Metagenomic insights into transferable antibiotic resistance in oral bacteria

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Abstract

Antibiotic resistance is considered one of the greatest threats to global public health. Resistance is often conferred by the presence of antibiotic resistance genes (ARGs) which are readily found in the oral microbiome. In-depth genetic analyses of the oral microbiome using metagenomic techniques reveal a broad distribution of ARGs (including novel ARGs) in individuals not recently exposed to antibiotics, including humans in isolated, indigenous populations. This has resulted in a paradigm shift from focusing on the carriage of antibiotic resistance in pathogenic bacteria to a broader concept of an oral resistome, which includes all resistance genes in the microbiome. Metagenomics is beginning to demonstrate the role of the oral resistome and horizontal gene transfer within and between commensals in the absence of selective pressure, such as an antibiotic. At the chairside, metagenomic data reinforces our need to adhere to current antibiotic guidelines to minimise the spread of resistance as it reveals the extent of ARGs without exposure to antimicrobials and the ecological changes created in the oral microbiome by even a single dose of antibiotics. The aim of this review is to discuss the role of metagenomics in the investigation of the oral resistome including the transmission of antibiotic resistance in the oral microbiome. Future perspectives, including clinical implications of the findings from metagenomic investigations of oral ARGs will also be considered.

Introduction

The success of any therapeutic is compromised by the potential development of resistance to that compound. This is illustrated by the declining efficacy of clinically used antibiotics. Significant reductions in mortality rates attributed to various infectious diseases, increases in life span and quality of life are now being tempered by the unprecedented rise in antibiotic resistance, representing one of the most serious global threats to modern medicine (Antimicrobial Resistance 2015). Dental practitioners are very aware of the significant clinical and financial burden of antibiotic resistance (Cope *et al.* 2014), however, the perception that antibiotic resistance is not a dental problem persists within the profession, justified by the

nature (short courses using a narrow range of antibiotics) and number of prescriptions - under 10% of scripts worldwide are prescribed for dental conditions (Sweeney *et al.* 2004, Bagg 2014).

Established definitions of antibiotic resistance are based on the likelihood of therapeutic failure in clinical isolates in mammalian populations (Berendonk *et al.* 2015). However, this definition fails to characterise the data provided by new approaches to studying antibiotic resistance, such as metagenomics (Martinez *et al.* 2015). The term 'metagenomics' was coined in 1998 to define the direct, function-based analysis of environmental samples containing a mixture of species (Handelsman *et al.* 1998). Next generation sequencing (NGS) technologies have advanced the metagenomic approach of studying microbes in their natural environments without the need for isolation and cultivation of individual species. Culture-based studies are unable to provide this comprehensive view of the oral microbiota, as less than one percent of bacteria, from some environments can currently be grown on solid culture media (Wade 2011). NGS studies of oral microbiota have revealed the huge diversity of bacteria in the oral environment with up to 1179 oral taxa found, of which about 68% were uncultured phenotypes (Dewhirst *et al.* 2010). This ecological community of commensal, symbiotic and pathogenic microorganisms that reside in the oral cavity in both planktonic and biofilm form is known as the oral microbiome (Lederberg and McCray 2001). The ability to isolate and analyse the entire metagenome of the oral and other microbiomes (Martinez *et al.* 2015) has resulted in a paradigm shift in our understanding of antibiotic resistance. The focus is no longer simply on understanding the carriage of resistance in cultivable, pathogenic bacteria but in the broader concept of pools of resistance genes within the commensal bacterial population and the potential of transfer of this resistance to pathogens. This collective has been labelled the resistome (Wright 2007). The resistome is the part of the metagenome of the oral microbiome which confers antibiotic resistance.

The aim of this review is to discuss the role of metagenomics in i) the comprehensive investigation of the oral microbiome; ii) investigating the distribution and diversity of antibiotic

resistance genes (ARGs); iii) transmission of antibiotic resistance in the oral microbiome and iv) increasing our understanding of the resistance profiles of specific human pathogens. Future perspectives, including clinical implications of the findings from metagenomic investigations of oral ARGs will also be considered.

