UiT
THE ARCTIC UNIVERSITY OF NORWAY

Department of Clinical Dentistry

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Presence and Levels of Antibiotic Resistance Genes in Saliva from Dental Students in Tromsø

Investigation of cfxA and erm(B) in Saliva Samples

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Master thesis in Odontology 20th May 2016

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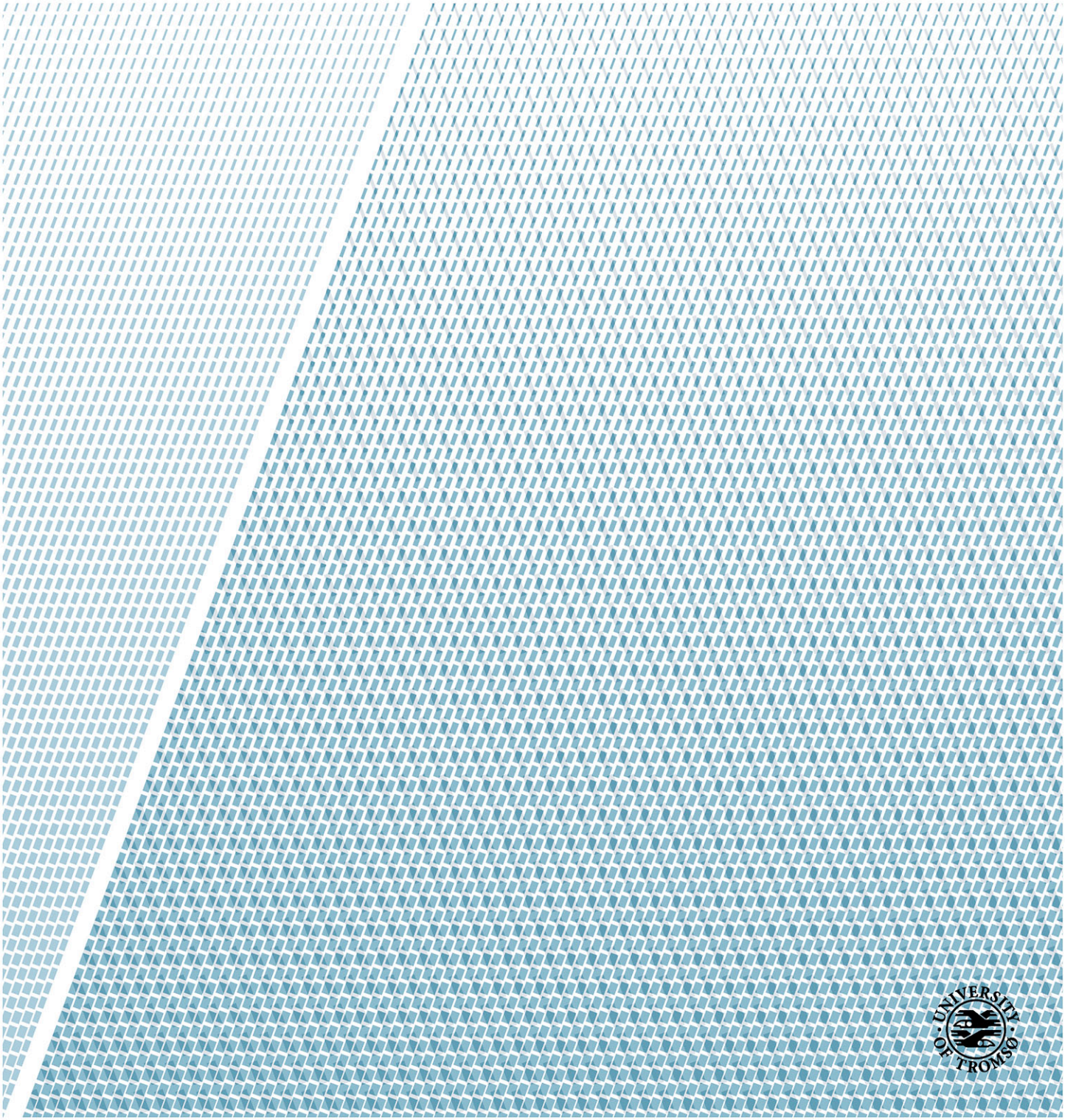

Acknowledgements

We wish to express our sincere gratitude to our supervisor Mohammed Al-Haroni, for providing us with the opportunity to work with this project. It would not have been accomplished without his encouragement, guidance and immense knowledge.

