The Oesophageal Microbiome and Cancer: Hope or Hype?

Bhaskar Kumar[a], Stephen Lam[a,d], Mina Adam[a], Rachel Gilroy[b], Mark J. Pallen[b,c,d]*

[a]Norfolk & Norwich University Hospitals NHS Foundation Trust, Norwich, UK
[b]Quadram Institute Bioscience, Norwich Research Park, Norwich, UK.
[c]School of Veterinary Medicine, University of Surrey, Guildford, Surrey, UK.
[d]University of East Anglia, Norwich Research Park, Norwich, UK.

*Correspondence: mark.pallen@quadram.ac.uk (M. J. Pallen)

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Abstract

The human oesophagus is home to a complex microbial community, the oesophageal microbiome. Despite decades of work, we still have only a poor low-resolution view of this community, which makes it hard to distinguish hope from hype when it comes to assessing links between the oesophageal microbiome and cancer. Here we review the potential importance of this microbiome and discuss new approaches, including culturomics, metagenomics and recovery of whole genome sequences, that bring renewed hope for an in-depth characterisation of this community that could deliver translational impact.

Hope, hype and the human microbiome

The human gut is home to a complex community of microbes, which, together with their genes and genomes, make up the gut **microbiome**. In recent decades, it has become clear that the gut microbiome plays a key role in setting the balance between health and disease in a wide variety of contexts [1,2]. In some cases, manipulation of a whole microbial community can be useful: for example, when faecal microbiota transplants are used to treat disease associated with *Clostridioides difficile* [3]. In other cases, targeting a single component has proven productive: for example, eradication of *Helicobacter pylori* from the gastric microbiome has played a decisive role in treating peptic ulceration and reducing the incidence of stomach cancer [Box 1]. However, there are also concerns that in some settings, claims for microbiome research are accepted too readily and thus verge on "hype" or **microbiomania** [4,5]. To guard against such hype, Hanage has suggested, when interpreting microbiome research, that we should ask whether any results reported really matter and reflect the real world and cannot be explained by other factors [5]. Hanage also suggests that we take care not to confuse correlation with causation and seek mechanistic explanations.

Until now, almost all the interest in gut microbiomes has focused on the lower gut and in particular on the large intestine, where the largest microbial biomass is found. However, spectacular advances in our understanding and control of *H. pylori* suggest that attention should also focus on the upper gut. So here we ask: what about the oesophagus? What can we say about the microbiome here and how might the hope/hype dichotomy play out in this context (Figure 1)?

The oesophagus: from health to cancer

The oesophagus is a fibromuscular tube about 25 cm long in adults that connects the pharynx to the stomach. Its internal surface is lined in health with a stratified squamous epithelium. **Gastro-oesophageal reflux disease**, caused by acid from the stomach entering the oesophagus, affects around 8% of the global population [6]. The inflammatory environment caused by the associated reflux oesophagitis is mutagenic, so that in ~7% of patients with reflux, the oesophageal epithelium undergoes **metaplasia** from squamous to columnar epithelium, leading to **Barrett's oesophagus** [7]. Barrett's oesophagus in turn is prone to malignant degeneration, with a risk of progression to adenocarcinoma of ~0.5% per patient, per year [8].

Oesophageal cancer is the sixth leading cause of cancer deaths, causing over 500,000 deaths per year globally [9]. There are two major forms of the disease. Oesophageal squamous cell carcinoma is causally linked to alcohol and tobacco and predominates in Central and South-East Asia and in China. Oesophageal adenocarcinoma is commoner in high-income countries, where it counts as the cancer with the fastest growth in prevalence. This type of oesophageal cancer is linked to acid reflux, Barrett's oesophagus and male gender. Long-term survival with oesophageal cancer remains dismally low, with fewer than 15% of those affected alive five years after diagnosis [10]. There is thus a desperate need to understand all the factors that contribute to this aggressive disease in the hope of improving diagnosis, treatment or better still prevention. Drawing on what we know from the role of microbes in cancer in other settings (Box 1), it is tempting to ask whether microbes and microbiomes might play a role here.