Antibiotic Resistance Phenotype

Antibiotic resistant phenotypes can either be intrinsic (due to a pre-existing physiological trait of the species) or acquired, via horizontal gene transfer (HGT) or by mutation. Intrinsic resistance is exemplified by vancomycin resistance in *Escherichia coli*. The vancomycin molecule is simply too large to pass through the porin channels in the outer membrane of the cell wall, thus rendering the antibiotic ineffective (Chen *et al.* 2009).

Bacteria within a biofilm (the majority of oral microbiota) show increased resistance to antibiotics compared with planktonic bacteria. This is due to the structure, physiology and resultant socio-microbiology of the biofilm (Høiby *et al.* 2010). The diverse ecological pressures in the oral cavity are a consequence of physical and chemical variations in this environment, which requires the individual members of the oral biofilm to adjust their metabolic and genomic activity in order to cope with these stresses. Thus, the nature of the oral biofilm permits, and may favour, complex bacterial interactions including HGT (Roberts and Kreth 2014). Acquired resistance represents a more flexible phenotype, and its prevalence is more immediately responsive to selection pressure (Martinez 2008). The majority of antibiotic resistance in human commensals and pathogens is acquired through HGT (Alekshun and Levy 2007, Hannan *et al.* 2010).

An ARG is defined as a specific gene which when expressed renders an otherwise susceptible host more resistant to a particular antibiotic. In fact, phylogenetic studies have determined that many ARGs have a long evolutionary history that predates the antibiotic era, as most antibiotics in use are naturally made by microbes (Aminov and Mackie 2007, D'Costa *et al.* 2011). It is suggested that the likely origin of ARGs in human commensals,

such as oral bacteria, are from the environment (Pehrsson *et al.* 2013), as diverse homologues of known resistance genes have been found to be broadly distributed across environmental locales (D'Costa *et al.* 2011). To date, the oral microbiota has been found to contain a broad distribution of ARGs, including in individuals with no recent exposure to antibiotics and isolated indigenous populations (Seville *et al.* 2009, Schmieder and Edwards 2012, Clemente *et al.* 2015, Rampelli *et al.* 2015). Antibiotic resistance genes found in an Amerindian population with no exposure to pharmacological grade antibiotics were thought to be either the result of HGT with antibiotic producing soil microbes or to have evolved in a soil dwelling ancestor of a human commensal (Clemente *et al.* 2015). In spite of our lack of understanding of the origins of resistance, the evidence is unequivocal; the introduction and widespread use of antibiotics has selected for ARGs (Roberts 1998). In fact, it has been demonstrated that in some circumstances the presence of low levels of antimicrobials in the environment is a key signal that promotes horizontal gene transfer of ARGs (Seier-Petersen *et al.* 2014, Berendonk *et al.* 2015).

Methods for identifying ARGs in the oral microbiome

Investigation of ARGs by culture-independent amplification-based methods, such as PCR and DNA microarrays, are limited by low throughput, limited availability of primers (generally targeting known pathogens and ARGs) and amplification bias. High-throughput sequencing based metagenomic analysis overcomes a number of these limitations, (Li *et al.* 2015) allowing for screening of ARGs in both culturable and non-culturable bacteria and importantly, the detection of novel ARGs. Furthermore, NGS is fast, robust and cost-effective (Thomas *et al.* 2012).

There are two different metagenomic approaches to investigating antibiotic resistance; sequence-based and functional studies (Schmieder and Edwards 2012, Mullany 2014). Sequence-based metagenomics involves the extraction and random (shot-gun) sequencing of DNA direct from an environment such as the oral cavity. The short sequence reads which overlap are assembled together to make longer contiguous sequences known as contigs,

which are compared to reference sequences in a database (Schmieder and Edwards 2012). This method can be used to detect and quantify ARGs in the microbiome as well as predict the function of these genes. In addition, high throughput sequencing can, if the assembly is satisfactory, suggest which bacteria within the sampled microbiome contain which ARGs. This is possible because long contigs that contain either a whole, or part of an ARG will also contain DNA flanking the ARG, which can be used to determine the likely bacterial source due to homology with sequenced genomes in the database. To demonstrate that targeted sequences actually cause resistance, functional metagenomic studies are required.

