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Antibiotic use and antibiotic resistance in dental practice

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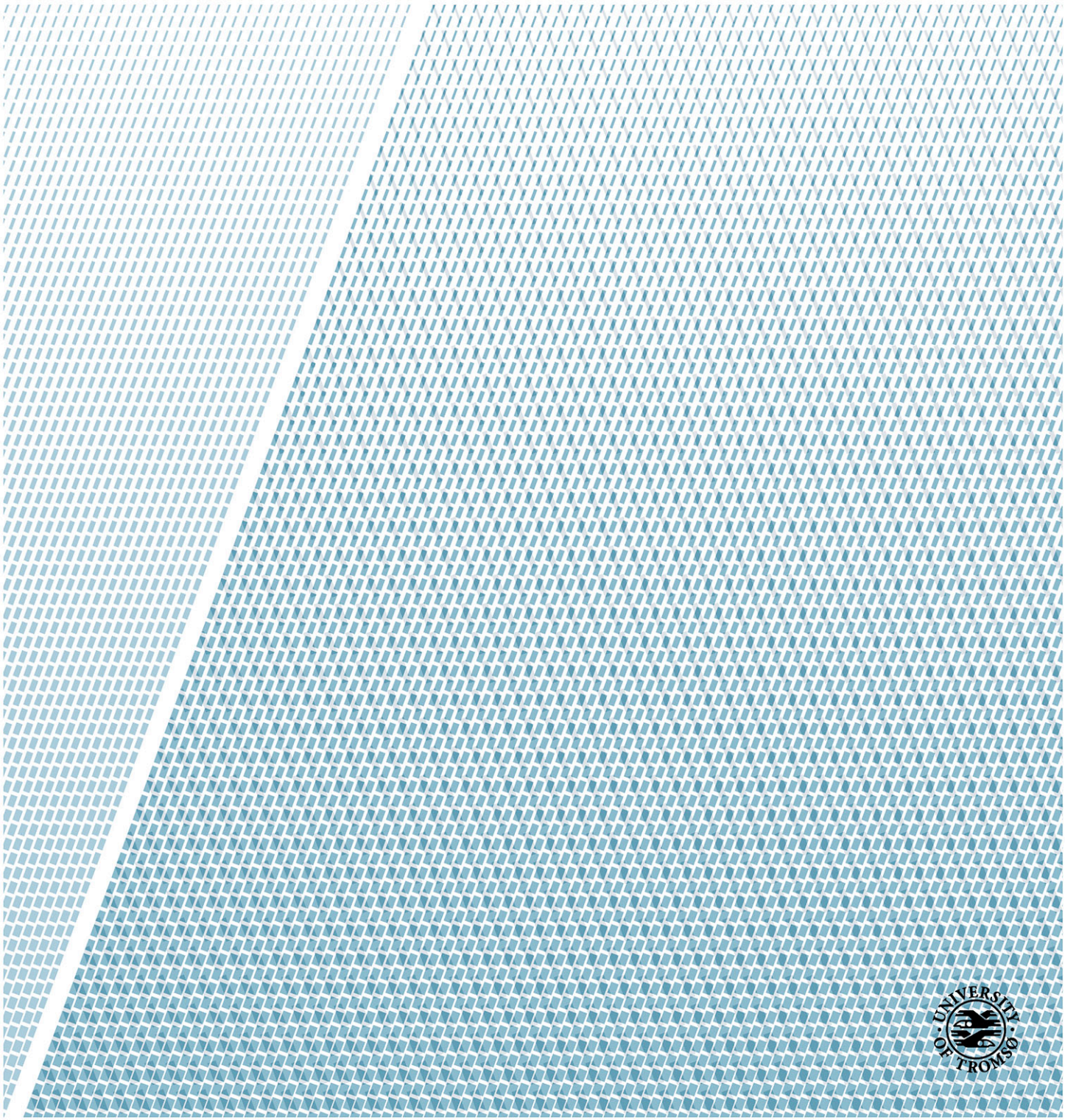

Table of content

Abstract

Antibiotic resistance in bacteria is an increasing problem in health care settings worldwide. After approximately 70 years of antibiotic use, the bacteria have developed mechanisms that let them survive antibiotic treatment. The use of antibiotics is an important factor in resistance development. Norwegian dentists prescribe approximately 5.3% of the total antibiotics consumed in the country.

Dentists tend to use mostly β-Lactam antibiotics, metronidazoles, macrolides, lincosamides and tetracylines. Bacteria can develop resistance to all kinds of antibiotics, by acquiring genetic traits that confer resistance to antibiotics. Horizontal gene transfer is the most effective way for bacteria to acquire new genes. Several genes are known to code for resistance against several antibiotics. Many of these genes can be found in bacteria residing in the oral cavity.

To ensure rational antibiotic prescribing practices, national guidelines for antibiotic use are developed in several countries. The Norwegian ones mention two main indications for the use of these drugs in dental practice. These are specified cases of acute odontogenic infections and in some forms of periodontitis. The United Kingdom also has guidelines for prescribing antibiotic in dental practice, but these contain some differences which are highlighted in this review.

The aim of this paper is to summarize and organize information about antibiotic use and antibiotic resistance to better understand the topic and the challenging situation the global society faces today; with a special focus on dental practice. An account to simplify and explain important terms used in the field of microbiology, essential to understand antibiotic resistance, are attempted. The information is mostly gathered from relevant articles published at PubMed, mainly consisting of recent publications from the last ten years. Other sources used in the current paper are published theses and internet resources from reliable organizations, such as WHO and the Norwegian FHI.

Keywords: Drug resistance, dentistry, horizontal gene transfer, prescription

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1. Introduction

Antibiotics today are an essential resource in the constant fight against infections. Antibiotics are commonly used in food production and human medicine, and in Norway in 2012 there was a total consume of about 60 tons, of which about 52 tons in human medicine (1). The development of antibiotic resistance, at a rapid pace, is acknowledged by the World Health Organization as an international health crisis. In 2015, the World Health Assembly developed their Global Action Plan on Antimicrobial Resistance, requesting the global society to act united against the situation (2). By the end of 2015, the Norwegian government published their National Action Plan against Antibiotic Resistance, with the main goal to reduce the antibiotic use by 30% within 2020 (3). Of the total amount of prescribed antibiotics in Norway in 2014, dentists prescribed about 5.3 %, which is a slight increase since 2004, but more important, there was an increasing prescribing of broad-spectrum antibiotics. The government's action plan proposed that the Norwegian Dental Association took action regarding the matter, and the Academic Committee for Recommended Use of Antibiotics in Dental Practice was founded (4). Media have, in the last few years, taken more and more interest in antibiotic use and overuse, and have highlighted the problem of antibiotic resistance to the public. Thus one can say that antibiotic resistance today is a relevant topic of discussion in all health disciplines and sectors.

The aim of this paper is to summarize and organize information about antibiotic use and antibiotic resistance to better understand the topic and the challenging situation the global society faces today; with a special focus on dental practice. An account to simplify and explain important terms used in the field of microbiology, essential to understand antibiotic resistance, are attempted. The new Norwegian antibiotic prescribing guidelines for dentists are compared to the one currently existing in the United Kingdom. The information is mostly gathered from relevant articles published at PubMed, mainly consisting of recent publications from the last ten years. Other sources used in the current paper are published theses and internet resources from reliable organizations, such as WHO and the Norwegian FHI.

1.2. Historical background

There are and have always been bacteria around us, and they are well known to occasionally cause different infections in humans and animals. Even though the cause of infection was unknown for humans in the old days, they've always tried to fight back with different remedies (5). The term ***antibiotic*** was first used as a noun in 1942, and was described as substances produced by microorganisms which are bacteriostatic or bactericidal to other microorganisms (6). *Bacteriostatic* antibiotics mean antibiotics that inhibit bacterial growth, while *bactericidal* antibiotics mean antibiotics that kill bacteria (7). Synthetic and semisynthetic derivatives substances that are designated antimicrobials or microbial agents, doesn't concur with the original antibiotic definition. However, the term antibiotic is still used, but nowadays it is extended to apply synthetic produced drugs with antimicrobial effects, as well.