The constraints of culture

An early hint that there might be an oesophageal microbiome came from the recognition that broad-spectrum antibiotics present a risk factor for oesophageal candidiasis [11]. Beginning in the 1980s, attempts to culture organisms from the oesophagus in cancer and in health revealed overlaps with the oral microbiota (*Haemophilus influenzae, Moraxella catarrhalis, Streptococcus* spp.*)*, but also organisms typically found in the lower bowel (*Escherichia, Klebsiella, Enterococcus,* plus anaerobes such as *Bacteroides* and *Clostridium*) [12–19]. Such efforts also showed overlaps between organisms colonising the oesophagus and causing local surgical infections, thereby guiding choice of regimens for antimicrobial prophylaxis and therapy [20–22].

Early attempts at defining the oesophageal microbiome by culture ran into technical and contextual constraints that prevented robust conclusions. Samples were prone to microbial contamination from the oral cavity. Furthermore, culture is often onerous and is ill-suited for the detection of microbes that do not grow readily under laboratory conditions. This has meant that sample sizes tended to be small and failed to capture the full diversity of relevant characters (e.g. age, sex, disease state, location within the oesophagus).

Two recent studies stand out as informative and provocative. A 2007 study comparing culture results from seven patients with Barrett's oesophagus and seven healthy controls reported recovery of 16 genera and 46 species of bacteria from oesophageal samples. *Campylobacter concisus* and *Campylobacter rectus* were grown from four of the patients with Barrett's oesophagus, but from none of the control subjects [23]. Crucially, confocal microscopy with sequence-based probes revealed cells of *Campylobacter* spp. within mucosal biofilms in mucosal samples.

A follow-up study from the same group in 2013 recovered over a hundred species from oesophageal samples [24]. They confirmed *Campylobacter concisus* as the dominant species in patients with reflux and Barrett's oesophagus and reported significant increases in IL-18 in samples colonised by *Campylobacter*. Taken together, the two papers suggested mechanistic links between colonisation with *Campylobacter concisus*, DNA damaging nitrosative and oxidative stress, pro-inflammatory effects and progression towards adenocarcinoma. A subsequent opinion piece provocatively asked "is *Campylobacter* to oesophageal adenocarcinoma as *Helicobacter* to gastric adenocarcinoma"[25]?

However, these—and all previous culture-based studies—predate the era of cheap and easy microbial genome sequencing. This means that no conclusions can be drawn on whether distinctive strains or species colonise the oesophagus or whether these encode specific determinants that induce pathology—as we know is the case in the microbial contribution to the pathogenesis of stomach and bowel cancer (Box 1).

The limitations of amplicon sequencing

More recently, attention has turned to sequence-based culture-independent approaches, focusing on molecular barcodes, in particular 16S ribosomal RNA gene sequences. In a recent review, Park and Lee summarise twenty-one studies on the oesophageal microbiome that rely on 16S rRNA gene sequencing [26]. Cumulatively, these studies document a wide range of taxa in this setting, largely confirming findings from culture that there are overlaps between the microbiomes of the oesophagus, mouth and intestines. However, almost all such studies rely on amplification and sequencing of small stretches of DNA, so cannot provide resolution down to species. A notable exception comes from Blaser's group, who documented cloning and sequencing of extended 16S rRNA gene sequences, allowing them to document at least 95 species-level operational taxonomic units in oesophageal samples [27,28]. Interestingly, they reported sequences from the bacterial lineage TM7 (now called Saccharibacteria), which also occur in the oral cavity and are now known to act as obligate epibionts of bacterial hosts [29]. They found *Campylobacter concisus* in one normal individual, but not among cases of reflux oesophagitis and Barrett’s oesophagus. Crucially, they showed the presence of bacteria adherent to the oesophageal mucosa by microscopy, confirming the existence of a resident rather than just a transient oesophageal microbiome.

Despite their low taxonomic resolution, cumulatively, studies using molecular barcodes suggest that the oesophageal microbiome undergoes changes along the route from health to reflux oesophagitis to Barrett's oesophagus to adenocarcinoma, typically accompanied by a decrease in microbial diversity. However, the results of these studies are not consistent: for example, in one study Proteobacteria were more prevalent and Firmicutes less prevalent in health than in Barrett's oesophagus [30], whereas others report a decrease in the relative abundance of streptococci and an increase in Gram-negative taxa as one moves away from health [26]. Curiously, none of these sequenceindependent studies identified an association between genus *Campylobacter* and disease progression.

