Inferring diet, disease, and antibiotic resistance from the ancient oral microbiome
Gwyn Dahlquist-Axe¹,* , Francesca Standeven¹,* , Camilla Speller², Andrew Tedder¹,§, Conor J. Meehan³,§
¹School of Chemistry and Biosciences, University of Bradford, Bradford, United Kingdom
²Department of Anthropology, University of British Columbia, Vancouver, Canada
³Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom
*§These authors contributed equally

Abstract
The interaction between a host and its microbiome is an area of intense study. For the human host, it is known that the various body site-associated microbiomes impact heavily on health and disease states. For instance, the oral microbiome is a source of various pathogens and potential antibiotic resistance gene pools. The effect of historical changes to the human host and environment to the associated microbiome, however, is less explored. In this review, we characterize several historic and prehistoric events which are considered to have impacted the oral environment and therefore the bacterial communities residing within. The link between evolutionary changes to the oral microbiota and the significant societal and behavioral changes occurring during the pre-Neolithic, Agricultural Revolution, Industrial Revolution, and Antibiotic Era is explored. While previous studies suggest the functional profile of these communities may have shifted over the centuries, there is currently a gap in knowledge that needs to be filled. Biomolecular archaeological evidence of innate antimicrobial resistance within the oral microbiome shows an increase in the abundance of antimicrobial resistance genes since the advent and widespread use of antibiotics in the modern era. Nevertheless, a lack of research into the prevalence and evolution of antimicrobial resistance within the oral microbiome throughout history hinders our ability to combat antimicrobial resistance in the modern era.

Keywords
Oral microbiome, ancient DNA, antibiotic resistance, evolution

Impact Statement
Understanding the evolution of microbiomes is becoming of utmost importance as we begin to use them for managing our own health and surroundings. We have had a profound impact upon the microbial communities that live in and on our bodies, driven by changes in our diet, lifestyle and pharmaceutical use. As the genomics era continues to progress, we can now sequence the genomes of both modern and ancient microbiomes, allowing us to chart this evolution over centuries and millennia. However, ancient DNA analysis is fraught with issues, even more so than when dealing with modern metagenomics. Our review outlines both our current knowledge base the oral microbiomes evolutionary path but also the issues faced by such research, and what is still left unexplored.

Introduction
Over 1.5 billion years, coevolution between microorganisms and their hosts have resulted in reciprocal adaptation and functional integration, as shown in our own interaction with the majority of microorganisms that populate our body surfaces (Kilian et al. 2016). In this sense, for as long as humans and their ancestors have existed, we have been living and evolving alongside the microbiota that live in and around our bodies, simultaneously shaping one another’s survival strategies. Therefore, studying the ancient human microbiome and its constituents can aid our understanding of the evolutionary networks belonging to both humans and microorganisms.

The human microbiome consists of numerous communities of bacteria, archaea, viruses, fungi, protists, and other microorganisms. Microbial communities are located at various areas of the human body including the nose (Liu et al. 2020); vagina (Auriemma et al. 2021); the oral cavity (Gao et al. 2018); the skin (Byrd et al. 2018); and the human gastrointestinal tract – the latter being the most widely research area of the human microbiome (Lloyd-Price et al. 2016b) and which maintains the largest concentration and complexity of microorganisms in the body (Yuan et al. 2021). Each microbial community is distinct in its compositions and functions within the human body (Ursell et al. 2012). The communities of bacteria across the human body engage in a variety of interactions with their environment allowing them to efficiently respond to changes. The adaptive potential of human microbiome communities is key to their survival in fluctuating environments (Davenport et al. 2017).

In host-associated microbiomes, microbiota perform critical tasks that determine and contribute to the host’s physiology, establishing a unique biological interaction known as symbiosis (de Bary 1879; Oulhen et al. 2016). Host-microbiome symbiosis is an important benefactor for general human health. It regulates the cardiovascular system, aids host defense functions, harbours inflammatory properties, has metabolic potential and antioxidant activity, supports a healthy digestive tract, and donates resistance to pathogen colonization (Kilian et al. 2016). Dysbiosis, however, in context of the microbiome refers to changes in the structure of the microbiome that can be linked to various diseases and infection (Salvucci 2019).

Despite adaptation to a niche, microbes still need to defend themselves against attack from the host and other microbes. One of these vital elements developed by bacterial communities to ensure survival in harsh conditions is antibiotic resistance. Antibiotic resistance genes (ARGs) are plentiful in the modern human oral cavity, increasing the likelihood of resistant bacterial infections (Almeida et al. 2020). Investigating the development of ancient bacteria and their acquired resistance mechanisms may assist in the present antimicrobial resistance (AMR) epidemic. It is understood that the molecular mechanisms that led to the evolution of resistant bacteria have existed since ancient times, some of which are forerunners of today’s resistance factors (Christaki et al. 2020). The study of microbial evolution is a valuable source to understanding emerging resistance mechanisms in clinical settings and further. The cataloguing of the vast environmental reservoir of resistance genes provides hints as to what types of resistance may evolve in clinical settings under selective pressure (Perry et al. 2016). Furthermore, understanding the genetics and evolutionary pathways of antibiotic resistance may improve the success of current treatments to antibiotic resistant infections as well as reduce the emergence of resistance in the future (Palmer and Kishony 2013). The discovery that ancient human microbiomes can be preserved within archaeological materials and human remains opens the possibility of examining the evolution of AMR. Ancient samples are unaffected by
modern antibiotic use, and can therefore be utilized as clean models to investigate the natural diversity of ARGs (Rascovan et al. 2016).

Through extensive study of the bacterial communities that constitute various aspects of the human microbiome, links have been established between specific internal and external factors and changes to the composition or abundance of species in the human microbiome as well as their function (Kim et al. 2015). Knowing that a change in host lifestyle can result in a change in the microbiome, it is important to understand what changes will occur given the introduction of new dietary resources, new environmental exposures, or access to new medicines, such as those that occurred during the pre-Neolithic Era, the Agricultural Revolution, the Industrial Revolution, and the Antibiotic Era. This review aims to address our current understanding of how oral bacterial communities responded to the changes that occurred across thousands of years of human societal evolution and how we can use this knowledge of the past to inform the present and future of bacterial research.

