in the form of highly curated libraries, involvement of regulatory bodies in quality control, and ever-reducing costs.

The idea that culture methods are incapable of appreciating the complexity of a bacterial ecosystem is not new – in the 1980s, environmental microbiologists noticed that less than 1% of bacteria in the natural ecosystem could be recovered using such means[50]. With the evolution and improvement of NGS platforms and bioinformatics, it is perhaps time to move on from traditional microbiology and begin implementing culture-independent technologies in day-to-day urological practice, with an aim to develop personalised therapeutic regimens.

References

1. Ehrlich GD, DeMeo PJ, Costerton JW. The Problem of Culture-Negative Infections. In: Ehrlich GD, DeMeo PJ, Costerton JW, Winkler, H, editors. Culture Negative Orthopedic Biofilm Infections. Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg, 2012:1-15.

2. Morales DK, Kolter R. Microbial Biofilms in Human Disease, Reference Module in Biomedical Sciences. Amsterdam: Elsevier, 2014
3. McCarty S, Woods E, Percival SL. *C*hapter Nine - Biofilms: From Concept to Reality. In: Percival SL, Williams DW, Randle J, Cooper T, editors. Biofilms in Infection Prevention and Control. Cambridge, Massachusetts: Academic Press, 2014:143-63
4. Jamal M, Ahmad W, Andleeb S, et al. Bacterial biofilm and associated infections. *J Chin Med Assoc* 2018;81:7-11. doi:10.1016/j.jcma.2017.07.012
5. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. Pseudomonas aeruginosa Displays Multiple Phenotypes during Development as a Biofilm. *J Bacteriol* 2002;184:1140-54. doi:10.1128/jb.184.4.1140-1154.2002
6. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2010;108:4554-61. doi:10.1073/pnas.1000087107
7. Naber KG, Schito G, Botto H, Palou J, Mazzei T. Surveillance Study in Europe and Brazil on Clinical Aspects and Antimicrobial Resistance Epidemiology in Females with Cystitis (ARESC): Implications for Empiric Therapy. *Eur Urol* 2008;54:1164-78. doi:10.1016/j.eururo.2008.05.010
8. Giesen LG, Cousins G, Dimitrov BD, van de Laar FA, Fahey T. Predicting acute uncomplicated urinary tract infection in women: a systematic review of the diagnostic accuracy of symptoms and signs. *BMC Fam Pract* 2010;11:78 doi:10.1186/1471-2296-11-78
9. Diagnosis of urinary tract infections, Quick reference tool for primary care for consultation and local adaptation. London: Public Health England, 2019. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/813033/PHE_UTI_diagnostic_flowchart.pdf (accessed 2 Jul 2019).
10. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of Coliform Infection in Acutely Dysuric Women. *N Engl J Med* 1982;307:463-8. doi:10.1056/nejm198208193070802
11. Tomas ME, Getman D, Donskey CJ, Hecker MT. Overdiagnosis of Urinary Tract Infection and Underdiagnosis of Sexually Transmitted Infection in Adult Women Presenting to an Emergency Department. *J Clin Microbiol* 2015;53:2686-92. doi:10.1128/jcm.00670-15

12. Bjerklund Johansen TE, Bonkat G, Cai T, Tandogdu Z, Wagenlehner F, Grabe M. Grey Zones in the Field of Urinary Tract Infections. *Eur Urol Focus* 2016;2:460-2. doi:10.1016/j.euf.2016.03.012
13. Smelov V, Naber K, Bjerklund Johansen TE. Improved Classification of Urinary Tract Infection: Future Considerations. *Eur Urol Suppl* 2016;15:71-80. doi:10.1016/j.eursup.2016.04.002
14. Bekeris LG, Jones BK, Walsh MK, Wagar EA. Urine culture contamination: a College of American Pathologists Q-Probes study of 127 laboratories. *Arch Pathol Lab Med* 2008;132:913-7. doi:10.1043/1543-2165(2008)132[913:UCCACO]2.0.CO;2.
15. Kass EH. Asymptomatic infections of the urinary tract. *Trans Assoc Am Physicians* 1956;69:56-64.
16. Kass EH. The role of asymptomatic bacteriuria in the pathogenesis of pyelonephritis. In: Quinn EL, Kass EH, editors. Biology of pyelonephritis. Boston: Little, Brown, 1960:399–412.
17. Mabeck CE. Studies in urinary tract infections. I. The diagnosis of bacteriuria in women. *Acta Med Scand* 1969;186:35-8.
18. Gallagher DJ, Montgomerie JZ, North JD. Acute infections of the urinary tract and the urethral syndrome in general practice. *Br Med J* 1965;1:622-6. doi:10.1136/bmj.1.5435.622
19. Stamm WE, Wagner KF, Amsel R, et al. Causes of the acute urethral syndrome in women. *N Engl J Med* 1980;303:409-15. doi:10.1056/NEJM198008213030801
20. Parsons CL. The role of a leaky epithelium and potassium in the generation of bladder symptoms in interstitial cystitis/overactive bladder, urethral syndrome, prostatitis and gynaecological chronic pelvic pain. *BJU Int* 2011;107:370-5. doi:10.1111/j.1464-410X.2010.09843.x
21. Baerheim A, Digranes A, Hunskaar S. Equal symptomatic outcome after antibacterial treatment of acute lower urinary tract infection and the acute urethral syndrome in adult women. *Scand J Prim Health Care* 1999;17:170-3. doi:10.1080/028134399750002593
22. Hilt EE, McKinley K, Pearce MM, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 2014;52:871-6. doi:10.1128/JCM.02876-13