Penicillin, as the first antibiotic, was discovered by Sir Alexander Fleming in 1928. It was first used to treat humans in the beginning of the 1940's, and the further development started at high rate, in the following years. Nowadays we have several different kinds of antibiotics, but the development of new effective ones, has nearly halted. Actually nearly every class of antibiotics that we use today was created before the 70's (5). The combination of an antibiotic resistance progress at a higher rate than the development of new antibiotics, has led to the problematic situation of antibiotic resistance the world is facing today (2). In total, 22% of the population in Norway was, at least once, dispensed some kind of antibiotics in 2015 (8).

2. The different kinds of antibiotics commonly used in dentistry today

2.1. β-Lactam antibiotics

β-lactam antibiotics is a group of antibiotics containing the β-lactam ring with various side chains. Penicillins; members of the β-lactam antibiotics, are the most frequently used antimicrobials in dentistry. They target the last step of the peptidoglycan synthesis in the bacterial cell wall, which is specific for most bacteria. Cell shape is maintained by peptide cross-linked glycan chains, and when the penicillin inhibits this process, the cells lose its stability and causes cell lysis. This makes β-lactams antibiotics bactericidal agents (9, 10). The penicillins can be divided into main groups according to their different antibacterial spectrums. The narrow spectrum penicillin G and penicillin V, as well as the broad-spectrum aminopenicillins, are of dental practitioners primary interest.

Penicillin V, phenoxymethylpenicillin, is sufficient acid stable to be orally administered, and is effective against most oral gram positive anaerobes and streptococci(11). Penicillin G, benzylpenicillin, is acid labile and has to be used intravenous or intramuscular, and thus not commonly prescribed in dental practice. The broad spectrum penicillins, the aminopenicillins, has a mode of action that is quite similar to phenoxymethylpenicillin, but includes Gram-negative microbes as well (11). Aminopenicillins is a generic term for ampicillin and amoxicillin (12). Their broader spectrum comes from the ability to penetrate the cell wall through porins of some gram negative bacteria (11).

Numerous bacteria produce enzymes named β-lactamases, which effectively abolish/inactivate the effect of β-lactam antibiotics. The production of β-lactamases account for most causes of antibiotic resistance against penicillins. The effect of β-lactamases is encountered by β-lactamase inhibitors, which according to the name, inhibits the effect of β-lactamases by binding and inactivation (11, 13). E.g. clavulanate, sulbactam and tazobactam, are all frequently used (13).

2.2. Metronidazole

Metronidazole is a bactericidal drug which is known to be effective against anaerobic bacteria in anaerobic conditions (14). It's known to be the second most used antibiotic by Norwegian

dentist (15). It inhibits nucleic acid synthesis, but need to have metabolic activation inside cells before it can have any bactericidal effect. The theory is that when the nitro group gets reduced inside the anaerobic cell, the reduced products can have cytotoxic effect as inactive intermediates. These intermediates are believed to target the organisms RNA, DNA or cellular proteins (16).

2.3. Macrolides and lincosamides

Macrolides: is a group of antibiotics that mainly target gram positive cocci, except enterococci (17). They are first and foremost bacteriostatic, but they can have a slight bactericidal effect as well (18). By binding to ribosomes, they inhibit protein synthesis, and do this in two ways. Either it binds to the narrow conduits, nascent peptide exit tunnel, and obstruct the passage of nascent peptides on their way out of the ribosome. This inhibits the translation in early stages, and prevents bacterial growth (18). The other mode of action is an inhibition of selective peptide bond formations between macrolide donors to specific acceptor substrates in the bacteria (18). If a patient is allergic to penicillins, the macrolide erythromycin may be an alternative antibiotic to use (19). Lincosamides mode of function is the same as macrolides, but is quite different in structure (11, 20). Clindamycin is a lincosamide and is effective against aerobe and anaerobe species (11). Clindamycin is the drug of choice for Norwegian dentists in cases of penicillin-allergy (21).

2.4 Tetracycline

Tetracycline – a broad spectrum antibiotic that can inhibit both gram positive and gram negative bacteria. It inhibits the protein synthesis of bacteria by binding to the ribosomes, mainly the 30S subunit, but some also at the 70S. While binding to this subunit, tetracycline overlaps an anticodon that is central in proofreading during the mRNA translation. This inhibition, leading to incomplete protein synthesis, halts the bacterial development, and is therefore a bacteriostatic drug group (11, 22).

3. Antibiotic use and guidelines

3.0.1. Measuring antibiotic consumption

In order to monitor the use of antimicrobials, there is a need to standardize the measurements, so that the gathered data can be reliable and comparable across different countries. One of the main challenges would be the variety of drug use on many areas, varying from livestock production to different clinical disciplines. Another challenge was that gathered data on drug utilization in countries, was in the beginning not comparable on a detailed level, because of variety in sources and forms. A central classification system used in systematizing drug surveillance is the Anatomical Therapeutic Chemical (ATC) was developed to enable methodical data gathering in drug consumption (2, 23).

The ATC system divides drugs into groups at five different levels, according to which organ systems they affect and their therapeutic and chemical characteristics. In addition to the ATC-system, a technical unit called Defined Daily Dose (DDD) was developed. It is a statistical unit for antibiotic consumption (15). It's defined by WHO as: "*The assumed average maintenance dose per day for a drug used for its main indication in adults"*(24). It does not give information about recommended or prescribed daily dose. WHO uses the ATC/DDD-system as a tool in their antibiotic utilization research and advise other researchers to use the same. Keeping to this system facilitates studies of drug consumption over periods of time, and between different countries and regions (2, 11, 23, 25).

3.1. The Norwegian guidelines (2013) for antibiotic use in dental health

In Norway in 2008, "The Antibiotic Centre for Primary Care" made, on request from The Norwegian Directorate of Health, the first national guidelines for the use of antibiotics in primary care. They were made as a reaction to the worldwide resistance problem, with the aim to minimize the development of resistance, by rational antibiotic use in primary health care. The guidelines have been updated and renewed once, this happened in 2013. It is made as a booklet with more than 300 pages, and this renewed version includes a section for dental use of antibiotics, as a separate chapter. This chapter was distributed with *Tannlege Tidende*, reaching every member of the Norwegian Dentist Association counting about 90% of the working dentists in the country (26).

The guidelines are evidence-based, with aim to maintain low antibiotic use, and more importantly, is to keep the use of broad-spectrum antibiotics at a minimum level. The guidelines are not legally binding, but it's said that in general they should be ruling, and in cases with bigger deviations, the choice of antibiotics needs a professional well-documented justification (27).

3.1.0. When do we use antibiotics, including types, doses and duration

Generally, there are two main indications where dentists can prescribe antibiotics to treat active infections. These are acute odontogenic infections and some cases of marginal periodontitis. In acute situations the choice of antibiotic has to be empiric based, and thus the choice should be a single drug or combination of two drugs that are likely to succeed. Bacterial samples should be taken in cases with evidence of systemic spread, for antibiotic sensitivity testing. This is done to gain some time in cases of no clinical effects of empirical therapy in the first 24-48 hours. With the antibiotic sensitivity testing results in hand; one can change to the right drug immediately, if needed. Dentists in Norway tend to prescribe narrow spectrum antibiotics and are generally considered conservative in their prescriptions (15, 27).

3.1.1. Acute odontogenic infections

Acute odontogenic infections can spread fast and lead to dramatic outcomes. For dental practitioners it's important to know which conditions they normally can treat themselves, and which ones they should refer to a hospital. Oral infections are not uncommon in a dentist's workday. Even quite serious cases can be seen and treated relatively easy in primary dental care. The oral infections primarily burst from either caries or periodontal diseases, and can result in the formation of abscesses, apical and marginal periodontitis, pulpitis, acute necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis and phlegmones. It's when these infections are left untreated; the more severe outcomes may occur, by extending from a simple odontogenic tissues into a more serious one and even might cause potentially life-threatening infections. In general, most cases of odontogenic infections are effectively treated by only incision and drainage (27). Antibiotic therapy should only be prescribed in severe cases where systematic development is evident. While awaiting sensitivity test results from the lab, empirical antibiotic treatment starts with:

Phenoxymethylpenicillin: 1 gram, four times a day, for 5 days.

For patients allergic to penicillins, the following regime is recommended;
Clindamycin: 300mg 4-5 times a day, for 5 days (27).