Functional metagenomics may involve random cloning of metagenomic DNA, such as from an oral microbiome sample, into a vector, which is then transferred into a suitable host such as *E. coli*. The vector is usually a plasmid, which is able to contain the inserts of fragmented metagenomic DNA. The transformed *E.coli* is plated onto an antibiotic containing medium. The plasmid inserts from the isolated resistant clones are sequenced to identify the genes that confer resistance and to determine if there are any flanking sequences which can be used to determine the likely source of DNA. An overview of this process is provided in Figure 1. While this is a more labour intensive method in comparison to sequence-based metagenomics, the major advantages are that no previous knowledge of resistance gene sequence is required, making it possible to identify novel ARGs by directly associating a genotype to resistance phenotype (Dantas and Sommer 2012, Pehrsson *et al.* 2013, van Schaik 2015). The main disadvantage of this technique is that it cannot be used to quantitatively investigate the resistome as a whole. This is because genes within a metagenomic library may not express in the surrogate bacterial host or if they do, the protein may not fold correctly or be transported to the appropriate part of the cell. Therefore, functional metagenomics, whilst being excellent for identifying new genes, will always underestimate the resistance potential of a metagenome (Clemente *et al.* 2015). Other considerations include whether the bacterial host has intrinsic resistance to an antibiotic, thus, excluding that antimicrobial from the investigation; for example *E. coli* has intrinsic

resistance to glycopeptides and macrolides. Finally, genes that may not normally be involved with resistance in their natural host, may interact with surrogate host genes and / or proteins in a novel way to confer resistance (Pehrsson *et al.* 2013). These problems can be overcome by using different vectors for library construction and different bacterial hosts in which to transform the library.

Metagenomic analysis provides vast amounts of information, which has resulted in a continual increase in the number of sequences available in databases specifically curating ARGs (see Table 2). A significant issue is that only a small proportion of sequences added to these databases have been functionally characterised. The inclusion of housekeeping and regulatory genes (for example, those that encode for antibiotic targets) increase the 'noise' in databases, as it is unlikely these genes confer clinical antibiotic resistance (Martinez *et al.* 2015, van Schaik 2015). A ranking system for ARGs has been proposed to provide some consensus to the definition of antibiotic resistance (Martinez *et al.* 2015) as well as to focus on the crux of the problem; which of these novel genes can be acquired and confer resistance to human pathogens?

Metagenomics of the oral resistome: distribution, diversity and discovery

Metagenomic analysis is advancing our understanding of the distribution and diversity of ARGs in the microbiome, in addition to being used to discover new ARGs. Culture and amplification-based genetic methods have previously established that individual oral species are resistant to a specific class or classes of antibiotics (Lancaster *et al.* 2003, Ready *et al.* 2003, Ready, Lancaster *et al.* 2004, Ready, Lancaster *et al.* 2006). However, these studies have not provided a broad view of the role of resistance genes amongst the whole microbiome (Roberts and Mullany 2010).

A key feature of the oral resistome revealed by metagenomic analysis is that ARGs are widespread in the oral microbiome (Diaz-Torres *et al.* 2006), even amongst antibiotic naive populations (Moraes *et al.* 2015). A recent functional metagenomic study of the oral microbiome found that ARGs are present in the absence of antibiotic selection pressure in

178 previously un-contacted Amerindians (Clemente *et al.* 2015). Twenty-eight functional ARGs
179 were found in this population. These included genes resistant to semi-synthetic and
180 synthetic antimicrobials, such as genes encoding for penicillin binding proteins that conferred
181 resistance to third generation cephalosporins. The Amerindians shared a common oral
182 resistome with populations exposed to antibiotics despite being naïve to anthropogenic
183 antibiotics - for example, the majority (79%) of ARGs in the Amerindian resistome aligned to
184 the Human Microbiome Project with over 95% nucleotide identity (Clemente *et al.* 2015).