The Oral Microbiome

The human oral microbiome consists of a vast array of microbial species whose functions and interactions with the host can influence many aspects of human health. The oral cavity is known to accommodate the second most diverse microbial community in the body, containing over 700 species of bacteria that populate the hard and soft tissues of the mouth (Kilian et al. 2016). Just one millilitre of saliva holds around $10^8$ microbial cells with an approximate range of 100-200 distinct bacterial organisms in a typically healthy microbiome (Willis and Gabaldón 2020a). A core bacterial community that is present in 66% or more of healthy human oral cavities includes such genera as Streptococcus, Granulicatella, Neisseria, and Haemophilus (Zaura et al. 2009). There are microenvironments within the oral cavity which will contain assemblages of bacterial species that may differ slightly in composition from that of the oral microbiome as a whole (Farrer et al. 2018). Microenvironments within the mouth, such as the teeth, tongue, cheeks, and gums, offer different physical and chemical properties to microbial communities (Mark Welch et al. 2020). The different properties associated with the different environments within the mouth, such as salivary flow (Proctor et al. 2018) and shedding versus non-shedding surfaces (Sedghi et al. 2021) (such as cheeks versus teeth), act as selective forces which allow certain species or communities of bacteria to more easily colonise one environment over another, resulting in localised bacterial communities.

On tooth surfaces, bacteria form multispecies communities that constitute a biofilm (Lamont et al. 2018a), which can mineralise into dental calculus if left untreated. Different species of bacteria within the same community often work together symbiotically to establish or enhance colonisation of various surfaces within the oral cavity. As different species of bacteria thrive in different nutrient-rich conditions, some species are expected to dominate whilst others diminish and vice versa depending on the nutrient availability in the oral cavity (Lamont et al. 2018a). Carbohydrates, and sugars specifically, supply many pathogenic species of oral bacteria with the nutrients necessary to establish colonisation and thrive (Lamont et al. 2018a). For this reason, dietary sugars are often blamed for instigating higher levels of dental disease both in ancient and modern humans, including caries and periodontal disease. While disease-causing bacteria in the oral cavity vary greatly,
a small number of the most predominant species identified in association with biofilm formation and/or caries initiation and progression include *Streptococcus mutans*, *Veillonella dispar*, and *Actinomyces gerencseriae* (Zhang et al. 2022). Bacteria associated with oral health, such as *Streptococcus mitis*, are more successful in an environment that is low in dietary sugars (Liu et al. 2012). These species can colonise surfaces within the oral cavity, and furthermore can discourage pathogenic species from binding and multiplying (Liu et al. 2012).

Genetic, behavioural, and environmental factors also contribute to determining which bacterial species will successfully colonise the oral cavity. Selective forces within the oral cavity, such as salivary flow and adhesion, shedding and colonization, and host and microbe interaction, exert selective pressures on microbial colonies (Welch et al. 2020). These forces have created habitat-specific subgroups of bacterial species whose genomes display adaptation to miniscule niches within the oral cavity (Utter et al. 2020). Bacteria are susceptible to physical and chemical attack, and therefore the biofilm production process can be disrupted through physical interruption (e.g. tooth brushing) as well as through chemical processes (e.g., fluoride exposure) (Lamont et al. 2018b). Physical and chemical attacks can also be introduced from within the bacterial community. Due to density and genetic diversity within a bacterial community, a large variety of aggressive behaviours have evolved among bacteria. Included amongst them are antibiotic production, toxin production, and mechanical weapons that can stab competitors (Granato et al. 2019). Conversely, bacteria can work together as a diverse community to detect and react to external stimuli, which may benefit the community as a whole, even if certain members of the community are harmed in the process (Li and Tian 2016). In the oral microbiome, bacterial communities are more likely to recruit a new species if a close relative of that species is not already included in the community (Duran-Pinedo et al. 2021). This suggests competition between closely related species and community level selection for diversity in oral bacterial communities (Duran-Pinedo et al. 2021), meaning that multiple species from the same genera are less likely to be found within the same oral community. This phenomenon makes it necessary to consider the ways in which oral bacterial communities respond to the loss or addition of new species over time and the functions they perform. For a bacterial community to thrive, a variety of functions must be performed by the members of the community, and so environmental changes that drives species loss or gain require a response from the community as a whole (Boon et al. 2014).

Both taxonomic composition and the functions performed by a microbial community are key factors in determining the health status of an individual. Some bacterial communities perform functions that cannot be carried out by human cells, such as production of certain vitamins (Das et al. 2019), creating a symbiotic relationship between human and microbes. However, multiple issues in both oral and general health can occur if that individual’s normal oral microbiota is disturbed (Kilian et al. 2016). Small numbers of opportunistic pathogenic bacteria can coexist alongside commensal bacterial without affecting the health of the host. Harmful bacteria can occasionally proliferate in enough numbers to overwhelm the population of beneficial bacteria. This is referred to as dysbiosis and is associated with several causes (Pizzorno and Murray 1998). Diseases directly associated with the oral cavity include caries, periodontitis, peri-implantitis, oral cancer and other mucosal diseases (Gao et al. 2018). Moreover, metagenomic studies have found correlations between a variety of organisms in the oral cavity and systemic disorders such as colorectal and pancreatic cancers, rheumatoid arthritis, and Alzheimer’s disease (Willis and Gabaldón 2020b) as well
as mental health (Wingfield et al. 2021). Lifestyle factors, such as diet or smoking, can contribute to the abundance of pathogenic bacteria and can affect whether the microbiome is in a state of symbiosis or dysbiosis with the human host (Ogunrinola et al. 2020).

**Genetic Approaches for Investigating Ancient Oral Microbiomes**

Early studies of bacteria preserved in dental calculus used microscopic analyses to identify bacterial species such as *Streptococcus mutans* in ancient oral microbiomes (Linossier et al. 1996). The discovery that dental calculus can preserve both ancient proteins and ancient microbial DNA which represents a portion of the ancient oral microbiome has allowed the fields of paleopathology, microbial ecology, and evolutionary biology to expand in new ways. Advancements in DNA sequencing technologies allowed for investigations of the ancient DNA (aDNA) preserved in dental calculus to increase in depth, from phylum-level characterizations of ancient oral microbiomes (Adler et al. 2013) to the discovery of genes with previously unknown metabolic functions (Klapper et al. 2023). However, it has been demonstrated that there is a difference in biofilm physiology between dental calculus and dental plaque (Velsko et al. 2019), making it important to consider that ancient dental calculus may not be an exact representation of the oral microbiome of the living human it was once hosted by.