Yamamura and colleagues used specifies-specific sequence-based approaches to investigate potential links between the species *Fusobacterium nucleatum* and oesophageal squamous cell carcinoma. They reported that squamous cell carcinoma tissues contained significantly more *F. nucleatum* DNA than normal oesophageal mucosa and found an association between *F. nucleatum* tumour DNA positivity, survival and response to chemotherapy [31–33]. In their most recent publication, they provide evidence that *F. nucleatum* confers chemoresistance to squamous cell carcinoma cells by modulating autophagy [34].

There is mounting evidence for an association between the oral pathogen *Porphyromonas gingivalis* and oesophageal squamous cell carcinoma [35]. Most recently, Chen and colleagues used 16S rRNA amplicons and immunohistochemistry to document links between the abundance of *P. gingivalis* in oral and oesophageal samples and disease severity, and linked this organism to cancer progression, invasion and stemness in animal models [36].

Grounds for fresh hope

While detection of microbes by amplification of DNA sequences has provided some useful insights, as with culture-based studies, such approaches have failed to deliver whole genome sequences, when analysis of genomes is crucial for the investigation of microbial diversity and of species- or strain-specific pathogenic potential.

So, how should we proceed with investigation of the oesophageal microbiome? A key lesson here can be learned from investigations of the lower gut microbiome. Here, two new approaches have recently proven highly productive. The first is **culturomics**, which combines high-throughput culture of isolates under a range of laboratory conditions with whole-genome sequencing to provide new taxonomic and functional insights [37,38]. The second is **metagenomics**, in which DNA is extracted from samples *en masse* and then sequenced at depth using high-throughput sequencing technology. Such an approach clearly has diagnostic potential [39,40] and even shallow metagenomic sequencing rivals barcode sequencing in probing taxonomic diversity [41].

A recent study by Deshpande and colleagues provides exciting proof-of-principle here in applying shotgun metagenomic sequencing to oesophageal samples, followed by reference-based phylogenetic profiling [42]. The study was able to confirm the presence of selected bacterial taxa in the samples. However, such reference-based analytical approaches often suffer from misclassification of reads that leads to reports of highly implausible organisms such as plague and anthrax bacilli on the New York subway[43]. This problem is evident from the study by Deshpande and colleagues, where their analyses reported the presence of parasitic worms such as *Trichuris, Trichinella*, and *Loa loa* in oesophageal samples, which could not be confirmed using 18S rRNA gene amplification. In addition, such phylogenetic profiling relies on a reference database and so can only report previously known organisms and can never uncover “unknown unknowns”, i.e. inhabitants of the oesophagus not seen elsewhere. Plus, as with studies on 16S rRNA gene sequences, reference-based phylogenetic profiling typically fails to provide genomic data that can deliver insights into the functional diversity or population structure of the microbial species that they identify.

Fortunately, these problems can be largely overcome by new sophisticated bioinformatics approaches to the binning of sequences, which are able to deliver **metagenome-assembled genomes** (MAGs) that approach the genomes from cultured isolates in quality and information content, particularly when long-read sequencing approaches are used [44,45]. Together, these two approaches, culturomics and metagenomics, have delivered many thousands of microbial genomes from the human gut, documenting a remarkable diversity of strains and species [46,47]. Our own preliminary attempts at binning sequences from the dataset deposited by Deshpande and colleagues confirms that MAGs can be recovered fairly easily from oesophageal **metagenomes**.

The stage is thus set for similar large-scale culture-based and metagenomics studies of the oesophageal microbiome. Only through the availability of large numbers of genome sequences from oesophageal microbes (and comparators sets from other contexts) will we be able to pin strains or species to pathogenic potential. For example, *Campylobacter concisus*, a key candidate for a role in the pathogenesis of oesophageal adenocarcinoma, is now known to constitute a diverse jumble of species, which are likely to differ in habitat and disease association—an issue which can only be resolved through genome sequencing [48].

Another key aspect in performing baseline and comparative studies of the oesophageal microbiome will be careful selection of approaches to sample collection [49]. When surveying the ileal pouch, minimal differences in microbial composition were reported between samples taken with a cytology brush or with biopsy forceps. However, brushing probably allows access to a larger surface area for sampling and proves less traumatic to the epithelium. The *Cytosponge*—a spherical mesh swallowed in a capsule and attached to a string—has proven a promising non-endoscopic device that yields ten-times more microbial DNA than endoscopic brushes or biopsies [50].