3.1.2. Periodontitis

Periodontitis with all of its forms, from mild to severe, is by some counted as perhaps the most common chronic disease in the world (28). It's an immunologic induced degradation of periodontal tissues. The important red complex, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are the bacterial species identified as significant pathogens in most cases of chronic periodontitis in adults (29). Mechanical scaling and root (SRP), with or without surgery, is the main treatment of chronic marginal periodontitis. Adequate oral hygiene on a daily basis is of course also a main factor, in disease control. But a small, but still significant portion, won't respond to the therapy. For these patients refractory to conventional therapy, studies show that antibiotics treatment as supplement to therapy can have positive effects on the clinical outcomes (30, 31). It's important to know that antibiotics won't suffice as a substitute of an inadequate SRP. Before antibiotics are added to the treatment, the operator should be satisfied with the patient's oral hygiene. Next should one look at the quality of the SRP, has that been optimal? If those factors are evidently good, a bacteria sample should be taken, and sent for laboratory analysis. The answer will determine the antibiotic sensitivity of the microbes, and therefore give us the information of what antibiotic to use as a supplement to clinical periodontal therapy (27, 30).

For cases of rapidly progressive periodontitis and infections related to *P. gingivalis, T. forsythia* and *T. denticola,* the following antibiotic regimes are recommended:
Amoxicillin 250 mg x 3 + Metronidazole 400 mg x 3, each for 5-10 days.
Or just Metronidazole alone.

For cases of aggressive periodontitis and infections related to *Aggregatibacter actinomycetemcomitans*, the following antibiotic regime is recommended:
Amoxicillin 250 mg x 3 + Metronidazole 400 mg x 3, each for 5-10 days.

For infections related to *Enterobacter*, for example *Escherichia coli*, *Enterobacter species* or *Klebsiella species*, the following antibiotic regime is recommended:
Ciprofloxacin 250-500 mg for 1-2 weeks.

For patients allergic to penicillins, where penicillin is recommended as a first choice, alternative treatment with following regime is recommended:
Clindamycin: 300mg 4-5 times a day, for 5 days.

3.2. Antibiotic prescribing practices among Norwegian dentists

A study regarding antibiotic prescriptions by Norwegian dentists, was published in 2007, and is based on a questionnaire sent to 10% of randomly selected Norwegian dentists (32). In average the dental practitioners prescribed 1.3% antibiotic prescriptions a week. The study concluded that Norwegian dental practitioners are conservative in antibiotic prescribing, and mainly prescribed the correct type of drugs. But only 3.4 % used laboratory services for analysis of bacterial infections, as basis for their prescriptions. It is found a trend of more frequently antibiotic prescribing among young dentists and dentists working in northern parts of Norway (32). Another study from 2007, based on the total antibiotic prescriptions from Norwegian dental practitioners in 2004 and 2005, is probably the first and only one that uses the DDD measurement unit system (15, 33). Norwegian dental practitioners prescribed 11 different antibiotics, which contributed to 8% of the total consumption of those antibiotics in Norway. Of the total of 131 128 prescriptions in 2004 phenoxymethylpenicillins amounted to 75%, metronidazole 6.3%, erythromycin 4.9%, amoxicillin 4.6%, klindamycin 3.7% and the last six antibiotics ranging from 2 - 0.08% (15, 33). The narrow-spectrum phenoxymethylpenicillin is the first choice of antibiotic in Norwegian dental practice. This implies a low prevalence of antibiotic resistance among oral bacteria in Norway (15, 33). In 2005 and 2006, 73.3% and 70,0% of the working Norwegian dentist issued antibiotic prescriptions (15).

3.3. Guidelines in the UK (UKGL) compared to the Norwegian ones (NGL)

UK got their first guidelines: “Antimicrobial Prescribing for General Dental Practitioners, in 2006. The second edition was published in 2012, and updated in 2014 (34). The main purpose

is described as a tool which can be *"useful in decision-making process and to be an aid to effective treatment planning and patient care"* (35). It's pointed out that there are just some cases where antimicrobials are indicated. Every choice is thoroughly documented with reference articles. Dose and dose frequency is stated for adults, and for children as well. They have more or less the same indications as in NGL, with acute infections and periodontitis as the main classifications. However, in Norway dentists are considered conservative, using the narrow-spectrum penicillin *phenoxymethylpenicillin*, while in the UKGL recommend the broad-spectrum penicillin *amoxicillin*. For the use of antimicrobial therapy in periodontitis there are some major differences. In the NGL, it is suggested that bacteria analysis should be utilized before prescribing antibiotics, however, in the UKGL it is suggested to refer to a specialist, in cases where the patient doesn't respond to normal treatment.

A quite important difference found between the two sets of guidelines regards the choice og antibiotics and duration of the treatment. In general, the UKGL recommends metronidazole as a first choice for acute odontogenic infections and shorter periods of antibiotic course, for example only 3 days regime of metronidazole for different odontogenic infections, whereas the NGL recommends 5 days of penicillin V. UKGL does only specify a minimum days of treatment when recommending metronidazole, which is the same as treatment durance; 3 days. When recommending other antibiotic regimes, the UKGL only give the maximum period of use, for example in cases of acute dento-alveolar infections where regimes of amoxicillin is suggested for *up to* five days. While the NGL suggests a 5-day regime. It should be stressed that not completing an antibiotic course, even though symptoms fades out, may lead to bacterial survival and recurrent of infections by mutated resistant bacteria (36).

Another difference that should be pointed out is that only the NGL suggests taking bacterial samples for sensitivity analysis. Both guidelines suggest alternatives in cases of patients allergic to penicillins (35).

4. Resistance

4.0.1. Sources of variation

An important concept in the field of antibiotics are genetic variations. As the term alludes it is genetic changes, which can occur by mutations, rearrangements and gene flow from immigration of nearby cell populations (Figure 1). On cell level there are two ways for genetic diversity to occur, known as mutations and rearrangements. Mutations are local chromosomal DNA sequence changes. Some of them can be highly beneficial for the bacteria, due to resistance development, which will be explained later on. Rearrangements are structural chromosomal changes, and come in the form of duplications, deletions or inversions and translocations. Generally all the rearrangements will be unfavorable or destructive, but they sometimes lead to beneficial alterations.

Interaction between cells and population is by far more important in genetic diversity, and therefore more in focus in this paper. First and foremost are horizontal gene transfers – basically DNA acquisition from nearby cells. Immigration is the concept describing how foreign genes spread among populations (37, 38).

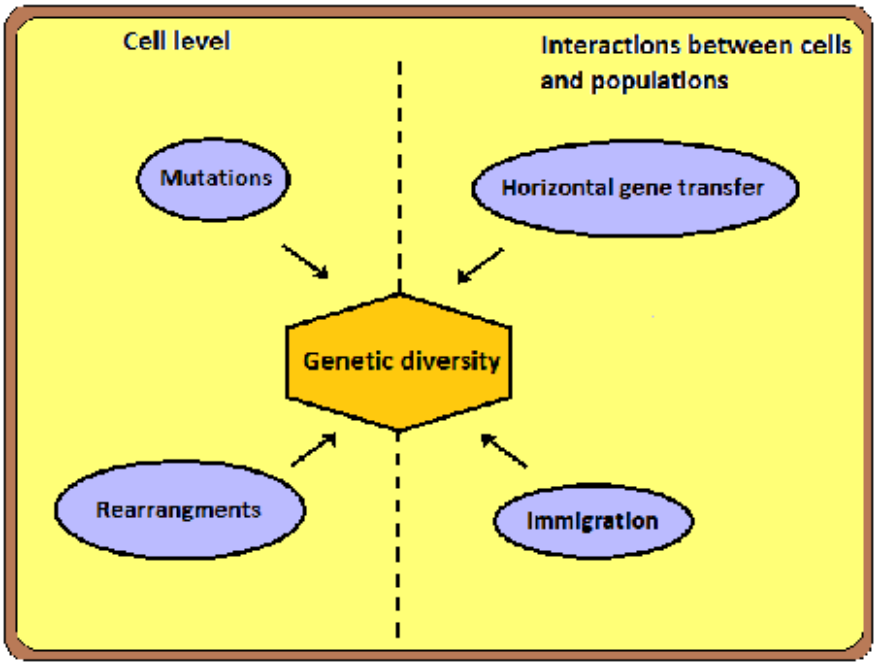

Figure 1: Factors causing genetic diversity, at both the cellular level and the environmental level.