We would also like to give a special thanks to Stanislav Iakhno, for facilitating the work with this thesis.

Also, we would like to thank the participants who have willingly contributed with their time.

Finally, we want to thank Mats Rydningen and Steinar Kolskog, for their love and dedication.

Abstract

Antibiotic resistance is a global health problem that threatens humankind. Extensive use of antibiotics has led to an increase in the prevalence of resistant bacterial strains.

Aims: The aim of this study is to investigate the prevalence and levels of antimicrobial resistance genes, i.e. *cfxA* and *erm(B)*, in saliva obtained from dental students, and to assess the relationship between the prevalence of these genes and commitment to hygiene procedures and habits by dental students in the clinics.

Materials and method: A questionnaire was used to report the demographic data, attitudes and hygiene practices of 1st- and 5th-year dental students. Together with the questionnaire whole saliva samples were collected from the study subjects. DNA was extracted from the samples followed by amplification and quantification of the resistance genes using droplet digital PCR (ddPCR).

Results: We detected the resistance genes in almost all the participants, with *cfxA* detected in 100% of the samples and *erm(B)* detected in 94%. However, our result suggested that there is no significant difference in level of resistance genes between the 1st- and 5th-year dental students. On the other hand, significant difference was found between participant who had a history of taking antibiotics in the past and levels of *erm(B)* resistance gene in their saliva.

Conclusion: It seems that the use of saliva samples as a biological sample companied with the sensitivity of ddPCR could be used as a diagnostic tool to reveal the presence and levels of resistance genes in a given individual. It also seems that the high levels of *cfxA* compared to that of *erm(B)* reflect the use of β-lactam antibiotics in the society. What we still do not know is the clinical aspect regarding the resistance gene. At what level of a particular resistance gene one could predict a failure of an antibiotic treatment aiming to affect a bacteria with that gene.

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1. Antibiotic resistance

1.1. History of antibiotics

The accidental discovery of the penicillin by Alexander Fleming in 1928 was a turning point in medical history that shaped the health of humankind. Its immense therapeutic potential led to a mass production in the 1940´s, and wide distribution of the medication. Antibiotics were used to cure a variety of infectious diseases affecting human and animals. However, the issue of antibiotic resistance became a concern right after antibiotic discovery. Some strains of bacteria developed mechanisms to withstand the effectiveness of antibiotics, an observation also made by A. Fleming, who warned against the irrational use of these drugs (1). Today, there are limited treatment options for bacterial infections caused by multidrug-resistant bacteria (MDR). The majority of the classes of antibiotics that we use today were discovered in the mid 20th century, and have later been chemically modified into new generation of synthetic and semi-synthetic drugs.

1.2. Classification of antibiotics

Antibiotics or antibacterial agents kill or inhibit bacteria growth. They are used to treat an existing bacterial infection or, less frequently, to prevent serious bacterial infections. One way of classifying antibiotics is by their sites of action. The five main bacterial targets sites are cell wall synthesis, protein synthesis, nucleic acid synthesis, metabolic pathways, and cell membrane function. Antibiotics that inhibit cell wall synthesis include β-lactams and glycopeptide antibiotics. The β-lactam antibiotics share a common molecular component, a four-atom ring known as β-lactam, e.g. penicillins and cephalosporins. Some antibiotics inhibit protein synthesis, such as tetracycline and macrolides including erythromycin. The nucleic acid synthesis is inhibited by quinolones and rifamycins. Antibiotics such as sulfonamides, trimethoprim and nitroimidazoles inhibit the synthesis of metabolites affecting the nucleic acid synthesis, such as folate. Other antibiotics like lipopeptides and polymyxins target the cytoplasmic membrane of bacteria (2).