185 Metagenomics has confirmed results from culture-based studies, that the tetracycline
186 resistant gene *tet*(M) predominates amongst the detected *tet* genes in the oral metagenome
187 (Seville *et al.* 2009). The *tet*(M) gene encodes a ribosomal protection protein and is often
188 contained on the Tn*916* conjugative transposon (Franke and Clewell 1981) which is a mobile
189 genetic element that integrates into the hosts' genome (Figure 2). The Tn*916* family is
190 widespread in both commensal and pathogenic oral bacteria (Roberts and Mullany 2009,
191 Roberts and Mullany 2010). This family of conjugative transposons contains a variety of
192 ARGs primarily to tetracyclines but also to other antibiotics, such as macrolides (Tn*1545*,
193 Tn*6002* and Tn*6079*), kanamycin (Tn*1545* and Tn*6003*) as well as antimicrobials such as
194 mercury (Tn*6009*) and antiseptics such as cetrimonium bromide (Tn*6087*) (Ciric *et al.* 2011),
195 which is commonly used in combination with ethylene diamine tetra-acetic acid (EDTA) as
196 an irrigant in endodontic therapy (Guerisoli *et al.* 2002). These additional resistance genes
197 are often located on, or associated with, smaller mobile genetic elements which have
198 inserted into Tn*916* (Figure 2).

199 The ubiquity of tetracycline resistance genes in the oral resistome may be explained by co-
200 selection. Co-selection, or co-carriage, refers to a genetic element which contains multiple
201 resistance determinants (Baker-Austin *et al.* 2006) such as the Tn*916*-like elements. A
202 variety of members from this family of conjugative transposons are present in oral
203 streptococci and contain elements with resistance genes in addition to *tet*(M) such as the
204 erythromycin resistance gene, *erm*(B) (Ciric *et al.* 2012). Hence, exposure to erythromycin

may co-select for tetracycline resistant bacteria (Salako *et al.* 2007). While sequencing technologies and improved PCR techniques have greatly advanced our understanding of these genetic elements, it also means that the presence of mobile genetic elements such as Tn*916* cannot be reported based on the detection of a few genes by PCR. Characterisation of the entire element is now required as both culture based (e.g. Tn*5386*) and metagenomic samples have shown that some oral streptococci have *tet*(M)-less Tn*916* elements (Seville *et al.* 2009, Santoro *et al.* 2014) (Figure 2).

Currently, there are only a limited number of metagenomic studies that have functionally identified novel oral ARGs. These include the tetracycline resistance genes; *tet*(37) (Diaz-Torres *et al.* 2003) and *tet*(32) (Warburton *et al.* 2009), *folP* which encodes for sulphonamide resistance (Card *et al.* 2014), as well as 95 unique β-lactamase genes, most of which were derived from commensal bacteria contained in saliva (Sommer *et al.* 2009). As discussed earlier, the majority of sequences are not functionally characterised, (Martinez *et al.* 2015) and further functional studies are required to verify the role of these putative ARGs.

Transmission of antibiotic resistance

Antibiotic resistance genes in the oral microbiome can be acquired via mutation of existing genes or by HGT. Mutation usually occurs in genes not classified as ARGs such as gyrase or topoisomerase and are usually not transferable. Whole genome sequencing and analysis of individual genes may be able to determine if the particular gene has been acquired through HGT. For example, *tet*(M) has been shown to be 95% identical at nucleotide level in a wide range of bacteria, indicating that this gene is very likely to have been acquired through HGT (Roberts and Mullany 2010, Roberts and Kreth 2014).

Horizontal gene transfer of ARGs occurs by movement of mobile genetic elements between bacteria; these include plasmids (pieces of DNA which usually exist separately from the chromosome), conjugative transposons (discussed above) and bacteriophages (bacterial viruses). The HGT of these mobile genetic elements occurs through multiple mechanisms that are not mutually exclusive. These mechanisms include conjugation (transfer of plasmids

and transposons), transformation (acquisition of extracellular DNA) and transduction (movement of chromosomal DNA by bacteriophages). Additionally, membrane vesicle mediated release of DNA is a more recently described process of HGT. This involves the release of membrane vesicles containing DNA from the cell surface of bacteria. These DNA containing vesicles can then be used by other bacteria as a substrate for DNA acquisition (Roberts and Kreth 2014).