Metagenomic characterizations of microbiomes have developed significantly in the past years with a move from 16S rRNA amplicon-based sequencing to whole genome sequencing (WGS). The benefits and hinderances of both 16S rRNA sequencing and WGS in modern contexts have previously been thoroughly analysed (Ranjan et al. 2016; Brumfield et al. 2020; Biegert et al. 2021). For reasons of cost and ease 16S rRNA sequencing is still popular for microbiome analysis, as it can rapidly and efficiently characterise the taxonomic profile of microbiome samples. 16S sequencing avoids the sequencing of human DNA, which can be seen as a contaminant in modern microbiome studies and thereby reducing the workload by lessening the production of data (Verma et al. 2018). In an archaeological context, however, the exclusion of human DNA can be considered a negative. Inclusion of human DNA in ancient studies can be especially important as sampling calculus from the teeth of human remains is a non-destructive technique and could replace destructive aDNA sampling techniques in certain situations. In such cases it would be integral to use WGS to ensure the inclusion of both bacterial and human aDNA.

Additional considerations when assessing data provided by 16S sequencing include evidence that ancient microbiome analyses using 16S sequencing have shown bacterial community profiles that don’t match biological expectations, as well as showing systemic taxonomic biases (Ziesemer et al. 2015b). Challenges related to 16S sequencing of modern microbiomes include less specific operational taxonomic unit detection and less precise beta diversity analyses than achieved when using deeper sequencing techniques (Pinto and Raskin 2012). Similar issues are likely to affect ancient samples as this approach requires high quality, well-preserved DNA whereas ancient human metagenomes are known to consist of highly degraded and fragmented aDNA (Ziesemer et al. 2015a). Furthermore, 16S rRNA variable region detection has a minimum threshold of 200 base pairs and many aDNA sequences do not meet that threshold and could therefore be misrepresented by 16S analysis (Stone and Ozga 2019).
Considering the obstacles involved when investigating aDNA, such as degradation, whole genome sequencing solved many issues that arose from 16S sequencing in studies of ancient human microbiomes, therefore shedding light on the origin and evolution of certain species, as well as virulence factors (Arriola et al. 2020). 16S rRNA sequencing has since been almost entirely replaced by WGS in ancient microbial research (Warinner et al. 2017). WGS provides high taxonomic and functional resolution (Hillmann et al. 2018), even for ancient materials. For example, analysis of the more than 5000-year-old mummified gut tissues of Otzi the Iceman using WGS successfully reconstructed the gut microbiome to the species level with additional analysis of functional profiles and virulence factors of genes in several pathogenic species (Lugli et al. 2017) (Figure 1). An earlier study of Otzi using 16S sequencing was able to determine bacterial phylogeny within the ancient gut environment but not bacterial function (Cano et al. 2000). This example shows how the progression of technology has impacted the specificity of data now available in the study of archaeogenomics and specifically ancient human microbiomes.

A significant factor hindering the success of ancient microbial DNA sequencing and subsequent analysis is contamination. Contamination of ancient oral microbial DNA preserved in dental calculus can be introduced by modern human intervention during excavation and laboratory analysis (Peyrégné and Prüfer 2020) as well as from soil and decomposition processes in the burial environment (Kazarina et al. 2019). However, even with the use of protective gear during excavation and sampling as well as decontamination protocols in labs, contaminant DNA can be present in sequenced aDNA and during subsequent analyses (Stone and Ozga 2019). The ability to identify contamination in aDNA sequences is vital to successfully study ancient microbiomes. Contamination is known to artificially inflate alpha diversity in ancient dental calculus samples, highlighting the importance of thorough decontamination (Farrer et al. 2021). Computational tools such as SourceTracker aid in this process by estimating the proportion of contaminants in a given sample based on a variety of potential source environments (Knights et al. 2011). There is also a selection of computational tools designed to remove contamination identified based on provided modern control samples, however the success of these methods in relation to ancient DNA is currently undocumented. Currently, genomic data gathered for ancient microbiome research is presented with the caveat that a certain degree of contamination is included.

Nevertheless, beyond the aforementioned issues regarding decontamination protocols, WGS has shown to be successful when reconstructing ancient microbiomes despite aDNA analysis still being a relatively young field of research (Fotakis et al. 2020; Neukamm et al. 2020; Granehäll et al. 2021; Ottoni et al. 2021). When considering research designs to fill current knowledge gaps about microbiome evolution such as AMR and niche adaptation in ancient periods, WGS should be implemented for the best chance at successfully identifying functions performed by ancient oral microbiomes.

The Evolution of Oral Microbiome Antimicrobial Resistance Mechanisms

When considering the functional genetic repertoire of oral microbial communities, AMR is an important factor. Understanding the role of AMR in the oral microbiome throughout human history is critical to understanding which factors may prompt an increase in ARGs. In southern Europe ARGs have been detected in historic and prehistoric individuals,
with one gene being more abundant in historic and prehistoric samples, and three genes being exclusive to modern day samples (Ottoni et al. 2021) (Figure 1). This differentiation in the gene that causes AMR suggests that factors impacting the evolution of ARGs in the human oral microbiomes can be inferred from archaeological evidence. Thus far, however, questions remain about how these variations of ARGs evolved and whether later versions evolved from earlier versions to adapt to new environments or if they evolved separately (Ottoni et al. 2021). Individuals from medieval Germany also show evidence of ARGs (Warinner et al. 2014), but a study of individuals from Mexico revealed no ARGs in ancient individuals, while they were identified in modern individuals (Bravo-Lopez et al. 2020). However, absence of evidence does not always mean evidence of absence and it is important to consider what causes ARGs to be readily identifiable in modern populations and less so in ancient individuals. These considerations include that ARGs are present in ancient individuals, but those DNA sequences are now too degraded to detect, as well as that these ancient sequences may go undetected if ARGs are not well represented in modern oral microbiome reference databases. Additionally, modern selective pressures, such as increased antibiotic usage in the environment and in clinical settings, have led to growing abundances of ARGs in contemporary oral microbiomes. Notably, the majority of ARGs that have been identified in past humans come from samples from the last century or more recently, suggesting that the most significant driver of ARG evolution is a relatively modern occurrence.