4.0.2. Fitness

Another central term in studying the biology of bacterial resistance is the concept of fitness, sometimes referred to as Darwinian fitness. Bacterial fitness is the measurement of the ability to survive and reproduce for a biological entity (37, 39). With fitness measurements it is possible to quantify data and statistically compare certain genotypes performances with other genotypes. Fitness commonly divides into absolute fitness and relative fitness. Absolute fitness can be described as the total genotypes performance, comprehending survival, successful reproduction and fecundity. While relative fitness is typically used for comparing bacterial populations (37). There are usually two ways relative fitness is measured; maximum exponential growth rate, or mixed competition with start and end ratios. Linked to antibiotic resistance, bacterial fitness determines the fate of a mutation. A resistant mutation that causes the least fitness cost, is the one that most likely will dominate after ended antibiotic treatment (37, 39, 40).

4.1. Resistance

"It is time to close the book on infectious diseases, and declare the war against pestilence won". Which is known as one of the most infamous quotes in the field of microbiology, supposedly spoken in 1967, and is credited to the United States Surgeon General, Dr. William Stewart. Whether or not he is being correctly quoted (41), the statement is unfortunately drastically wrong. Infections remain as one of the main causes for human morbidity and mortality in the world, due to the organism's ability to adapt and adjust to survive antibiotic challenges (42). This is known as antibiotic resistance, and makes microbes resistant against antimicrobial drugs which originally were effective treatment against the diseases and infections caused by it. The problem is aggravated by the massively use and abuse of antibiotics, which speeds up the development of resistance rapidly all over the world. Bacterial resistance is now reported from all around the world, where diseases and infections that used to be easily treated with antibiotics, now will be much harder to treat. Treatment will take longer time, requires stronger antibiotics and is considerably more expensive to manage. That is if the infections are even possible to treat, especially with the spread of multi drug resistance like Methicillin-resistant *Staphylococcus aureus* (MRSA). In the EU region alone, the resistance trouble is estimated to take 25000 humans life a year, with high economic cost (11, 36, 43).

4.1.1. Natural and acquired resistance

Antibiotic resistance among bacteria can be either intrinsic [natural] or acquired. Intrinsic or innate resistance is basically the ability for the bacteria to naturally withstand antibiotic agents, because they either don't have the structures upon which antibiotics can act on, they lack the essential metabolic processes needed for antimicrobial activation, or they have mechanisms that expulse the antibiotic before it reach its target (11, 44)

Acquired resistance in bacteria, is traits gained during the lifetime, and happens by genetic alteration. There are two different ways for the bacteria to achieve alterations that result in antibiotic resistance. It can either do it by chance through mutations in the existing genome, that makes it withstand the antibiotic agents, or by receiving already resistant genes from other nearby bacteria, a process called horizontal gene transfer (11).

4.2. Horizontal gene transfer (HGT):

This is by far the most effective way for a bacterial population to acquire resistance genes, and HGT is sometimes referred to as bacterial sex. Studies have revealed that approximately 75% of every bacterium's genome is a result of horizontal gene transfer throughout the time of evolution (37, 45). Bacteria are known to have three common ways to transmit resistant genes to other bacteria, named transformation, transduction, and conjugation. There are also two other mechanisms, nanotube- and vesicle-mediated gene transfer, but their importance are not fully researched yet (37).

4.2.1. Transformation: Is a process were extracellular "naked" DNA segments with resistance genes, are taken up by the bacterial cell, from the environment. For this to happen, the bacterial cell has to be in a physiological state named competence (46). A competent cell can bind naked DNA strands to the cell wall, before uptake across the cell membrane. The exogenous DNA has to be similar to the bacterial DNA for incorporation in the genome (a process called recombination) (Figure 2). Natural transformation will only occur in bacterial cells that naturally competent.(11, 37, 46)

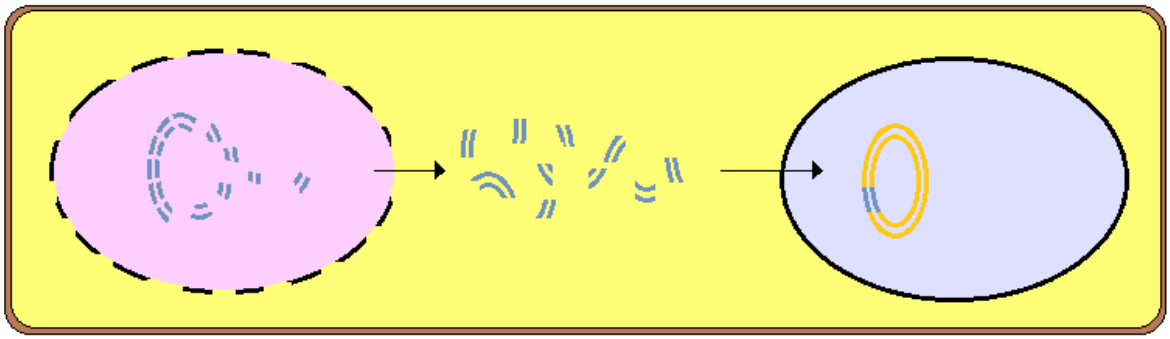

Figure 2: Transformation: lysis of an antibiotic resistant cell, where a DNA fragment coding for resistance, gets picked up by a competent cell and its recombination in the host DNA.

4.2.2. Transduction: Somewhat similar to transformation, but require a virus for extracellular DNA transport. Bacteriophages, a bacteria-infecting virus, attach to bacteria and releases DNA into the cytoplasm and integrate into the host genome. They may become dormant, or they can be triggered to replicate themselves and start lytic growth. Bacterial DNA that contains antibiotic resistance gene(s) randomly becomes packed inside bacteriophages. The bacteriophages will be released when the host dies, and may attach to a new host, releasing its newly adopted resistance gene (Figure 3). Transduction is quite limited as a source of gene transfer, because of the host specificity of the bacteriophages. It therefore most commonly occurs between highly related bacteria in within the same population (37, 45, 47).

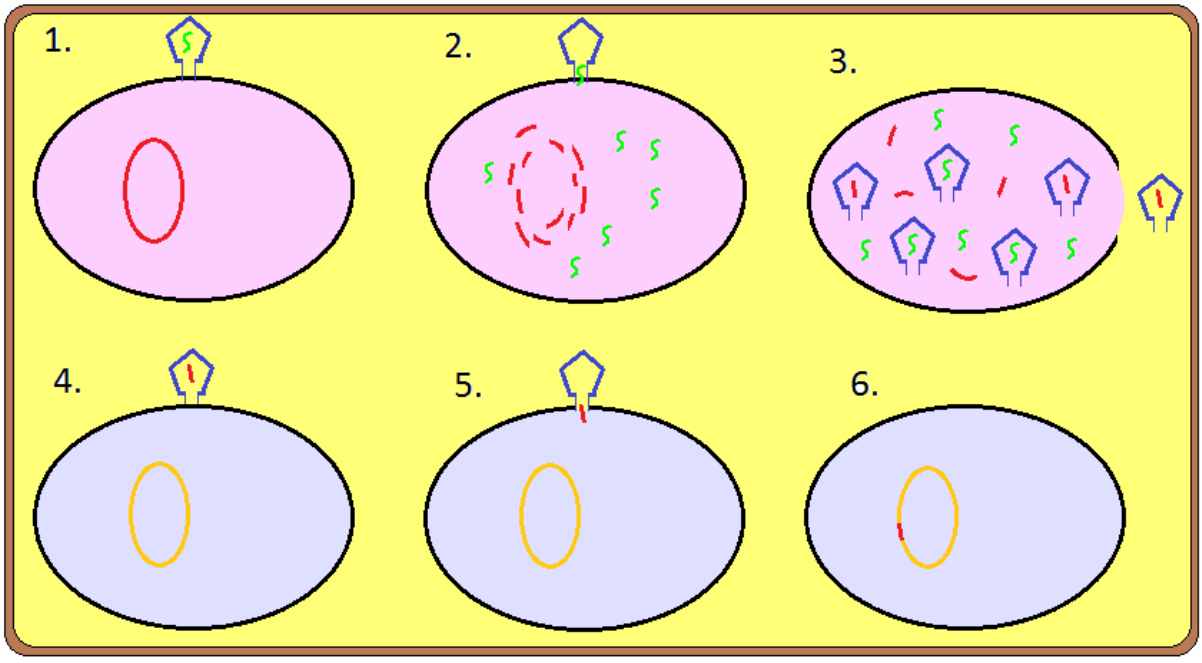

Figure 3: Transduction: 1. A virulent phage attaches to a bacteria and 2.releases its DNA into the cell, which replicates and 3. phage coats are synthesized and randomly pick up hosts resistance gene. 4. the phage carries the gene to another host, 4. where it attaches and 5. releases the gene 6. which gets incorporated in the new hosts genome.