Another potential challenge when taking samples for metagenomics, as illustrated in the only shotgun metagenomics study to date, is contamination with human DNA, which can swamp microbial DNA [42]. How far this will prove an intractable problem with oesophageal samples remains to be seen. However, in the recent proof-of-principle study applying shotgun metagenomic sequencing to oesophageal samples, it proved possible to enrich for microbial DNA using a commercial microbiome enrichment kit [42].

Another challenge is recruitment of enough patients and samples to provide sufficient statistical power for robust conclusions. Although recent years have seen a steady increase in the rates of gastroscopy for cancer screening and diagnosis [51], the COVID-19 crisis clearly represents a set back here [52]. However, a planned return to pre-pandemic rates is likely to facilitate collection of samples at scale.

Although observational and comparative studies have the potential to rule in or rule out microbes involved in oesophageal oncogenesis, only mechanistic and intervention studies can prove causality. Studies on the interactions between human cells and microbial candidates such as *F. nucleatum* and *C. concisus* have paved the way here [24, 25, 31–33]. However, a fuller understanding is likely to benefit from the recent development of animal and organoid models of oesophageal cancer to replicate the prolonged and multifactorial pathogenic processes that occur in vivo [53,54]. A recent study in mice provides a promising start here in showing, through microbiome transplants, that oesophageal carcinogenesis on a high-fat diet depends on the intestinal microbiome, although taxonomic resolution was hampered by a dependence on 16S amplicon sequencing [55].

Another approach to determine whether particular microbes play a role in pathogenesis of oesophageal cancer might be to administer antibiotics active against them and see what effect this has on disease progression. Given the slow and uncertain progress from oesophagitis to metaplasia to neoplasia, prevention studies might prove logistically difficult. Intervention studies with administration of antibiotics to patients with cancer might prove more tractable, as could epidemiological studies determining whether there is any association between progression to cancer and prior antibiotic use (including *H. pylori* eradication therapy).

Concluding remarks

After decades of studies, summarised in repeated reviews [26,56–59], it is perhaps all too easy to dismiss any contribution of the oesophageal microbiome to progression to cancer as mere hype. However, we take the opposite view: the arrival of new approaches including advances in microbial genomics, metagenomics, bioinformatics and the study of pathogenesis, means that fresh hope burns bright and there are compelling questions to be addressed (see **Outstanding Questions**). The challenge now is to assemble relevant interdisciplinary teams and recruit enough samples and sequences to provide a definitive answer—and even if turns out that there is no link between the microbiome and cancer (or any other pathology) in the oesophagus, we will learn a lot of exciting microbiology along the way.

Box 1: Microbes and Cancer

Helicobacter pylori is classified by the World Health Organisation as a type I carcinogen. Pathways to oncogenesis appear complex and multifactorial. However, not all strains of *H. pylori* are oncogenic and key virulence factors such as CagA vary in distribution from strain to strain [60]. Eradication of *H. pylori* using antimicrobial agents and a proton pump inhibitor lessens the risk of peptic ulceration and stomach cancer. However, at least in some populations, gastric carriage of *H. pylori* is inversely related to metaplastic and neoplastic changes in the oesophagus [60].

Several other bacteria have been implicated in cancer, although so far there are no accepted interventions to prevent, reduce or mitigate the effects they might have on the initiation or progression of cancer:

- ***Fusobacterium nucleatum*** has been implicated in progression of colorectal cancer, bringing an increased risk of recurrence and of chemoresistance [61]. Through at least two mechanisms, *F. nucleatum* can increase cell proliferation in cancer cells localize to tumours and adversely influence the microenvironment and even live within metastases.
- ***Escherichia coli*** produces two cytotoxins that damage DNA and so potentially induce or promote cancer in the host: cytolethal distending toxin and colibactin [62,63].
- **Blood-borne infection with *Streptococcus gallolyticus subsp. gallolyticus*** (formerly known as *S. bovis*) has long been linked to colorectal cancer [64]. As around 65% of patients diagnosed with invasive infection have a concomitant colorectal neoplasia, it can be seen a cancer biomarker. However, it remains unclear whether this organism plays a causal role in oncogenesis or merely benefits form the tumour microenvironment.