AMR in the human oral microbiome is a growing concern in modern dentistry and medicine (Ahmadi et al. 2021). ARGs are plentiful in the oral cavity, raising the likelihood of resistant bacterial infections (Almeida et al. 2020). For example, a high proportion of resistance genes have been identified in oral microbiota, most likely originating from streptococci, which are abundant in the oral cavity (Caselli et al. 2020). ARGs among oral bacteria not only jeopardise antibiotic therapy for oral infections, but they also prevent the treatment of other diseases that may occur in the body due to bacterial translocation (Almeida et al. 2020). For instance, oral commensal streptococci serve as a reservoir of resistance genes that are utilised by the pathogen Streptococcus pneumoniae, potentially obstructing treatment of serious systemic infections (Jensen et al. 2015). Based on a study that found an overlap in the oral cavity and stool samples in nearly half (45%) of individuals in the Human Microbiome Project (Segata et al. 2012), oral bacterial translocation to the rest of the body, particularly to the gut, is thought to be a common occurrence (Olsen and Yamazaki 2019). Understanding how widespread the concern of bacterial translocation was among ancient individuals would inform our knowledge of modern occurrences of oral to gut translocation and AMR. The rarity of these studies pertaining to ancient individuals may be due to the scarcity of archaeological materials that provide us with information on the gut, such as preserved intestinal content and coprolites, which only survive intact in very dry or extremely cold environments (Warinner et al. 2015). Furthermore, the coprolite and dental calculus of a single individual cannot be matched. However, by comparing skeletal remains with materials from ancient cesspits from the same site, population-level comparisons of the gut and oral microbiomes are possible.

It is acknowledged that some areas of AMR and oral microbiome research are understudied and, unlike the gut, the oral microbiome is understood to be an unexplored source of ARGs (Carr et al. 2020). The use of databases and consistent classification of ARGs is essential to their study (Alcock et al. 2020). Despite the widely acknowledged need to collect data on the prevalence and type of antimicrobial resistance due to the rising global
AMR threat, population studies with adequate number of subjects are limited (Caselli et al. 2020). Therefore, it is necessary for research to provide advanced characterisation of healthy oral microbial communities to be used as references for future comparative studies (Caselli et al. 2020). Despite these concerns, research regarding ARGs in oral microbiomes has thus far revealed a plethora of findings. Recent work on modern microbiomes has focused on the complexity of the oral microbiome and the presence of ARGs in periodontal disease (Almeida et al. 2020). Other research has identified ARGs of the β-lactamase class in neonate oral microbiome samples, suggesting that early neonate oral microbiota is regulated by maternal antibiotic treatment during childbirth (Gomez-Arango et al. 2017). Notably, one investigation has uncovered ARGs in the oral microbiome of a secluded, previously uncontacted Yanomami Amerindian community, despite having been isolated for possibly over 11,000 years in South America with no known exposure to antibiotics (Carr et al. 2020). Therefore, to understand ARG evolution in human oral microbiomes over time, researchers must explore the disparity between innate ARGs and exogenous factors that lead to their presence in the oral microbiome.

The analysis of ARGs identified in ancient oral microbiota is likewise rarely explored. Studying ancient resistance mechanisms in oral bacteria could present significant benefits given the current global AMR crisis. Further investigation should focus on ancient dental calculus as a useful source of ARGs. Their abundance gives insight to acquired ARGs in the oral cavity prior to the widespread use of modern antibiotics. Researchers may be able to develop strategies to block ARGs from propagating if more is discovered about why they exist in the oral cavity independent of the antibiotic era.

The Oral Microbiome Though the Ages

Pre-Agricultural Hominids

In recent years, the analysis of ancient oral microbes from archaeological samples has provided biomolecular insights into hunter-gatherer diets and diseases. Hunter-gatherers have been described as individuals who do not intentionally modify the gene pool of exploited resources, as opposed to those who rely primarily on an agricultural or pastoralist subsistence (Panter-Brick et al. 2001). Dental morphology is considered to be an indicator of diet, and previous studies have argued that excessive tooth wear is a sign of disease and the type of diet consumed by ancient individuals, and the pattern of wear can also provide further information on nutrition (Forshaw 2014). For example, the high level of dental wear and visible pathology is thought to correspond with the high meat content and hard fibre observed in the diets of hunter-gatherer populations, as well as from the inclusion of foreign particles in foodstuffs such as fine particles of stone in grains from grinding or grit present in shellfish (Forshaw 2014). Furthermore, since they consumed fewer simple carbohydrates, ancient hunter-gatherers often had a low prevalence of caries (Forshaw 2014). The effects of diet on the modern gut microbiome have received more investigative attention than the oral microbiome thus far (Lloyd-Price et al. 2016a), and therefore more is currently known on this subject compared to the impact of diet on the human oral microbiome. Some correlations have been found between diet and the modern oral microbiome, specifically linking vitamin B, C, and E intake to increased presence of bacterial species in the fusobacteria class (Kato et al. 2017). Understanding how oral microbiomes
responded to hunter-gatherer diets is an important step in understanding the evolution of oral bacterial communities and their functions in the human oral cavity.

Advancements in biomolecular techniques have provided further insights into the components of ancient oral microbiomes and has shed light on the evolutionary links between ancient humans, Neanderthals, and primates. Using a precise molecular clock to pinpoint the divergence of a nearly complete genome of the archaeal commensal *Methanobrevibacter oralis* in Neanderthal and modern human calculus, researchers discovered that commensal species were transported between the two hosts during subsequent encounters, possibly in the Near East (Weyrich *et al.* 2017). Furthermore, findings revealed that the oral microbiota of Neanderthals were more comparable to that of ancient chimps than contemporary humans and included less potentially harmful Gram-negative organisms linked to secondary enamel colonisation, enhanced plaque development and periodontal disease (Weyrich *et al.* 2017). Additionally, (Fellows Yates *et al.* 2021) compared 124 dental biofilm metagenomes from individuals including Neanderthals and Late Pleistocene to modern humans, chimpanzees, gorillas, and New World howler monkeys. This study discovered taxonomic and functional disparities among *Homo* and chimp (*Pan troglodytes*) oral microbiomes, but also many connections between Neanderthals and modern humans, such as an apparent adaptation to host diet. This research also revealed evidence of common genetic diversity in the oral bacteria of Neanderthal and Upper Palaeolithic humans that is not evident in later modern human populations (Fellows Yates *et al.* 2021). However, (Charlier *et al.* 2019) warn of the limits of comparing ancient tooth samples to modern individuals due to variables that can affect microbiome development and skew the molecular clock, such as the advent of refined sugar consumption and changes in oral hygiene practices causing issues in the reconstruction of microbial phylogenies from microbiome data. Furthermore, it has been noted that data collected from a diseased microbiome should be viewed with care since they are not always typical of that species in a non-diseased state. Therefore, the *M. oralis* genome detected in the study by Weyrich *et al.* (2017) may have been altered by the abscess-induced dysbiosis (Charlier *et al.* 2019).