At present there are a limited number of metagenomic studies investigating how HGT mechanisms transmit ARGs within the oral microbiome. Results to date are highly indicative that gene transfer occurs in the oral cavity; however, more metagenomic data is required to gain a better understanding of the situation.

Metagenomics and oral species with systemic relevance

While metagenomics continues to broaden our understanding of resistance through investigation of the resistome, the clinical imperative remains with pathogenic bacterial species and their antibiotic resistance profiles. Some strains of oral commensals such as the viridans group streptococci (VGS) cause opportunistic infections at distant sites including the heart (infective endocarditis) (DeSimone *et al.* 2015). The potential for the development of infective endocarditis from oral microbes does not legitimise a “blanket cover” approach to antibiotic prophylaxis guidelines as changes to international recommendations demonstrate. In fact, the evidence from metagenomic studies on the resistance profiles of oral species capable of causing infective endocarditis and their demonstrable ARG transmission provides further impetus for reducing antibiotic prophylaxis. Current guidelines in the UK and USA have greatly reduced the number of patients who require antibiotic prophylaxis, resulting in declining dental antibiotic prescription rates (Dayer *et al.* 2015, DeSimone *et al.* 2015).

Over the past decade high rates of resistance have been observed in commensal and pathogenic VGS (includes mitis, anginosus, salivarius, mutans and bovis groups) to antibiotics such as β-lactams, clindamycin and erythromycin (Chaffanel *et al.* 2015). The

mef(A/E) gene confers erythromycin resistance and is often on the mobile genetic element, MEGA, itself associated with Tn*916*-like elements, which has been previously implicated in the conjugative transfer of ARGs between VGS and major streptococcal pathogens such as *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Chaffanel *et al.* 2015*)*.

ARGs have also been found in gram-negative commensals such as *Neisseria subflava*, *Veillonella parvula* and *Haemophilus parainfluenzae.* Functional sequencing of saliva samples was able to determine the source of DNA of the ARGs (Card *et al.* 2014). The ARG *folP* was recovered from the chromosomes *of N. subflava* and *V. parvula* and the ampicillin resistance genes *acrA* and *acrB* were recovered from the chromosomes of *H. parainfluenzae* (Card *et al.* 2014). It has been demonstrated that *N. subflava* is capable of exchanging DNA with related and other pathogenic species such as *Neisseria gonorrhoeae* and *Neisseria meningitides* as well as *Haemophilus influenzae* (Pachulec and van der Does 2010*).* While these gram-negative commensals are not associated with distant site infections, metagenomics studies have detected a link between the oral resistome and the resistome of pathogens.

Oral commensals can also cause other systemic conditions including pulmonary infections such as aspiration and community acquired pneumonia (Yamasaki *et al.* 2013). Molecular analysis of specimens from patients with community-acquired pneumonia found known common causative pathogens such as *S. pneumoniae*, *H. influenzae* as well as relatively high rates of oral bacteria such as *Neisseria* spp. and VGS (Yamasaki *et al.* 2013).

Thus, antimicrobial resistance of oral commensals are of significant concern as it may compromise current therapeutic regimes for systemic infections. This is due to ARGs carried by opportunistically pathogenic commensals such as VGS and also via the exchange of ARGs by commensals to related and other pathogenic species. Furthermore, the role of as yet uncultivated oral bacteria (over a third of the oral microbiome) in disease processes is not understood as their virulence potential cannot be investigated (Vartoukian *et al.* 2016). A very recent study has successfully cultivated novel bacterial strains from three previously-

uncultivated taxa using a specifically developed supplemented culture medium (Vartoukian *et al.* 2016). This development as well as further metagenomic data is required to elucidate the role of oral species in systemic infections and then to compare the resistance profile of the oral resistome and the clinical isolates.

The metagenome and the future

Research

The shift towards metagenomics has significantly advanced our understanding of the amount and diversity of ARGs in the oral microbiome. In the future, complete oral metagenomes will be sequenced (Roberts and Mullany 2010, Sommer *et al.* 2010) allowing for comprehensive characterisation of the resistome of an individual. The information generated by this approach will enable the creation of a complete resistance profile of the individual oral microbiome. Without this fundamental knowledge, our understanding of the origins and evolution of ARGs is restricted (Martinez *et al.* 2015). However, new metagenomic approaches may be required as current methods for functional identification of novel resistance genes are relatively low through-put and time consuming (Schmieder and Edwards 2012).