We have focused primarily here on bacteria. However, many viruses are known to play a role in carcinogenesis in humans, including *inter alia* human papillomavirus in cervical cancer, hepatitis B and C viruses in liver cancer and Epstein-Barr virus in Burkett's lymphoma. Al-Zimaity and colleagues have recently reviewed the emerging evidence implicating human papillomavirus, Epstein-Barr virus and perhaps also human polyoma virus in the pathogenesis of oesophageal cancer [65].

Glossary

Barrett's oesophagus: a clinical condition characterised by metaplasia in the lower oesophagus, with a change from a stratified squamous epithelium to a columnar epithelium similar to that seen in the intestine. This change is considered to be a premalignant condition predisposing to oesophageal adenocarcinoma.

Culturomics: an approach that allows extensive assessment of the microbial composition of a habitat by high-throughput culture under a range of laboratory conditions, typically followed by whole-genome sequencing.

Gastro-oesophageal reflux disease: a common clinical condition, where stomach contents, particularly acid flow back into the oesophagus causing inflammation and pain and predisposing to Barrett's oesophagus.

Metagenome: a set of sequences from multiple genomes obtained after extraction and sequencing of DNA from a sample without laboratory culture

Metagenome-assembled genome: a microbial genome sequence obtained from a metagenome after binning of reads based on coverage and composition.

Metaplasia: transformation of one differentiated cell type to another differentiated cell type, as occurs in Barrett's oesophagus.

Microbiome: A community of microbes, together with their genes and genomes, which inhabit a particular environment.

Microbiomania: a term popularised and defined by American evolutionary biologist Jonathan Eisen as the overselling of the impact (beneficial or detrimental) of microbiomes without supporting evidence.

Oesophageal cancer: a malignancy that typically presents with difficulty or pain in swallowing and weight loss. Subdivided into oesophageal squamous-cell carcinoma, which is more common in the developing world and oesophageal adenocarcinoma, which is more common in the developed world.

Figures

Figure 1 (Key Figure): Old and new approaches to investigation of the oesophageal microbiome. Old approaches were limited in throughput and taxonomic resolution, while new approaches deliver high taxonomic functional and taxonomic profiles.

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References

1 Lynch, S.V. and Pedersen, O. (2016) The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* 375, 2369–2379

2 Mullish, B.H. *et al.* (2021) The gut microbiome: what every gastroenterologist needs to know. *Frontline Gastroenterol* 12, 118–127

3 van Nood, E. *et al.* (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 368, 407–415

4 Adee, S. (2016) Moonshot or mania. *New Scientist*, 3074,16-17

5 Hanage, W.P. (2014) Microbiology: Microbiome science needs a healthy dose of scepticism. *Nature* 512, 247–248

6 GBD_2017_Gastro-oesophageal_Reflux_Disease_Collaborators (2020) The global, regional, and national burden of gastro-oesophageal reflux disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 5, 561–581

7 Marques de Sá, I. *et al.* (2020) The global prevalence of Barrett's esophagus: A systematic review of the published literature. *United European Gastroenterol J* 8, 1086–1105

8 Theron, B.T. *et al.* (2016) The risk of oesophageal adenocarcinoma in a prospectively recruited Barrett's oesophagus cohort. *United European Gastroenterol J* 4, 754–761

9 Sung, H. *et al.* (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71:209-249.

10 Coleman, H.G. *et al.* (2018) The Epidemiology of Esophageal Adenocarcinoma. *Gastroenterology* 154, 390–405

11 Scherr, S.A. *et al.* (1980) Chronic candidiasis of the oral cavity and esophagus. *Laryngoscope* 90, 769–774

12 Lau, W.F. *et al.* (1981) Oesophageal microbial flora in carcinoma of the oesophagus. *Aust N Z J Surg* 51, 52–55

13 Finlay, I.G. *et al.* (1982) Microbial flora in carcinoma of oesophagus. *Thorax* 37, 181–184

14 Mannell, A. *et al.* (1983) The microflora of the oesophagus. *Ann R Coll Surg Engl* 65, 152–154

15 Sjöstedt, S. *et al.* (1988) Microbial colonization of tumors in relation to the upper gastrointestinal tract in patients with gastric carcinoma. *Ann Surg* 207, 341–346