Studying biomolecular components of ancient oral microbiomes has also provided further depth in understanding of ancient diets. aDNA isolated from 5700-year-old chewed birch pitch from southern Denmark was used to infer hunter-gatherer characteristics such as biological sex, geographical heritage, appearance, and disease (Jensen *et al.* 2019) (Figure 1). The study identified *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, a group of bacteria associated with severe types of periodontal disease. As a result, ancient mastics have been credited as a valuable source of information on the development of the human oral microbiome, which may be used to supplement studies of ancient dental calculus (Jensen *et al.* 2019). Nevertheless, calculus is more readily available in the archaeological record, and is a more attractive target for dietary reconstruction as it is linked with more well-preserved archaeological skeletons than other potential sources of aDNA (Table 1) (Mann *et al.* 2018).

Table 1: Description of archaeological materials sampled to study ancient oral microbiomes.

<table>
<thead>
<tr>
<th>Material type</th>
<th>Description</th>
<th>Sampling strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental calculus</td>
<td>Calcified dental plaque, also called tartar, is the result of untreated/built-up dental</td>
<td>Dental calculus is sampled from archaeological human</td>
</tr>
</tbody>
</table>
Many populations outside of Europe remained hunter-gatherer societies for much longer than populations in Europe. Many indigenous North American populations did not adopt agricultural practices until after they were colonised by Europeans in the 1500-1700s (Lightfoot Kent 2004), while some indigenous communities maintained some of their traditional subsistence activities even as colonial practices were forced upon them (Gamble 2015). Pre-European contact indigenous populations in what is now the San Francisco Bay area of California displayed site-level diversity in oral bacterial species, with individuals from one site displaying higher abundances of *Enterococcus faecalis* and *Enterococcus faecium* and individuals from another site displaying higher abundances of *Pseudopropionibacterium porpionicum* (WADA et al. 2018). Additionally, the more recently formed village of those included in the study showed a lower alpha diversity when compared with a nearby, more established village site. This may speak to the nature of the inhabitants of the more recently established village, in that they were possibly of lower social status with access to less diverse dietary resources or that individuals already suffering from dietary distress chose to establish a new village site (WADA et al. 2018). Such insights into the connection between oral microbiome composition and host behaviour is an important step to understanding how certain functions, such as metabolites or AMR, developed within ancient and modern microbiomes alike.

Dental calculus has revealed a great deal regarding pathology and diet of ancient hunter-gatherers. A combined genomic and proteomic analysis on the oral microbiome composition of two Palaeolithic hunter-gatherer individuals from San Teodoro cave in Italy showed a diet of rich animal proteins (Scorrano et al. 2021). This multi-omics approach also allowed for identification of bacterial composition in both individuals, such as *Actinomyces*, *Streptococcus*, and *Propionibacterium*, which are commonly linked with the modern oral microbiome (Scorrano et al. 2021). Other studies have identified different diets between distinct gatherer groups via dental calculus through the analysis of shotgun sequencing data (Weyrich et al. 2017). These findings suggest that one group consumed a largely meat-based
diet, while the other utilised mostly herbaceous foodstuffs, specifically a type of moss called *Physcomitrium patens*. However, due to doubtful interpretations of this plant species, critics have called for cleaner databases, decontaminated genomes, and more efficient mapping algorithms for metagenomics datasets to be developed in the future to enable effective taxonomic estimations from ancient metagenomic samples (Haas et al. 2022). However, Weyrich et al. (2017) not only determined potential diet and disease, but also offered fascinating theories concerning ancient hominin behaviours. One individual with a tooth abscess and a possible gastrointestinal infection showed evidence of self-medication by ingesting poplar (*Populus trichocarpa*), a plant containing the natural pain reliever salicylic acid — the active component in aspirin — as well as the natural antibiotic generating *Penicillium sp.* from the moulded plant (Weyrich et al. 2017). From investigations of ancient diet and disease during times of cultural transition via oral microbiomes preserved in the archaeological record, we are able to better understand events that held the potential to inflict selective pressure upon oral microbial communities. Investigations of ARGs in hunter-gatherer communities would be beneficial for the understanding of the ancient origins of resistance mechanisms in bacteria before the use of modern antibiotics.

The Agricultural Revolution

Different populations across the globe transitioned from hunter-gatherer to an agricultural subsistence strategy at varying times (Figure 1). Several dietary, environmental, and lifestyle differences accompanied these transitions and in turn impacted the human oral microbiome. Non-genetic methods of investigation can provide useful evidence of dietary practices in ancient individuals, which can in turn inform our understanding of changes in composition or abundance in ancient human oral microbiomes. For example, stable isotope and microbotanical evidence demonstrate a transition from freshwater fish and terrestrial carnivores as the main sources of dietary sustenance during the Late Mesolithic in the Central Balkans to heavier reliance on terrestrial resources and the cultivation of crops during the transition to agriculture (Jovanović et al. 2019; Jovanović et al. 2021). This knowledge of ancient diet can inform our understanding of changes in oral disease prevalence, as well as potential changes in ARGs in the oral cavity. There are currently few studies that investigate the relationship between AMR and agricultural transitions in the past. However, it is important to consider how our modern agricultural systems contributed significantly to the spread of AMR and how this could be mirrored in ancient agricultural systems. For example, farm waste discharge, manure spreading, animal feed, livestock, and deadstock have been associated with the spread of AMR in modern settings within a large and complex network of transmission pathways connecting humans to a variety of environments (Christaki et al. 2020). It is currently unknown to what extent these transmission pathways may have existed in ancient agricultural settings but would be a worthy route of investigation to further our understanding of ARGs and AMR prior to the antibiotic era.