1.3. Antibiotic resistance

Antibiotic resistance is the ability of a bacterium to resists the action of an antibacterial agent at a concentration equivalent to a normal dosage. Resistance could be due to internal resistance (inherited) or could be an acquired one. Acquired resistance is gained by

undergoing a permanent genetic change, for example through horizontal gene transfer or mutations that render susceptible bacteria into a resistance one (3). There are three main mechanisms by which bacteria can resist antibiotic agents: drug inactivation, altering target and altering uptake.

1.3.1. Drug inactivation

Resistance genes allow bacteria to produce enzymes that modify and inactivate antibiotics before reaching the target site. These enzymes include β-lactamases that hydrolyse the β -lactam ring in antibiotics such as penicillin and cephalosporins (4).

1.3.2. Target alteration

Bacteria may also be able to alter the antibiotic target by the acquisition of genes encoding a different target enzyme. This mechanism leads to a lower affinity for the antibiotic and thus development of resistance.

1.3.3. Uptake alteration

Bacteria can lower the permeability of the cell wall by altering the function or numbers of protein structures needed by antibiotics to enter the cell. Also, the outer cell membrane of gram-negative bacteria has a bilayer of hydrophobic lipid bilayer creating a barrier (5). Efflux pumps are another bacterial defence mechanism that functions by transporting toxic substances out of the bacterial cell. The efflux pumps are energy dependent and may be either drug-specific or able to exert its function on a number of different substances. Overexpression of these pumps reduces the drug concentration within the cell and cause antibiotic resistance (6).

1.4. Antibiotic resistance in Norway

"Norwegian surveillance system of antimicrobial drugs resistance" (NORM) (7) concluded in a recent report that antibiotic resistance is a limited problem in Norway. However, NORM also emphasizes that the situation may change quickly if preventive measures are not followed. An effective measure to reduce the occurrence of antibiotic resistance among bacteria is to minimise the use of antibiotics to absolute necessity. In Norway, medical doctors, dentists and veterinaries have the legal right to prescribe antibiotics. Dentists contribute with approximately 8% of the all the prescriptions in the country (8). Consumption of antibiotic in any society can be measured in defined daily doses (DDDs) per 1000 inhabitants per day. The most frequently prescribed (measured in DDDs) antibiotic by

dentists is phenoxymethylpenicillin (it counts to 72% of all antibiotics prescribed by dentists). Amoxicillin and clindamycin are the second and third most prescribed accounting for approximately 11% and 6%, respectively. Looking at the consumption of antibiotics in Norway measured by DDDs for the treatment of human infections in 2014, penicillin accounted for 42% of total antibiotic consumption whereas tetracycline accounted for 18%. Macrolides (e.g. erythromycin) and lincosamides made up 9% of the total consumption (7). In addition to human use of antibiotics, a number of antibiotics are also used as growth promoters in the food industry, which is hugely disputed in the scientific community.

1.5. Resistance genes

There are a number of different genes encoding resistance to the most commonly used antibiotics in dental practice. Genes of interest in this study were *cfxA* and *erm(B),* which respectively corresponds to antibiotic resistance against β-lactam antibiotics and erythromycin.

A number of genes encode β-lactamases, one of which is the *cfxA* gene. β-lactamases are divided into subclasses A-D, and *cfxA* belongs to class A utilizing serine for β-lactam hydrolysis. This gene is prevalent in gram-negative bacterial species (9).

Erm(B) is a gene that confers resistance to the macrolide antibiotic erythromycin by encoding the enzyme rRNA adenine N-6-methyltransferase. This enzyme methylates adenine in a specific position in bacterial rRNA, hence causing an alteration of the target (10).

2. Oral bacteria

The oral cavity is inhibited with diverse microflora, consisting of viruses, fungi, protozoa archaea and bacteria. The archaea represent only a few species while the bacteria are the dominating microorganisms with hundreds and probably thousands of different bacterial species. The bacterial communities found in the human mouth shows high complexity and is the second most complex and miscellaneous after the bacterial community found in the colon (11). In the recent years, the use of next-generation sequencing (NGS) technique allows the discovery of a higher number of bacterial diversity present in the mouth, and other parts of the human body (12). Over 600 human oral bacteria and phylotypes have been identified and classified into taxonomic system provided by a public available database, the Human Oral Microbiome Database (HOMD) (13).