4.2.3. Conjugation: The most studied form of the HGT (47), and is the transferal of mobile genetic elements by cell-to-cell contact, mediated by a conjugative pilus or a pore (Figure 4). Conjugation can transfer small DNA fragments and up to large chromosomes within species, as well as between some different species. Conjugative genetic elements are plasmids and transposons. Depending on the plasmid, some are self-transmissible while others are not, and are termed mobilizable. Bacterial cells in dental biofilm show increased plasmid spread through conjugation, due to the high density of cells. It is even suggested that biofilm construction is stimulated by conjugation. (46-48)

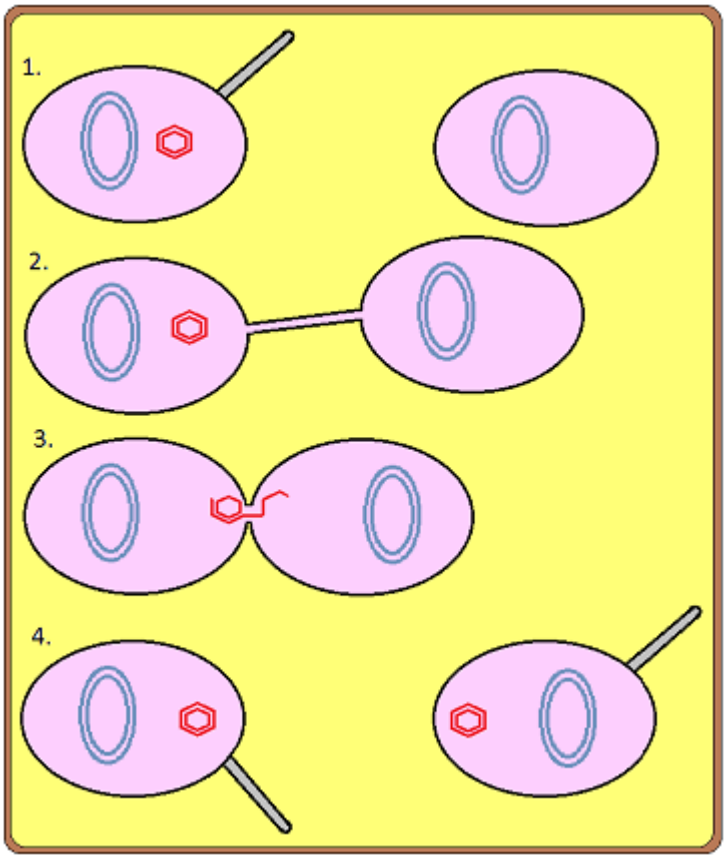

Figure 4: Conjugation process. 1. Donor cell with a conjugative resistant element 2. attaches to recipient nearby cell by a pilus and draws it to cell wall contact. 3 the genetic element sends one strand to the other cell, and both single strands synthesizes a complementary strand, and 4. the cells disengage.

4.3. Resistance in oral bacteria

The properties of the oral cavity, with its variety in bacterial habitats, make it a suitable place for lots of different bacteria (11). Older articles suggest that there are more than 700 different species of microorganisms estimated to be found in the oral cavity, consisting of bacteria, fungi, viruses, protozoa and archaea (49). Newer and more sensitive techniques, which also

detect rare species of low concentrations, have identified more than 1000 different bacterial species in dental plaque alone (50). With this high density of bacteria, the possibility of bacterial interactions resulting in gene transfer and genetic diversity, are high. Studies have shown that HGT is commonly occurring internally in dental biofilm, as well as between bacteria passing through the mouth and oral bacteria (46).

4.3.1. Genes responsible for antibiotic resistance in oral bacteria

Several genes known to cause antibiotic resistance in bacteria, have been reported to be found in bacteria residing in the oral cavity, or recovered from oral infections. Most of these studies investigated resistance occurrence in oral bacteria, but are based on cultivable bacterial species, and therefore, doesn't take into account the large numbers of uncultivable bacteria present in the oral cavity. Several studies have shown a panel of resistance genes present in oral bacteria. These are genes that are responsible of resistance against common antibiotics prescribed by dentists. For example, *cfxA2*, *cfxA, bla(TEM*) have been reported to be present in oral bacteria, which are genes responsible for resistance against β-lactam antibiotics (51-53). *Nim(B)* are found in a few studies of periodontal abscesses (54), and it is a gene coding for resistance to Metronidazole (55). *Erm(B)* and *erm(F)* are genes detected among oral bacteria and these genes are associated with resistance against Erythromycin (56, 57). Genes coding for tetracycline resistance are known to be common in the oral flora. These genes are often found in mobile genetic elements, making them easy to spread. Several tetracycline resistance genes are detected among oral bacteria, for example *tet(M), tetO, tetQ, tetW* (51, 58, 59). The nucleotide sequence of each gene mentioned herein is listed in the appendix I.

4.3.2.The bacterial resistance mechanisms:

When bacteria have received new genes that confer antibiotic resistance, it exploits the information to protect itself from hostile antibiotic agents. The mechanisms of antibiotic resistance are either based on target modification or by reducing the concentration of active free antibiotic molecules. On one hand, we have those termed passive mechanisms of resistance, meaning those who don't affect the antibiotic itself, by tampering with the pathways the antibiotics uses to reach their target sites. On the other hand, we find those termed active mechanisms of resistance, which works directly on the antibiotic agent (42, 60). The biochemical mechanisms that the bacteria exhibit for antibiotic resistance can be divided into four major groups (figure 5):

1. Producing an alternative target: normally by producing an enzyme that is resistant to the effect of the antibiotic, and keep producing the original sensitive target. The original target will be inhibited, while the alternative enzyme "bypasses" the antibiotic's effect and the bacteria survive (61, 62). For example, methicillin resistant *S. aureus* produces an "alternative penicillin binding protein (PBP2a)", in addition to the main target "penicillin binding protein". PBP2a is not affected by the antibiotic, and the bacterial cell continues to function normally (61).

2. Disallowing antibiotics to be in or reach the cytoplasm, either by preventing cell wall passage in the first place, by changing the membrane composure making it less permeable, or by pumping it out at a higher rate than the entering pace. The latter is so-called efflux, and is mediated by energy-requiring efflux-pumps (42, 60, 63, 64). For example, tet proteins in *E. coli* is responsible for tetracycline efflux pumps (60).

3. Changing the antibiotic targets and/or target overproduction. Antibiotic agents that actually reaches the target, but is inhibited binding because of alterations in the target site structure, while the target still functions properly (42, 60, 63). For example, alteration in the penicillin-binding proteins, which lowers the affinity of β-lactam antibiotics, or vancomycin targets, by the products of *van* genes, changes their pentapeptide precursor, resulting in a target with lower affinity for vancomycin (63).

4. Antibiotic modification, which is different active ways of directly changing the antibiotic agents. Either by enzymatic modifying, by adding chemical groups prohibiting target binding. Or bacterial inactivation by enzymes, e.g. β -lactamases, which destroys the active component (β -lactam ring) of the penicillin (42, 60, 63).