Investigation of the impact of the transition to agriculture in the Balkans and Italian Peninsula on the human oral microbiome revealed a functional shift in bacterial genes (Ottoni et al. 2021). There was not a significant difference in the functional profiles of foragers and farmers from this region, however foragers had an enrichment of a pathway associated with the breakdown of carbohydrates, indicating a highly plant-based diet (Ottoni
et al. 2021). Migration regions and the relatively slow uptake of agriculture may be reasons for the lack of differentiation between foragers and farmers in southern Europe and therefore inclusion of modern studies where such variables can be controlled for can clarify these issues. Three pairs of modern communities practising hunter-gatherer and traditional farming subsistence strategies living in close proximity in the Philippines provide the opportunity to control for many of the unknown variables that come with archaeological study. These populations in the Philippines showed specific differences in oral microbiome composition and gene function between hunter-gatherer and traditional farmers (Lassalle et al. 2018). The oral microbiomes of the hunter-gatherer populations contained species linked to oral pathogens despite the hosts’ good oral health (Lassalle et al. 2018), suggesting a different niche for these species in this context. Furthermore, hunter-gatherers had additional gene functions linked to vitamin B production, which could be driven by less vitamin B5 in the hunter-gatherer diet (Lassalle et al. 2018). The inclusion of modern individuals with typical western diets shows oral microbiomes with gene functions linked to pH regulation that buffers biofilms against acidification (Lassalle et al. 2018), potentially caused by the increase in sugar consumption in the western diet and the resulting changes in the oral environment.

The above examples illustrate how the changes to diet and lifestyle brought about by the advent of agriculture likely impacted the human oral microbiome, but variables beyond our control in archaeological samples may mask some of these changes. Additionally, the overarching similarities between the oral microbiomes of hunter-gatherers and traditional farmers in the Philippines indicates that factors such as exchange of resources and physical interaction may confound the results of both modern and archaeological studies (Weyrich 2021). These findings are further substantiated by a similar study of modern hunter-gatherers in the Philippines. This study illustrated a similar shift in the oral microbiome between those relying on the fish and meat heavy diet of hunter-gatherers to those relying on the rice-rich diet of farmers, representing an agricultural revolution (Dobon et al. 2023).

With the understanding that functional profiles of oral microbiomes were potentially impacted for ancient populations transitioning from hunter-gatherer subsistence to agricultural subsistence, it is possible that AMR was one of the functions also changing in prevalence at this time. The current scarcity of data on this topic prevents deeper understanding but would be a worthwhile avenue of investigation in the future.

Analysis of several ancient populations has demonstrated that the adoption of agriculture did not always have a significant impact on the oral microbiome. In Japan, hunter-gatherer subsistence was practiced until approximately 3000 years ago when agriculture was introduced by mainland Asian immigrants (Eisenhofer et al. 2020). The geographical and cultural separation of Japan from mainland Asia before this admixture potentially allowed a distinct oral microbiome to develop among this population. Individuals from approximately 1000 BCE and 1600-1870 CE show no significant differences in diversity of species, composition of species, or abundance of species (Eisenhofer et al. 2020). However, agricultural practices in Japan are not identical to those in Europe and therefore the transition to crop cultivation and animal husbandry could have had very different interactions with the oral microbiomes of individuals in Japan. While in this case the transition to agriculture did not have any detectable or significant impacts on the oral microbiome, this study provides useful evidence that all cultures globally may not respond to changes in subsistence strategy the same way, which may in turn impact the oral microbiome in unique ways or not at all. In contrast to this example from Japan, one study
has detected notable differences in the oral microbiome of European populations transitioning to agriculture and beyond (Adler et al. 2013), which will be explored in more detail in the discussions of the Industrial Revolution.

As another example of agricultural transitions outside of Europe, Mexico experienced colonisation by Europeans which instigated a change in cultural and subsistence practices (Roca-Rada et al. 2020) (Figure 1). Comparisons between pre- and post-colonial Mexican populations can demonstrate possible changes in the oral microbiome initiated by colonial presence. The oral bacteria species *Tannerella forsythia* presents as an example of transmission dynamics, as post-colonial Mexican individuals had *T. forsythia* strains that resemble both ancient European and modern strains, while pre-Colonial individuals had a separate strain unrelated to modern or ancient European strains (Bravo-Lopez et al. 2020). Additionally, no ARGs were detected in the pre-colonial individuals but were identified in the post-colonial individuals, suggesting that the events that followed colonialism in Mexico were not drivers off AMR in the oral microbiome, but rather a more recent selective pressure (Bravo-Lopez et al. 2020).

The Industrial Revolution

In Britain, the technological advancements that were introduced during the Industrial Revolution allowed for new and diverse foods to be mass produced and widely distributed, as well as bring in new ingredients through increased trade (Kelly and Ó Gráda 2013) (Figure 1). Modern investigations of the impact of diet on the human microbiome can inform predictions of how these changes during the Industrial Revolution may have impacted the oral microbiome of individuals at that time. There is some disagreement regarding whether increasing certain components in overall diet can lead to an identifiable change in bacterial abundance within the human microbiome (Xu and Knight 2015; Kato et al. 2017; Eisenhofer et al. 2020), indicating that this concept requires further study. Further research on the effects of dietary changes brought on by the Industrial Revolution on the number of bacterial species in the human oral microbiome could clarify this discrepancy during the Industrial Era.

It is firmly established by stable isotope studies (O'Donoghue et al. 2021), written records such as cookbooks (Glasse 1747; Glasse 2015), and archaeological investigations of human remains (Rando et al. 2014) that the food resources available and the average individual’s diet during the Industrial Revolution was remarkably different than earlier and later diets. The potential link between processed foods and the oral microbiome is a necessary consideration, as the Industrial Revolution brought with it increased manufacturing and availability of processed foods and drinks (Hudson 2014). Food additives, such as emulsifiers, preservatives, and artificial sweeteners, have been shown to alter the gut microbiome resulting in the multiplication of pro-inflammatory organisms and repression of anti-inflammatory organisms (Kamm 2020). Considering the evidence provided from studies of modern individuals, it is reasonable to conclude that the dietary shift that occurred during the Industrial Revolution in Britain had impacts on the abundance or composition of bacterial species in the human oral microbiome at that time, potentially forcing the affected bacterial communities to adapt the way they function as individuals and as communities.
The first amplicon metagenomic study of archaeological dental calculus investigates microbial ecology shifts associated with industrialization. The amplicon metagenomic study of 34 European individuals dating from the Mesolithic to the Medieval period was able to demonstrate that the human oral microbiome underwent a shift at the onset of the Industrial Revolution (Adler et al. 2013). Notably, a rise was identified in the cariogenic species *Streptococcus mutans* between the Medieval period and the modern era. (Adler et al. 2013) (Figure 1). While identifying changes or stability in the oral microbiome associated with specific time periods is important, it is hasty to assign them a specific cause, such as the introduction of industrialisation. Due to the abundance of socio-economic changes occurring during a short period of time during the Neolithic and Industrial Revolution and the incomplete evidence of the archaeological record, it is impossible to control for variable and therefore determine if the cause of a certain change in the oral microbiome was due to the introduction of animal husbandry, new dietary resources, new trade routes, or a myriad of other factors (Box 1).