16 Gagliardi, D. *et al.* (1998) Microbial flora of the normal esophagus. *Dis Esophagus* 11, 248–250

17 Norder Grusell, E. *et al.* (2013) Bacterial flora of the human oral cavity, and the upper and lower esophagus. *Dis Esophagus* 26, 84–90

18 Zilberstein, B. *et al.* (2007) Digestive tract microbiota in healthy volunteers. *Clinics (Sao Paulo)* 62, 47–54

19 Norder Grusell, E. *et al.* (2018) The cultivable bacterial flora of the esophagus in subjects with esophagitis. *Scand J Gastroenterol* 53, 650–656

20 Sjöstedt, S. (1989) The upper gastrointestinal microflora in relation to gastric diseases and gastric surgery. *Acta Chir Scand Suppl* 551, 1–57

21 Sharpe, D.A. *et al.* (1992) The relevance of the microbiological flora of the upper alimentary tract to postoperative infection in major oesophageal surgery. *Eur J Cardiothorac Surg* 6, 403–5; discussion 406

22 Brook, I. (2006) The role of anaerobic bacteria in mediastinitis. *Drugs* 66, 315–320

23 Macfarlane, S. *et al.* (2007) Microbial colonization of the upper gastrointestinal tract in patients with Barrett's esophagus. *Clin Infect Dis* 45, 29–38

24 Blackett, K.L. *et al.* (2013) Oesophageal bacterial biofilm changes in gastro-oesophageal reflux disease, Barrett's and oesophageal carcinoma: association or causality. *Aliment Pharmacol Ther* 37, 1084–1092

25 Kaakoush, N.O. *et al.* (2015) Is Campylobacter to esophageal adenocarcinoma as Helicobacter is to gastric adenocarcinoma. *Trends Microbiol* 23, 455–462

26 Park, C.H. and Lee, S.K. (2020) Exploring Esophageal Microbiomes in Esophageal Diseases: A Systematic Review. *J Neurogastroenterol Motil* 26, 171–179

27 Pei, Z. *et al.* (2004) Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci U S A* 101, 4250–4255

28 Pei, Z. *et al.* (2005) Bacterial biota in reflux esophagitis and Barrett's esophagus. *World J Gastroenterol* 11, 7277–7283

29 Bor, B. *et al.* (2019) Saccharibacteria (TM7) in the Human Oral Microbiome. *J Dent Res* 98, 500–509

30 Liu, N. *et al.* (2013) Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. *BMC Infect Dis* 13, 130

31 Yamamura, K. *et al.* (2016) Human Microbiome Fusobacterium Nucleatum in Esophageal Cancer Tissue Is Associated with Prognosis. *Clin Cancer Res* 22, 5574–5581

32 Yamamura, K. *et al.* (2017) <i>Fusobacterium nucleatum</i> in gastroenterological cancer: Evaluation of measurement methods using quantitative polymerase chain reaction and a literature review. *Oncol Lett* 14, 6373–6378

33 Yamamura, K. *et al.* (2019) Intratumoral Fusobacterium Nucleatum Levels Predict Therapeutic Response to Neoadjuvant Chemotherapy in Esophageal Squamous Cell Carcinoma. *Clin Cancer Res* 25, 6170–6179

34 Liu, Y. *et al.* (2021) Fusobacterium nucleatum confers chemoresistance by modulating autophagy in oesophageal squamous cell carcinoma. *Br J Cancer* 124, 963–974

35 Olsen, I. and Yilmaz, Ö. (2019) Possible role of Porphyromonas gingivalis in orodigestive cancers. *J Oral Microbiol* 11, 1563410

36 Chen, M.F. *et al.* (2021) Porphyromonas gingivalis promotes tumor progression in esophageal squamous cell carcinoma. *Cell Oncol (Dordr)* 44, 373–384

37 Lagier, J.C. *et al.* (2018) Culturing the human microbiota and culturomics. *Nat Rev Microbiol* 16, 540–550

38 Bilen, M. (2020) Strategies and advancements in human microbiome description and the importance of culturomics. *Microb Pathog* 149, 104460

39 Loman, N.J. *et al.* (2013) A culture-independent sequence-based metagenomics approach to the investigation of an outbreak of Shiga-toxigenic Escherichia coli O104:H4. *JAMA* 309, 1502–1510

40 Pallen, M.J. (2014) Diagnostic metagenomics: potential applications to bacterial, viral and parasitic infections. *Parasitology* 141, 1856–1862