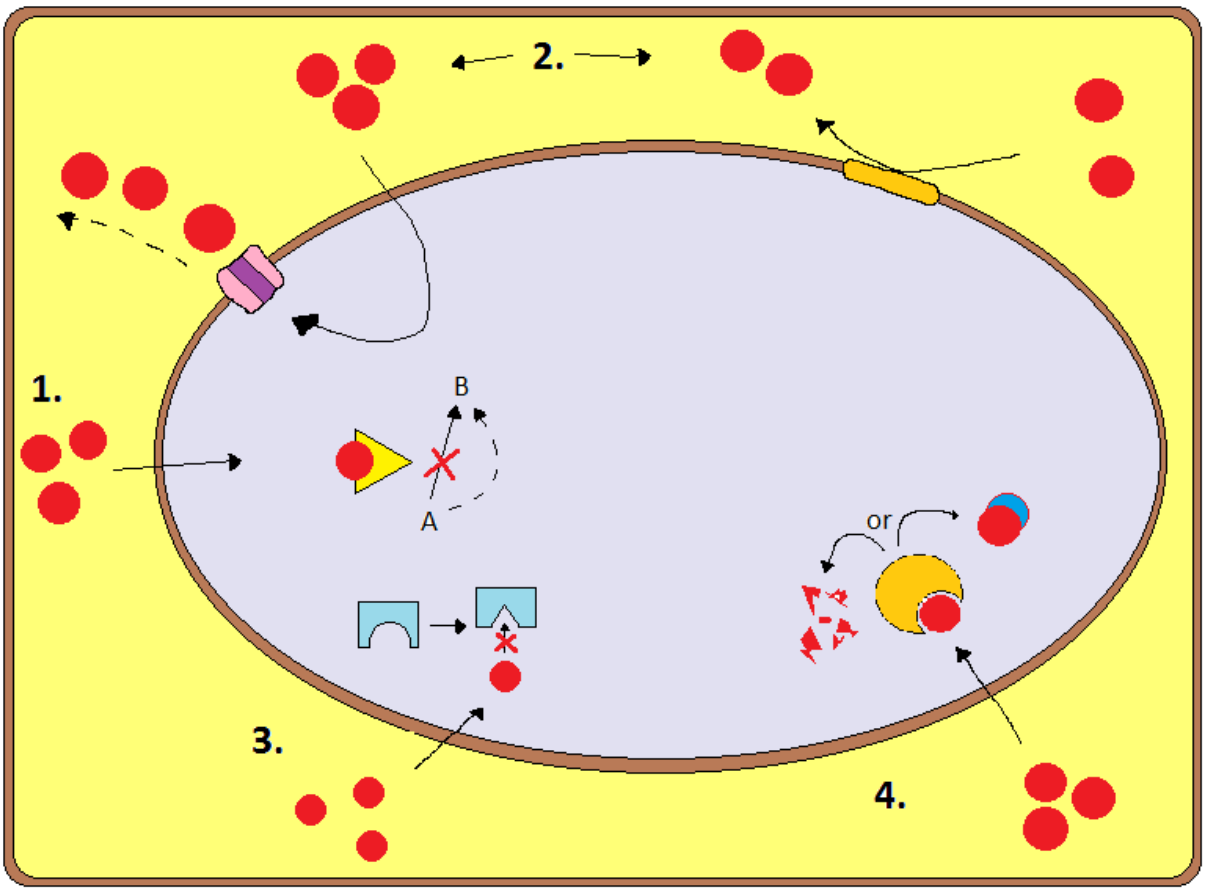

Figure 5: Depiction of the five different resistance mechanisms mentioned in this paper. Numbers corresponds to the paragraph in front of the figure.

4.4. Multidrug resistance

It is known that bacteria can develop more than one of the abovementioned mechanisms, making it even harder to fight. A bacterium that is resistant to two or more antibiotics is termed multidrug resistant. The multidrug resistance is known to generate in two different ways, ether by receiving several genes, coding for resistance to different antibiotics. For example: different plasmids spread through horizontal gene transfer from different bacteria. The other way is by increased expression of highly effective efflux pumps, which single-handedly targets and extrudes several antibiotics – so called multidrug efflux pumps (65, 66).

4.5. Collateral damage

As resistance among bacteria is increasing, scientist constantly tries to find new strategies to encounter the development of resistance. Recent research has suggested that there is a way to make existing antibiotics more efficient, termed collateral sensitivity. The concept was found in cancer research, where cancer cell which became resistant to one drug agent, seemed to be

more susceptible to other agents. The rationale assumption was that this could affect antibiotic resistance as well, especially in patients which are treated with several antibiotics in a cycling pattern. In brief, when bacteria have developed resistance to the initial drug, they are succeeded by another drug which they now will be more vulnerable to. A big challenge with collateral sensitivity is to map the effect of one antibiotic to the sensitivity of the other for a given species. When mapping of the *E. coli* alone, it was laboratory tested against 23 known antibiotics, before they could map out the collateral sensitivity network. This sensitivity network then can be used as a tool to predict which antibiotic that should be used after each other. Needless to say that what works for *E. coli* will not necessarily work on other species (37, 67).

4.6. How to fight antibiotic resistance

Globally there has been reported increasing numbers of infection-cases where bacterial isolates are resistant to all known and available antibiotics. Even though these cases are quite few in Norway, it is an increasing problem elsewhere in Europe (1). The spread of resistance is acknowledged as a worldwide problem, and it's agreed upon that there is a need for a united worldwide collaboration to encounter the situation. The World Health Assembly conducted in 2015, the Global Action Plan on Antimicrobial Resistance, where antibiotic resistance is included and addressed in a global scale. As mentioned earlier, use of antibiotics is the main driving force for resistance development. Once resistance is originated, it can epidemiologically spread through interactions among human beings, between humans and animals, through natural environment, water, food, import and travelling (2, 68).

4.6.1. Essential ways to encounter the global progress of antibiotic resistance:

- Reducing the use (2, 15, 27).
- Information of optimal use (69): for example choose narrow-spectrum antibiotics rather than broad-spectrum whenever it is possible (2, 15, 27, 70).
- Confine the spread of already resistant bacteria by proper infection control measures (2).

- Surveillance of antibiotic use, globally and international and implementation of antibiotic resistance monitoring programes (2, 69).
- Develop new types of antibiotics (2).

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Appendix I

Following are the exact nucleotide sequences of antibiotic resistance-causing genes found in oral bacteria. They are copied from PubMed Gene bank, and found by the nucleotide-search function, by searching with the gene name.

DNA sequences of some genes found in oral bacteria, coding for antibiotic resistance

cfxA2:
Prevotella denticola strain NI-106 CfxA2-like beta-lactamase (cfxa2) gene, complete cds
Accession AF504913

ORIGIN

```
        1 atggaaaaaa acagaaaaaa acaaatcgta gttttgagta tagctttagt ttgcattttc
       61 atcttggtat tttcattgtt ccataaatca gcgacaaaag atagcgcaaa tcctccttta
      121 acaaatgttt tgactgatag catttctcaa attgtctcag cttgtcctgg cgaaattggt
      181 gtggcggtta ttgttaataa cagagatacg gttaaggtca ataataagag tgtttatcct
      241 atgatgagtg tgtttaaggt tcatcaggta ttagctcttt gtaatgactt tgacaataaa
      301 ggaatttcac ttgatacctt agtaaatata aatagggata aacttgaccc taagacttgg
      361 agtcctatgc tgaaagatta ttcagggcca gtcatatcat tgacagtgag agatttgctg
      421 cgttatactc ttactcagag tgacaacaat gcaagcaacc ttatgtttaa ggatatggtt
      481 aatgtcgctc aaacagatag ttttatagcc acactcattc ctcgttcaag ttttcagata
      541 gcttatacgg aagaggaaat gtcggctgac cataacaagg cttactctaa ctatacatct
      601 cctcttggtg ctgcaatgtt gatgaatcgt ttgtttactg aaggtcttat cgatgatgag
      661 aaacaaagtt tcattaagaa tacgttaaaa gaatgcaaaa caggtgtaga taggatagca
      721 gctccacttc ttgataaaga aggggttgtt atagcgcata agacaggttc aggtgatgtt
      781 aatgaaaatg gtgttcttgc agctcacaat gatgttgcct atatatgtct gcctaataat
      841 atcagttata ccttagcggt atttgttaag gatttcaagg gaaatgaatc acaagcgtca
      901 caatatgttg cgcatatatc agctgtagta tattctttat taatgcaaac ttcagtaaaa
      961 tcttaa
```