A study of individuals from Italy dating from the 11th to 19th century showed that the oral microbiomes of these ancient humans consisted of the same commensal bacteria species as modern day human oral microbiomes (Santiago-Rodriguez et al. 2019). This study further suggests that bacterial species presence and absence remained stable across time and within different locales, but that factors such as time-period, geographic location, sec, and socioeconomic status of an individual could affect relative abundance of such bacterial species in the oral microbiome. However, these possibilities could not be investigated due to the limited sample size of the study. An attempt was made to investigate the link between specific bacteria and lifestyles or dietary habits but was unsuccessful, possibly again, due to the limited sample size. Given an appropriately sized sample and consideration of the many complicated factors involved in interpreting ancient contexts, the prospect of linking specific bacterial assemblages to certain lifestyle factors or dietary inclusions in ancient populations may still be a viable route of investigation.

Thus far, the study of ARGs in the oral microbiome of industrial age individuals in Europe is an under researched topic, as no studies were found which attempt to identify such genes, primarily owing to a lack of suitable genomic data. Currently, research regarding the evolution of ARGs in the human oral microbiome identifies few of these genes in ancient humans and a significant increase in their frequency in the oral microbiomes of individuals who lived after the advent of widespread antibiotic use. It will be important to determine what role the Industrial Revolution played in the large-scale emergence of ARGs. The evidence outlined above suggesting that the Industrial Revolution provided many opportunities for oral bacterial communities to adjust their composition or function could also suggest that there may have been opportunities for oral bacterial species to develop ARGs. Increased availability of personal hygiene products such as soap (Pears 1859) and foodstuffs with antibacterial properties such as garlic (Petrovska and Cekovska 2010; Karic et al. 2020) could have contributed to an environment of increased selective pressure for microbial species during the Industrial Revolution. This, in turn, would lead to an increased rate of AMR in bacterial communities as they fight to survive in the new, harsher environment. While there is currently no evidence to suggest that the Industrial Revolution was any more of an instigator of AMR than the Neolithic Revolution, the lack of research in this area indicates a gap that needs filling to understand the complete history of AMR evolution and factors that may contribute to combating these genes in the modern era.
The Antibiotic Era

Since the discovery and widespread use of antibiotics to treat human infection in the 1940s (Figure 1), antibiotics have been used globally for the treatment of human infection, animal husbandry, and agricultural pests, among others (Fymat 2017). However, antibiotic misuse, overuse, and underuse tends to increase the antibiotic load in the environment, leading to the evolution of antibiotic resistance in the microbial community (Bombaywala et al. 2021). Factors such as the unnecessary prescribing of antibiotics (Imanpour et al. 2017) and their excessive use in animal stock feeds (Fair and Tor 2014) are particularly known to have exacerbated the spread of antibiotic resistance. On a molecular level, AMR can arise from mutations or the acquisition of resistance-conducting genes via horizontal gene transfer in bacteria, with the latter being a major factor in the current AMR crisis (Von Wintersdorff et al. 2016).

Modern dental hygiene practices, technology, dietary changes, and antibiotics are all thought to have contributed to the human oral microbiome composition in the antibiotic era and can have both positive and negative effects on disease, dysfunction, and microbial diversity. Oral health self-management based on adequate toothbrushing, flossing, and frequent maintenance of dentures (Schensul et al. 2020), as well as mechanical plaque removal have been found to be the most effective methods of disease prevention (Rosier et al. 2014). However, modern dental hygiene techniques can be potentially harmful, as pathogens such as Acinetobacter baumannii, Staphylococcus aureus, and Candida albicans can reside on toothbrushes and are thought to raise the host’s risk of infectious diseases, neurological disorders, cardiovascular disease, and cancer (Shang et al. 2020). It is possible that increased mobile phone usage has influenced the modern oral microbiome as it is linked elevated salivary flow rate and parotid gland secretion, as well as reduced protein production (Bhargava et al. 2012; Jeevitha and Anuradha 2020).

Changes in diet, the Industrial Revolution, and the excessive use of antibiotics are all thought to have contributed to the reduction in biodiversity and higher abundance of pathogenic bacteria in the modern oral microbiome (Baroudi et al. 2016). Evidence provided by Adler et al. (2013) demonstrated how a carbohydrate-rich Neolithic farming diet and industrialised processing of wheat and sugar were two of the most significant dietary transitions in human evolution that have affected the human oral microbiome, such as the increase in Streptococcus mutans. However, there is little archaeological data from the Industrial Revolution to show that processed grains and sugar caused a string of dietary habits that resulted in less diverse and more diseased oral microbiomes today, and therefore additional research is required.

In a study of modern individuals in the United States designed to include the natural variation of oral microbiomes found in the general human population, the most abundant genera in both youths and adults was Streptococcus, followed by Haemophilus (Burcham et al. 2020). Furthermore, the pathogenic genera Treponema was associated with poor oral health habits in adults as well as obesity in youths (Burcham et al. 2020). To investigate the microbial components of a healthy oral microbiome in modern individuals, Caselli et al. (2020) conducted a study of 20 individuals from Italy with good oral health status. Streptococci, Neisseria, Prevotella, and Haemophilus were the most abundant genera across all oral sites sampled. Genera typically associated with oral pathogens, such as Actinomyces, Veillonella, and Fusobacterium, were less prevalent in all sites except subgingival plaque.
(Caselli et al. 2020). Additionally, a high proportion of strains harbouring resistance genes were identified, likely originating from the abundant streptococci (Caselli et al. 2020). *Streptococcus* and *Actinomyces* have also been identified in the oral microbiomes of pre-Neolithic individuals (Gabriele et al. 2021), illustrating the stability of certain components of the human oral microbiome over time. Furthermore, pathogenic species *Tannerella forsythia* and the genera *Treponema* have been identified in ancient individuals (Jensen et al. 2019) allowing for inferences to be made about the health status of these ancient individuals.