41 Hillmann, B. *et al.* (2018) Evaluating the Information Content of Shallow Shotgun Metagenomics. *mSystems* 3,

42 Deshpande, N.P. *et al.* (2018) Signatures within the esophageal microbiome are

associated with host genetics, age, and disease. *Microbiome* 6, 227

43 Gonzalez, A. *et al.* (2016) Avoiding Pandemic Fears in the Subway and Conquering the Platypus. *mSystems* 1, e00050–16

44 Chen, L.X. *et al.* (2020) Accurate and complete genomes from metagenomes. *Genome Res* 30, 315–333

45 Ciuffreda, L. *et al.* (2021) Nanopore sequencing and its application to the study of microbial communities. *Comput Struct Biotechnol J* 19, 1497–1511

46 Forster, S.C. *et al.* (2019) A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat Biotechnol* 37, 186–192

47 Almeida, A. *et al.* (2019) A new genomic blueprint of the human gut microbiota. *Nature* 568, 499–504

48 Gemmell, M.R. *et al.* (2018) Comparative genomics of Campylobacter concisus: Analysis of clinical strains reveals genome diversity and pathogenic potential. *Emerg Microbes Infect* 7, 116

49 Huse, S.M. *et al.* (2014) Comparison of brush and biopsy sampling methods of the ileal pouch for assessment of mucosa-associated microbiota of human subjects. *Microbiome* 2, 5

50 Elliott, D.R.F. *et al.* (2017) A non-endoscopic device to sample the oesophageal microbiota: a case-control study. *Lancet Gastroenterol Hepatol* 2, 32–42

51 Ravindran, S. *et al.* (2020) National census of UK endoscopy services in 2019. *Frontline Gastroenterology* doi: 10.1136/flgastro-2020-101538

52 Ho, K.M.A. *et al.* (2021) Predicting endoscopic activity recovery in England after COVID-19: a national analysis. *Lancet Gastroenterol Hepatol* 6, 381-390,

53 Li, X. *et al.* (2018) Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. *Nat Commun* 9, 2983

54 Reichenbach, Z.W. *et al.* (2019) Clinical and translational advances in esophageal squamous cell carcinoma. *Adv Cancer Res* 144, 95–135

55 Münch, N.S. *et al.* (2019) High-Fat Diet Accelerates Carcinogenesis in a Mouse Model of Barrett’s Esophagus via Interleukin 8 and Alterations to the Gut Microbiome. *Gastroenterology* 157, 492–506.e2

56 Corning, B. *et al.* (2018) The Esophageal Microbiome in Health and Disease. *Curr Gastroenterol Rep* 20, 39

57 Baba, Y. *et al.* (2017) Review of the gut microbiome and esophageal cancer: Pathogenesis and potential clinical implications. *Ann Gastroenterol Surg* 1, 99–104

58 May, M. and Abrams, J.A. (2018) Emerging Insights into the Esophageal Microbiome. *Curr Treat Options Gastroenterol* 16, 72–85

59 Okereke, I. *et al.* (2019) Associations of the microbiome and esophageal disease. *J Thorac Dis* 11, S1588–S1593

60 Piscione, M. *et al.* (2021) Eradication of Helicobacter pylori and Gastric Cancer: A Controversial Relationship. *Front Microbiol* 12, 630852

61 Brennan, C.A. and Garrett, W.S. (2019) Fusobacterium nucleatum — symbiont, opportunist and oncobacterium. *Nature Reviews Microbiology* 17, 156–166

62 Dubinsky, V. *et al.* (2020) Carriage of Colibactin-producing Bacteria and Colorectal Cancer Risk. *Trends Microbiol* 28, 874–876

63 Graillot, V. *et al.* (2016) Genotoxicity of Cytolethal Distending Toxin (CDT) on Isogenic Human Colorectal Cell Lines: Potential Promoting Effects for Colorectal Carcinogenesis. *Front Cell Infect Microbiol* 6, 34

64 Pasquereau-Kotula, E. *et al.* (2018) Significance of Streptococcus gallolyticus subsp. gallolyticus Association With Colorectal Cancer. *Front Microbiol* 9, 614

65 El-Zimaity, H. *et al.* (2018) Risk factors for esophageal cancer: emphasis on infectious agents. *Ann N Y Acad Sci* 1434, 319–332