(1)

cfxA
Citrobacter freundii genes for CFXA, CFXB, complete cds
Accession AB076667
ORIGIN

```
        1 taattgacgc tatctaatgt catcaagaat ttacttaaca ttctttcaaa atatacttaa
       61 taaaaatatc aacataccta acacaggtag tattaatgaa taaaaaaata atcatgattt
      121 tatctgtatt agttttccca ttaacaacat tagctgagag gaccccaaac gaagaaaaaa
      181 cagttgtagg atatgcagat cataatggtc aattatataa catcacagca atatatggta
      241 ggactatata ctataacgta ccaaggggaa ctctaacaat taacactacg gatgctactg
      301 gggcgcgaat acaagtttct tttgattatg cagattatgt tcgagaagcg tttaatgaat
      361 gggccccggc aggtataagt gtacaagaag ttcctgggcc tggaatggaa gcacgagttg
      421 tcacattcgg tacatccaat tatgcagata attcattagg cagtacaatc tttgatccgt
      481 caggaaactc aagattaaga attgacttag gatcatttaa taaaattatt atgaaaaatt
      541 ttgacaaact caagtctaga aaagcgatcc cagaaaacat gagtcctgag gaatacataa
      601 aactaaaatt aagaattacc ataaagcatg agatagggca catccttggc ctgttacata
      661 ataatgaaag tggtgcatac ttcccacatg gtgtcggaca ggaaatagca cgctgtaggc
      721 tcctgaacca ggctccatcg attatgttaa atggcagtaa ctatgattat atagagcgtt
      781 tagctcatta tttaggacgg ccggttaccg agtccgatat tggtccttca agaaatgaca
      841 ttgaaggggt tcgggtaatg agaagaggag gaagttgggg ttcgctaact aatcgatttt
      901 cctgccttgg tttaggattg gcattttcac gatcaggagg agacctgtaa ttaattatcc
      961 agcataactt tcaccgactt aaataatatt gtttaggtaa gatatgaaaa ttaaagctta
     1021 taaaattatg gcctatggtt tcctgatatt gccgctgtat tctaaagcgg cacctcaaaa
```

```
     1081 tatcacagag ctatgtagtg agtatcataa cactcaaatt tatgaactaa acaaagaaat
     1141 taagacgtat actgagtctt tagctggcta cagagaaatg gttattattt catttgcaaa
     1201 tggtgcaaca tttcaggtag aagttcccgg cagtcagcat ttagaatctc aaaagagacc
     1261 attagagaga atgaaggata cgctaagagc agcatatttc acaggaataa aagtcagcaa
     1321 gctttgtgtc tggaataata agacaccaaa ttctatcgct gcaatcgaat tgagtaacta
     1381 agttctaaca gcttataatt aaaggtga
```

(2)

bla(TEM)
Kiella pneumoniae plasmid pKP_STM19 beta lactamase TEM (**blaTEM**) gene, partiallebs cds
Accession JN193524
ORIGIN

```
        1 cattttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa gatgctgaag
       61 atcagttggg tgcacgagtg ggttacatcg aactggatct caacagcggt aagatccttg
      121 agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt ctgctatgtg
      181 gngcggtatt atcccgtntt gacgccgggc aagagcaact cggtcgccgc atacactatt
      241 ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga
      301 cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcn gccaacttac
      361 ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac atgggggatc
      421 atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca aacgacgagc
      481 gtgacaccac gatgcctgna gcaatggcaa caacgttgcg caaactatta actggcgaac
      541 tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat aaagttgcag
      601 gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa tctggagccg
      661 gtgagcgtgg gtctcgcggt atcattgcag cactggggcc agatggtaag ccctcccgta
      721 tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat agacagatcg
      781 ctgagatagg tgcctcactg attaagc
```

(3)

nim(B)
B.fragilis insertion sequence IS1168 and nimB gene for 5-Nitroimidazole antibiotic resistance protein.
Accession X71443

ORIGIN

```
        1 gatcatagat gccagttttg tcgttgcccc acgccagcgc aacactcgtg aggagagtgc
       61 gaagataaag gagggcaagg gcgacgaact gtggaatgac aatcctcaca agaaattcca
      121 taaggatgtg gatgcccgtt ggacgaagaa gcgcggagac acgttttacg gctacaagca
      181 gcatgtcaag gtagacaaag gcaacaaggt aatcctctcg tatgcaacaa cgcctgccaa
      241 tgtgcatgat tccaaaggtt ttgagcagct gcttgatgag tcagacaagg ataaggattt
      301 gtatcttgac gcaggatatg ccgggcagga gtcaaccgtg aaggagcatg gcatgaatcc
      361 gataatctgc gagaaaggcc gtcggaatca tcctctgaca gaaaaacaga aggctgagaa
      421 caggcgcaag tccaagaccc gttgccttgt cgagcacgta ttcggttttg aagagcaaag
      481 tatgcacggc cttattgtca ggacaatagg gattgtacgg gcaaaggcga atgtggcgat
      541 gacaaatttg acctacaaca tcttccgcta catccagatt gtgtgcaata aacgtgaatt
      601 ggcgattgcc caataaaaag agacggggtg tatcctcaaa aatgcaaaaa tccctcattt
      661 ttttttgggg ggggagatta aattgcttaa ctttacgcaa gattatcttg ctcaagacag
      721 ttaaagggtt ctgctgtctt atgcaagaaa taaaaaatgg aattgataga acccacctta
      781 ataacagatt gatatgttta gagaaatgcg acgtaagcgg caattattgc caacagaaga
      841 aagcgttgcc atccttgaaa ggatgacgaa cggaacattg gctcttcatg gggacgatgg
      901 ttacccgtat gccgttccca tcagttatgt atatgctgat ggcaaaatat atttccatag
      961 tgccatgaaa ggtcataaag tggatgccat tttgcagaat gacaaggtat cattctgcgt
     1021 ggtagaacag gatgacatca gaccgtctga gtttaccact tactttcgaa gtgtgatagt
     1081 ctttggcaaa gcccacatat tgacggatga actcgaaaaa cgtgttgctt tgggtttatt
     1141 ggcagacaag tattcgtatg gcgaagctgg catggaggct gaaatagcca aagggttcaa
```

```
     1201 tcatttgtta atagtgaaaa ttgcaattga gcatattaca ggcaaggaag ccatagaact
     1261 gaccaaaaat aggaatgacc gtccttgaca ttgcgaccaa cgggaggaca gcaagcggtt
     1321 ttggtctgcc caaaacacaa acttgctacc attgaaaaac agagttctga cttttgagta
     1381 acttaccagc ttttggataa gttcaaggat acgttgcgct tctggctgaa tc
```

(4)

erm(B)
Lactobacillus salivarius strain CHS-1E ErmB (ermB) gene, partial cds
Accession HQ651923

ORIGIN

```
        1 gtaacgtcta ttgaattaga cagtcatcta ttcaacttat cgtcagaaaa attaaaactg
       61 aatactcgtg tcactttaat tcaccaagat attctacagt ttcaattccc taacaaacag
      121 aggtataaaa ttgttgggaa tattccttac catttaagca cacaaattat taaaaaagtg
      181 gtttttgaaa gccgtgcgtc tgacatctat ctgattgttg aagaaggatt ctacaagcgt
      241 accttggata ttcaccgaac actagggttg ctcttgcaca ctcaagtctc gattcagcaa
      301 ttgcttaagc tgccagcgga atgctttcat cctaaaccaa aagtaaacag tgtcttaata
      361 aaacttaccc gccataccac agatgttc
```

(5)

erm(F)
Bacteroides fragilis partial ermF gene, allele ermFS, strain 3Bac (79a)
Accession FR692331

ORIGIN

```
        1 gacacagctt tggttgaaca tttacgaaaa ttattttctg atgcccgaaa tgttcaagtt
       61 gtcggttgtg attttaggaa ttttgcagtt ccgaaatttc ctttcaaagt ggtgtcaaat
      121 attccttatg gcattacttc cgatattttc aaaatcctga tgtttgagag tcttggaaat
      181 tttctgggag gttccattgt ccttcaatta gaacctacac aaaagttatt ttcgaggaag
      241 ctttacaatc catataccgt tttctatcat actttttttg atttgaaact tgtctatgag
      301 gtaggtcctg aaagtttctt gccaccgcca actgtcaaat cagccctgtt aaacattaaa
      361 agaaaacact tatttttga ttttaagttt aaagccaaat acttagcatt tatttcctgt
      421 ctgttagaga aacctgattt atctgtaaaa acagctttaa agtcgatttt caggaaaagt
      481 caggtcaggt caatttcgga aaaattcggt ttaaacctta atgcccaaat tgtttgtttg
      541 tctccaagtc aatggttaaa ctgttttttg gaa
```