Resistance in ancient oral microbiomes prior to the use of modern antibiotics had not been thoroughly investigated until a study by Warinner et al. (2014) explored ARGs in ancient dental calculus dating to the medieval period. This study uncovered resistance genes to aminoglycosides, β-lactams, bacitracin, bacteriocins and macrolides, in addition to genes for multidrug efflux pumps. *Tannerella forsythia*, on the other hand, was found to be devoid of resistance genes. This demonstrates that this pathogen had not yet acquired resistance mechanisms in the distant past, as evidenced by a wide gap in the genome reconstruction performed by the research. This genome corresponded to a complete conjugative transposon carrying putative tetracycline resistance gene that was missing in the reconstructed ancient *T. forsythia* (Warinner et al. 2014). This study provides evidence for the absence of its resistance gene prior to widespread antibiotic use and its evolved presence after, implying that the antibiotic era was a contributing factor for this evolutionary trait. A more recent study proposed that patterns of distinct metabolic pathways in contemporary samples were caused by changes in the functional activity of the oral microbiome since the mass manufacture of antibiotics (Ottoni et al. 2021). These findings, in addition to other processes underpinning the acquisition and loss of resistance mechanisms, may be investigated more thoroughly in the future by collecting more sequencing data from ancient and modern calculus (Ottoni et al. 2021) (Box 2).

**Conclusion**

The established understanding of the human oral microbiome is based on knowledge of individual bacterial species’ behaviour, how bacteria interact with other bacteria within the oral cavity, and how bacterial communities respond to external influences. This examination has shown how the significant societal and behavioural changes occurring during the pre-Neolithic, Agricultural Revolution, Industrial Revolution, and Antibiotic Era likely influenced the evolution of the human oral microbiome. Furthermore, the genomic and functional profile of these communities may have shifted over the centuries, but there is currently a gap in knowledge that needs to be filled. Compared to other habitats and microbiomes, there is a deficit in ARGs databases and study on ARGs in the ancient human oral microbiome. There is a particularly significant deficiency of research regarding ARGs from the Industrial Revolution. Given the current AMR crisis, it is worthwhile to investigate ancient resistance mechanisms in bacteria. Further research should concentrate on ancient dental calculus as a valuable source of ARGs, as the extent of their abundance sheds light on acquired ARGs in the oral cavity prior to the widespread use of modern antibiotics. Whole genome sequencing provides the high-quality data necessary to study these genes and their functions in ancient oral microbiomes. Overall, understanding the nature, richness, and evolution of resistance in the past offers predictive and comparative value for the future.
Funding
Gwyn Dahlquist-Axe is funded by the University of Bradford School of Chemistry and Biosciences and Francesca Standeven is funded by the University of Bradford Faculty of Life Sciences. No other authors have external funding to acknowledge.

Author Contributions
G.D, F.S, A.T and C.J.M conceived of the review. G.D and F.S led the drafting and compilation of sources. A.T. and C.J.M led the editing. All authors contributed the final draft of the review and the approved manuscript.

Conflicts of Interest
The authors declare no conflicts of interest


preservation of endogenous human and microbial DNA in dental calculus and dentin. *Scientific Reports* 8 (1), 9822.


Pears, F. (1859) *The skin, baths, bathing, and soap*. The author.


Figure Legends

Figure 1. Timeline of notable events in human history pertaining to the evolution of the oral microbiome.
Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola identified in hunter gatherers dating to 5700 BCE (Jensen et al. 2019)

Clostridium perfringens and Pseudomonas veronii identified in Otzi the Iceman dating to 3000 BCE (Lugli et al. 2017)

Different strains of Tannerella forsythia identified in Mexican individuals – pre/post 1500s CE (Bravo-Lopez et al. 2020)

AMR genes detected in oral microbiomes of Neolithic individuals (Ottoni et al. 2021)

Advent of agriculture in Europe – 4000 BCE

Medieval period Europe – 500 – 1400 CE

Industrial Revolution in England – 1750 CE

Advent of the Neolithic era in Europe – 10000 BCE

Widespread use of antibiotics – 1940s CE

ADVENT OF AGRICULTURE IN EUROPE – 8000 BCE

ADMIRAL CONNECTORS DETECTED IN ORAL MICROBIOMES OF NEOLITHIC INDIVIDUALS (OTTONI ET AL. 2021)

DIFFERENT STRAINS OF TANNERELLA FORSYTHIA IDENTIFIED IN MEXICAN INDIVIDUALS – PRE/POST 1500S CE (BRAVO-LOPEZ ET AL. 2020)

CLOSTRIDIUM PERFRINGENS AND PSEUDOMONAS VERONII IDENTIFIED IN OTZI THE ICeman DATING TO 3000 BCE (LUGLI ET AL. 2017)

AMR GENES DETECTED IN ORAL MICROBIOMES OF NEOLITHIC INDIVIDUALS (OTTONI ET AL. 2021)

ADVENT OF AGRICULTURE IN EUROPE – 4000 BCE

MEDIEVAL PERIOD EUROPE – 500 – 1400 CE

INDUSTRIAL REVOLUTION IN ENGLAND – 1750 CE

ADVENT OF THE NEOLITHIC ERA IN EUROPE – 10000 BCE

WIDESPREAD USE OF ANTIBIOTICS – 1940S CE

8000 BCE

6000 BCE

4000 BCE

2000 BCE

0 CE
Box 1: Description of preservation bias and issues affecting the study of microbiomes from archaeological materials.

Investigation of archaeological material is accompanied by the issue of preservation bias, meaning that not all materials will survive in the archaeological record (Jackes 2011) and therefore many variables are impossible to control for because we are unable to determine every detail of an ancient individual’s life. Consequently, it may be impossible to control for factors such as diet or profession. Ancient humans were likely to have participated in an array of activities that would be considered factors able to affect the oral microbiome and it is therefore difficult to determine the exact cause of any potential detected changes in the oral microbiome.

Box 2: Further details regarding ARG data collection, storage, and sharing and the issues present with this type of work.

The use of databases and consistent classification of ARGs is essential (Alcock et al. 2019), and there are various bioinformatics software, databases, and data sharing resources available for identifying, predicting, cataloguing, and analysing molecular sequences of ARGs (McArthur and Tsang 2017). The Comprehensive Antibiotic Resistance Database (CARD), for example, is a regulated tool that provides reference DNA and protein sequences, detection models, and bioinformatics tools on the molecular bases of bacterial antibiotic resistance (Alcock et al. 2020). Despite the widely acknowledged need to collect data on the prevalence and type of drug resistance due to the rising global AMR threat, population studies and adequate number of subjects are limited (Caselli et al. 2020). As a result, it is necessary for investigations to provide advanced characterisation of healthy oral microbial