23. Pearce MM, Hilt EE, Rosenfeld AB, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *MBio* 2014;5:e01283-14. doi:10.1128/mBio.01283-14
24. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic Escherichia coli. *Annu Rev Microbiol* 2010;64:203-21. doi:10.1146/annurev.micro.112408.134258
25. Fairfax MR, Salimnia H. Diagnostic molecular microbiology: a 2013 snapshot. *Clin Lab Med* 2013;33:787-803. doi:10.1016/j.cll.2013.08.003
26. Davenport M, Mach KE, Shortliffe LMD, Banaei N, Wang TH, Liao JC. New and developing diagnostic technologies for urinary tract infections. *Nat Rev Urol* 2017;14:296-310. doi:10.1038/nrurol.2017.20
27. Abdeldaim GM, Strålin K, Korsgaard J, Blomberg J, Welinder-Olsson C, Herrmann B. Multiplex quantitative PCR for detection of lower respiratory tract infection and meningitis caused by Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis. *BMC Microbiol* 2010;10:310. doi:10.1186/1471-2180-10-310
28. Tsalik EL, Jones D, Nicholson B, et al. Multiplex PCR to diagnose bloodstream infections in patients admitted from the emergency department with sepsis. *J Clin Microbiol* 2010;48:26-33. doi:10.1128/JCM.01447-09
29. Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham DW. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *J Med Microbiol* 2013;62:1823-7. doi:10.1099/jmm.0.064428-0
30. García LT, Cristancho LM, Vera EP, Begambre O. A New Multiplex-PCR for Urinary Tract Pathogen Detection Using Primer Design Based on an Evolutionary Computation Method. *J Microbiol Biotechnol* 2015;25:1714-27. doi:10.4014/jmb.1406.06079
31. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43:491-8. doi:10.1038/ng.806
32. Behjati S, Tarpey PS. What is next generation sequencing? *Arch Dis Child Educ Pract Ed* 2013;98:236-8. doi:10.1136/archdischild-2013-304340
33. Mendes RE, Jones RN, Woosley LN, Cattoir V, Castanheira M. Application of Next-Generation Sequencing for Characterization of Surveillance and Clinical

Trial Isolates: Analysis of the Distribution of β-lactamase Resistance Genes and Lineage Background in the United States. *Open Forum Infect Dis* 2019;6:S69-78. doi:10.1093/ofid/ofz004