(6)

tet(M)
Streptococcus pyogenes strain 2133-99 TetM (tetM) gene, partial cds
Accession EF363197

ORIGIN

```
        1 aaaagtaata tagggattga taaccttata gaagttatta ctaataaatt ttattcatca
       61 acacatcgag gtccgtctga actttgcgga aatgttttca aaattgaata tacaaaaaaa
      121 agacaacgtc ttgcatatat acgcctttat agtggagtac tacatttacg agattcggtt
      181 agagtatcag aaaaagaaaa aataaaagtt acagaaatgt atacttcaat aaatggtgaa
      241 ttatgtaaga ttgatagagc ttattctgga gaaattgtta ttttgcaaaa tgagtttttg
      301 aagttaaata gtgttcttgg agatacaaaa ctattgccac agagaaaaaa gattgaaaat
      361 ccgcaccctc tactacaaac aactgttgaa ccgagtaaac ctgaacagag agaaatgttg
      421 cttgatgccc ttttggaaat ctcagatagt gatccgcttc tacgatatta cgtggattct
      481 acgacacatg aaattatact ttctttctta gggaaagtac aaatggaagt gattagtgca
      541 ctgttgcaag aaaagtatca tgtggagata gaactaaaag agcctacagt catttatatg
      601 gagagaccgt taaaaaatgc agaatatacc attcacatcg aagtgccg
```

(7)

tet(O)
Enterococcus faecalis strain e291 TetO (tetO) gene, complete cds
Accession AY660532

ORIGIN

```
        1 ggaggaaaat cacatgaaaa taattaactt aggcattctg gctcacgttg acgcaggaaa
       61 gacaacatta acggaaagtt tattgtatac cagtggtgca attgcagaac tagggagcgt
      121 agatgaaggc acaacaagga cagatacaat gaatttggag cgtcaaaggg gaatcactat
      181 ccagacagca gtgacatctt ttcagtggga ggatgtaaaa gtcaacatta tagatacgcc
      241 aggccatatg gatttttgg cggaagtata ccgttcttta tccgtattag acggagcagt
      301 attattagtt tctgcaaagg atggcataca ggcacagacc cgtatactgt ttcatgcact
      361 acagacaatg aagattccga caatttttt catcaataaa attgaccaag aggggattga
      421 tttgccaatg gtatatcgag aaatgaaagc aaagctttct tcggaaatta tagtgaagca
      481 aaaggttggg cagcatcccc atataaatgt aacggacaat gacgatatgg aacagtggga
      541 tgcggtaatt atgggaaacg atgaactatt agagaaatat atgtcaggga aaccgtttaa
      601 aatgtcagaa ctggaacagg aagaaaacag gagattccaa aacggaacgt tatttcccgt
      661 ttatcacgga agcgctaaaa acaatctggg gattcggcag cttatagaag tgattgccag
      721 taaattttat tcatcaacgc ctgaaggtca atctgaacta tgcgggcagg tttttaagat
      781 tgaatattca gagaaaaggc ggcgttttgt ttatgtgcgt atatatagcg gaacattgca
      841 tttgagggat gttattagaa tatctgaaaa agagaaaata aaaatcacag agatgtgtgt
      901 tccgacaaac ggtgaattat attcatccga tacagcctgc tctggtgata ttgtaatttt
      961 accaaatgat gttttgcagc taaacagtat tttggggaac ggaatactgt tgccgcagag
     1021 aaaatttatt gaaaatcctc tccctatgct ccaaacaacg attgcagtaa agaaatctga
     1081 acagcgggaa atattgcttg gggcacttac agaaatttca gatggcgacc ctcttttaaa
     1141 atattatgtg gatactacaa cgcatgagat tatactttct tttttgggga aagtgcagat
     1201 ggaagtcatt tgtgccatcc ttgaggaaaa atatcatgtg gaggcagaaa taaaagagcc
     1261 tactgttata tatatggaaa gaccgcttag aaaagcagaa tataccatcc acatagaagt
     1321 cccgccaaat cctttctggg cttctgtcgg gttgtccata gagccgctcc ctattggaag
     1381 cggagtgcag tatgaaagca gagtttcact tggatattta aatcaatcgt tccaaaatgc
     1441 ggttatggag ggggttcttt atggctgcga gcaggggctg tatggatgga aagtgacaga
     1501 ctgtaaaatc tgttttgaat atggattgta ttatagtcct gtaagtaccc ccgcagactt
     1561 tcggctgctt tcccctatcg tattggagca ggctttaaaa aaagcaggga cagaactatt
     1621 agagccatat ctccactttg aaatttatgc accgcaggaa tatctctcac gggcgtatca
     1681 tgatgctcca aggtattgtg cagatattgt aagtactcag ataaagaatg acgaggtcat
     1741 tctgaaagga gaaatccctg ctagatgtat tcaagaatac aggaacgatt taacttattt
     1801 cacaaatggg cagggagtct gcttgacaga gttaaaagga taccagccag ctattggtaa
     1861 atttatttgc caaccccgcc gcccgaatag ccgtatagat aaggttcggc atatgttcca
     1921 caagttagct taacagcttg caaaagt
```

(8)

tet(Q)
Prevotella buccalis partial tetQ gene, strain 10Pre (55b)
Accession FN546888

ORIGIN

```
        1 atagagcatg accccaaagg acataaaaga agttttctaa aaataattga cggaagtctg
       61 agacttcgag acgttgtaag aatcaacgat tcggaaaaat tcatcaagat taaaaatcta
      121 aaaactatca atcagggcag agagataaat gttgatgaag tgggcgccaa tgatatcgcg
      181 attgtagagg atatggatga ttttcgaatc ggaaattatt taggtgctga accttgtttg
      241 attcaaagat tatcgcatca gcatcccgct ctcaaatcct ccgtccggcc agacaggccc
      301 gaagagagaa gcaaggtgat atccgctctg aatacattgt ggattgaaga cccgtctttg
      361 tccttttcca taaactcata tagtgatgaa ttggaaatct cgttatatgg tttaacccaa
      421 aaggaaatca tacagacatt gctggaagaa cgattttccg taaaggtcca ttttgatgag
      481 atcaagacta tatacaaaga acgacctgta aaaaaggtca ataagattat tcagatcgaa
      541 gtgccgccca acccttattg ggccacaata gggctgactc ttgaaccctt accgttaggg
      601 acagggttgc aaatcgaaag tgacatctcc tatggttatc tgaaccattc ttttcaaaat
```

```
      661 gccgtttttg aagggattcg tatgtcttgc caatccgggt tacatggatg ggaagtgact
      721 gatctgaaag taacttttac t
```
(6)

tet(W)
Bifidobacterium longum bv. Longum strain R29 TetW (tetW) gene, partial cds
Accession DQ988359

ORIGIN
```
        1 ggaggtgtac cgctctttgg ctgttttaga tggggccatc ttggtgatct ccgctaaaga
       61 tggcgtgcag gcccagaccc gtattctgtt ccatgccctg cggaaaatga acattcccac
      121 cgttatcttt atcaacaaga tcgaccaggc tggcgttgat ttgcagagcg tggttcagtc
      181 tgttcgggat aagctctccg ccgatattat catcaagcag acggtgtcgc tgtccccgga
      241 aatagtcctg gaggaaaata ccgacataga agcatgggat gcggtcatcg aaaataacga
      301 taaattattg gaaaagtata tcgcaggaga accaatcagc cgggaaaaac ttgtgcggga
      361 ggaacagcgg cgggttcaag acgcctccct gttcccggtc tattatggca gcgccaaaaa
      421 gggccttggc attcaaccgt tgatggatgc ggtgacaggg ctgttccaac cgattgggga
      481 acaggggagc gccgccctat gcggcagcgt tttcaaggtg gagtatacag attgcggcca
      541 gcggcgtgtc tatctacggc tatacagcgg aacgctgcgc ctgcgggata cggtggccct
      601 ggccgggaga gaaaagctga aaatcacaga gatgcgtatt ccatccaaag gggaaattgt
      661 tcggacagac accgcttatc cgggtgaaat tgttatcctt cccagcgaca gcgtgaggtt
      721 aaacgatgta ttaggggacc caacccggct
```
(9)

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