Metagenomics will also enable further understanding of the transmission of antibiotic resistance by providing information on mobile genetic elements and HGT (Schmieder and Edwards 2012). This will lead to insights into triggers for the transmission of antibiotic resistance and how resistance may be controlled or even stopped (Roberts and Mullany 2010). This will have an impact on the clinical decisions made by dental practitioners and reinforce their role in antibiotic stewardship.

Clinical Implications

Ongoing inappropriate prescription and use of antibiotics in dentistry will undoubtedly have an impact in the clinical setting, as resistance patterns will result in difficulties with the management of oro-facial infections or even failure of therapy (Bagg 2014, Cope *et al.*

2014). The accumulation of metagenomic data indicating the presence of ARGs in commensal oral bacteria emphasises the importance of appropriate surgical management and further underscores the importance of limiting antibiotic use in the dental clinical setting.

Metagenomics is likely to have a bigger impact in the clinical setting, beyond expanding our knowledge of resistance. Application of the metagenomic approach for clinical diagnostics has already begun and has applications to combat antibiotic resistance (Schmieder and Edwards 2012). At a population level, large amounts of sequence-based metagenomic data will be able to combine information about ARG abundance, microbial community composition and metabolic pathway information. In-depth data such as this has the potential to inform the development of therapeutic guidelines for antibiotic use based on the impact of antibiotics on the overall composition and function of the oral microbiome, which may assist in reducing the selection for resistance (Schmieder and Edwards 2012). A recent dual centre randomised placebo controlled trial in the UK and Sweden used sequence-based metagenomics to demonstrate that the oral microbiome was more ecologically stable than the gut microbiome in terms of species composition following a single course of antibiotics (Zaura *et al.* 2015). At an individual level, repeated sequencing of the oral metagenome has been used to evaluate changes in the oral resistome over time, providing a window into an individual's oral health and response to antibiotic treatment (Schmieder and Edwards 2012). The metagenomic approach can be used to develop 'genome-inspired personalised medicine' that will allow the prescription of an antibiotic with the appropriate spectrum of activity to the targeted bacteria and/or disease, rather than an empirical course of broad-spectrum antibiotics (Schmieder and Edwards 2012).

Summary

Antibiotic resistance is a natural phenomenon that predates clinical antibiotic use (D'Costa *et al.* 2011), thus, the historical focus on resistance being confined to pathogenic bacteria has by necessity been broadened. Recent metagenomic studies of the human oral microbiome reveal a greater presence of ARGs than has been previously recognised (Diaz-Torres *et al.*

2006) and that oral commensal bacteria are reservoirs of ARGs (Penders *et al.* 2013, Port *et al.* 2014). Antibiotic resistance in the oral biofilm is mainly acquired through HGT and the biofilm is likely to be an ideal environment for transfer. Functional metagenomics reveals that many ARGs in the oral microbiome are located on mobile genetic elements, which facilitate HGT. Further research utilising sequence-based and functional metagenomics will provide a more detailed understanding of the diversity of the oral resistome, its interplay with commensal and possibly pathogenic bacteria in the oral cavity and eventually impact on clinical decision-making in the dental setting to manage this significant public health issue.

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Nil

Conflict of Interest

None of the authors have any financial interest in the subject matter or materials discussed in this manuscript.

Figure Legend

Figure 1: Schematic representation of functional metagenomics used to identify ARGs (purple rectangle). **A & B:** Extraction of genomic DNA from oral saliva and biofilm sample. **C:** The genomic DNA is sheared. **D & E:** The DNA is ligated into plasmid vectors to create a library of metagenomic DNA. **F:** The library is transformed into a bacterial host such as *E. coli*. **G:** *E. coli* is plated on antibiotic containing agar plates in order to identify the resistant clones (H). **I:** The plasmid from the isolated resistant clones are extracted and sequenced so the ARG can be identified. **J**: The **blue arrows** indicate the phylogenetic markers elsewhere on the insert which may be used to identify the host chromosome, thus identifying the likely bacterial host of the ARG.