34. McDonald M, Kameh D, Johnson ME, Bjerklund Johansen TE, Albala D, Mouraviev V. A Head-to-Head Comparative Phase II Study of Standard Urine Culture and Sensitivity Versus DNA Next-generation Sequencing Testing for Urinary Tract Infections. *Rev Urol* 2017;19:213–20. doi:10.3909/riu0780
35. Lynch SV, Pedersen O. The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* 2016;375:2369-79. doi:10.1056/NEJMra1600266
36. Hilt EE, McKinley K, Pearce MM, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 2014;52:871-6. doi:10.1128/JCM.02876-13
37. Heytens S, De Sutter A, Coorevits L, et al. Women with symptoms of a urinary tract infection but a negative urine culture: PCR-based quantification of Escherichia coli suggests infection in most cases. *Clin Microbiol Infect* 2017;23:647-52. doi:10.1016/j.cmi.2017.04.004
38. Dixon M, Stefil M, Skinner C, et al. Comparative value of chronic urinary tract infection (UTI) diagnosis between standard culture sensitivity and next generation sequence (NGS) in urine samples. *Eur Urol Suppl* 2019;18:e378. doi:10.1016/s1569-9056(19)30283-0
39. Lewis DA, Brown R, Williams J, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol* 2013;3:41. doi:10.3389/fcimb.2013.00041
40. Liss M, Reveles K, Wheeler A, Arellanes A, Tseng T. MP77-02 ANTIBIOTIC SELECTION USING NEXT GENERATION SEQUENCING VERSUS URINE CULTURE FOR STEWARDSHIP PRIOR TO SURGERY USING AN INTERPROFESSIONAL MODEL. *J Urol* 2019;201: e1131-2. doi:10.1097/01.ju.0000557314.00940.68
41. Shrestha E, White JR, Yu SH, et al. Profiling the Urinary Microbiome in Men with Positive versus Negative Biopsies for Prostate Cancer. *J Urol* 2018;199:161-71. doi:10.1016/j.juro.2017.08.001
42. Sfanos KS, Markowski MC, Peiffer LB, et al. Compositional differences in gastrointestinal microbiota in prostate cancer patients treated with androgen

axis-targeted therapies. *Prostate Cancer Prostatic Dis* 2018;21:539-48. doi:10.1038/s41391-018-0061-x

43. Liss MA, White JR, Goros M, et al. Metabolic Biosynthesis Pathways Identified from Fecal Microbiome Associated with Prostate Cancer. *Eur Urol* 2018;74:575-82. doi:10.1016/j.eururo.2018.06.033
44. Mouraviev V, Dixon M, Stefil M, et al. Individual, DNA-guided, antibacterial prophylaxis prior to transrectal prostate biopsy based on results of next generation sequencing (NGS) of rectal swabs can be considered as a promising targeted approach to prevent severe urinary tract infection. *Eur Urol Suppl* 2019;18:e54. doi:10.1016/s1569-9056(19)30039-9
45. Borghesi M, Ahmed H, Nam R, et al. Complications After Systematic, Random, and Image-guided Prostate Biopsy. *Eur Urol*. 2017;71:353-65. doi:10.1016/j.eururo.2016.08.004
46. Lehmann LE, Hauser S, Malinka T, et al. Rapid qualitative urinary tract infection pathogen identification by SeptiFast real-time PCR. *PLoS One* 2011;6:e17146. doi:10.1371/journal.pone.0017146
47. Motro Y, Moran-Gilad J. Next-generation sequencing applications in clinical bacteriology. *Biomol Detect Quantif* 2017;14:1-6. doi:10.1016/j.bdq.2017.10.002
48. Didelot X, Bowden R, Wilson DJ, Peto TEA, Crook DW. Transforming clinical microbiology with bacterial genome sequencing. *Nat Rev Genet* 2012;13:601-12. doi:10.1038/nrg3226
49. Dixon M, Stefil M, McDonald M, et al. Metagenomics in diagnosis and improved targeted treatment of UTI. *World J Urol* 2019. doi:10.1007/s00345-019-02731-9
50. Hugenholtz P, Pace NR. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. *Trends Biotechnol* 1996;14:190-7. doi:10.1016/0167-7799(96)10025-1

Table 1. *Summary of studies using culture-independent methods and their main findings*

Study	**Design**	**Population type**	**Main findings**
McDonald et al., 2017[34]	Randomised	Acute cystitis	NGS was superior to culture in detection of organisms. Treatment based on NGS results was also more effective
Pearce et al., 2014[23]	Observational	Urgency urinary incontinence	EQUC was superior to both standard culture and NGS with regard to microbial detection. NGS did, however, detect several bacterial genera that EQUC did not reveal
Heytens et al., 2017[37]	Observational	Acute cystitis	Quantitative PCR out-performed standard culture in detection of organisms
Dixon et al., 2019[38]	Observational	Chronic UTI	NGS detected microbes more readily than standard culture methods
Lewis et al., 2013[39]	Observational	Asymptomatic, healthy individuals	NGS detected and successfully identified 63 bacterial genera that would not have been detected by standard culture

			methods
Liss et al., 2019[40]	Observational	Patients undergoing ureteroscopy	Compared to standard culture methods, NGS more readily detected urinary microbes
Shrestha et al., 2018[41]	Observational	Prostate cancer	NGS allowed the discovery that microbes associated with urogenital infection are more frequently seen in men with prostate cancer than in those without prostate cancer