Figure 2: Schematic representation (adapted from Roberts and Mullany, 2010) of multiple Tn*916*-like elements. Tn*916* containing the tetracycline resistance gene *tet*(M) is located at the top of the figure and all other elements have been aligned to the *tet*(M) gene for comparison. The core Tn*916* genes are shown in light blue and red and are present in all elements illustrated. The name and original bacterial host are shown on the left of the image. The names of the genes are located above the arrows which show the size and orientation of the genes. The scale bar in top right corner represents one kilobase (kb). The key below the figure shows the colours that are associated with each category of genes (first line) and the second line shows other (smaller) mobile genetic elements and the resistances these carry.

377 **Tables**

Glossary	
Taxa	A category or group such as phylum, genus or species
Phylogenetics	The study of evolutionary history and the way different organisms and species are related to each other.
Phenotype	Observable/detectable/measurable characteristic of an organism which is a manifestation of its genotype.
Genotype	The genetic constitution of a cell or organism as distinct from its phenotype or expressed features
Metagenome	The totality of genomes of all microbiota (culturable and not yet culturable) found in a given location such as the oral cavity.
Microbiome	The ecological community of all microorganisms that reside in a niche.
Resistome	All the resistance genes within a microbiome.
PCR	Polymerase Chain Reaction: a method used for *in vitro* amplification of DNA which results in millions of copies of a template. The PCR products are of sufficient quantity to be utilised in a range of laboratory procedures.
DNA microarray	An analytical tool where DNA is arranged in a regular pattern on a small membrane or glass slide.
Through-put	Number of samples being analysed, e.g. PCR is a low throughput technique while NGS is high throughput.
Genomic DNA	Total DNA in the cell - both chromosomal and extrachromosomal (on plasmids).
Vector	Common term for a plasmid that can be used to transfer DNA sequences from one organism to another.
Human Microbiome Project	A project launched in 2008 by the US government which has the goal of identifying and characterising the microbes associated with health and disease in the human microbiome (hmpdacc.org).
Mobile genetic elements	Segments of DNA with the ability to move from one position in a genome to another e.g. transposons, introns and insertion sequences (ISs). Some also have the ability to undergo horizontal gene transfer between cells e.g. conjugative plasmids and conjugative transposons.
GenBank	An annotated database of all publicly available DNA sequences run by the National Institute of Health, USA

378 Table 1: Description of Terms

Box 1: ARG-specific Databases	
These databases aim to unify the publicly available information on antibiotic resistance by annotating each gene and resistance type with information such as resistance profile and mechanism of action. The database can be used as a compendium of antibiotic resistance factors as well as to identify the resistance genes of newly sequenced genes, genomes, or metagenomes.	
Antibiotic Resistance Gene-Annotation (ARG-ANNOT) http://en.mediterranee-infection.com/article.php?laref=283&titre=arg-annot-	Maintained but concise database with excellent sensitivity and specificity for the identification of known ARGs.
Antibiotic Resistance Genes Database (ARDB) http://ardb.cbcb.umd.edu/	The first database to compile information about ARGs. No longer recommended as it is not maintained and contains a large number of housekeeping and regulatory genes.
Comprehensive Antibiotic Resistance Database (CARD) http://arpcard.mcmaster.ca/	Curated set of reference genes involved in antibiotic resistance from a variety of organisms, genomes and plasmids.
RESfams http://www.dantaslab.org/resfams/	A curated database used to quantitatively analyse the relationship between environmental and human-associated resistomes.
RESfinder https://cge.cbs.dtu.dk/services/ResFinder/	Curated database that uses whole shot-gun sequencing data to identify acquired antimicrobial resistance genes in bacteria specifically, horizontally acquired ARGs, not resistance mediated by mutations.
Resistance Determinants Database (RED-DB) http://www.fibim.unisi.it/REDDB/	An updated repository of reference gene sequences.

Table 2: List of ARG Databases

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