A mechanism that contributes to the diversity of human microflora is horizontal gene transfer between bacterial species. The horizontal gene transfer allows bacteria to take up DNA from the environment by three major mechanisms transduction, conjugation and transformation. In the similar way, antibiotic resistance genes are spread between bacteria, especially by conjugation and transformation (14). In transduction bacterial viruses (bacteriophages) take DNA from one cell and incorporating it into a new host cell upon infection. Conjugation is a transfer of DNA directly between two bacteria by specific structures in the cell membrane. Connection between the two bacteria is established and provides a bridge where the DNA from one bacterium could be exchanged to the new host. Mobile genetic elements could be mobile plasmids or transposons, which integrate in the host chromosomes by recombination. Transformation is when bacteria take up DNA directly from the surrounding environment (15). It involves binding of DNA to the cell surface, transfer one strand of the DNA across the cell membrane and integrate the new DNA through recombination to chromosomal DNA. The ability to transfer DNA between oral bacterial strains promotes a better adaption to the environment of the mouth and improves the survivability of the bacteria (14).

2.1. The mouth as a habitat

The oral cavity is warm and moist and provides favourable growth conditions for different microorganisms. This is reflected in the rich diversity of microorganisms found in the oral cavity. Saliva is helping to maintain the oral pH in neutral level, which is suitable for the growth of many microorganisms. In general, the mouth is aerobic. However, the oxygen

which is present is rapidly used by early colonizers that are aerobic or facultative anaerobic, in this way making conditions more appropriate and suitable for obligate anaerobes (12). Obligate anaerobes are especially found on tooth surfaces in dental plaque biofilms, hosting acidogenic and aciduric bacterial species. The oral cavity itself is a major source of nutrition with endogenous and exogenous nutrients, suitable for bacterial growth. Endogenous nutrients like peptides, proteins and glycoproteins are found in saliva and gingival crevicular fluid, while the exogenous nutrients originate from the daily dietary intake. Despite a wide-range of diet, the fermentable carbohydrates are the only class of nutrients that influence the microbial ecology within the mouth (16).

3. Oral Biofilm

To understand the role of the oral bacteria in health and disease it is essential to view the microbial community as one entity. Communities in oral cavity appear as microbial biofilms on teeth surfaces, mucosal surfaces, gingival crevices and tongue, all in contact with saliva. Biofilms are highly organized matrix-enclosed communities of microorganisms that develop on different surfaces, with constituent organisms becoming phenotypically distinct from their unattached counterparts (17, 18). Several studies have shown that the composition of the microflora, which constitutes the biofilm, varies remarkably at different oral structures and sites (19-21). The development of microbial community begins with adhesion of early or primary colonizers to a surface. The early colonizing organisms then provide a new surface and more favourable conditions for succeeding organisms to attach. Primary and early colonizers in the oral cavity often include *Streptococcus* and *Actinomyces* species. These provide a conditioning film for the subsequent early colonizers, such as *Veillonella* and different strains of *Actinomyces* and influence the succeeding stages of biofilm maturation (18, 22, 23).

Initial adhesion of bacteria to dental surfaces is mediated by saliva components adsorbed by these surfaces. These molecules are primarily originating from saliva, but in the subgingival region molecules derived from gingival crevicular fluid have also been documented (24). The different surfaces present different salivary receptors and, therefore, the specific components adsorbed will depend on the surface composition (18). Molecules are adsorbed to the distinct surfaces within seconds, immediately after exposure to the oral environment. Primary colonizers adhere to the surface initially by weak and reversible adhesion. Subsequently,

irreversible and strong adhesion is established between specific molecules on the microbial cell surface, called adhesins, and complementary molecules called receptors, present in the conditioning film. Later on, further accumulation of bacteria will occur by co-adhesion. Secondary and late colonizers adhere when cell surface adhesins are binding to new receptors provided by attached bacteria. The attached bacteria will multiply and increase the volume of the biofilm and exopolymers are synthesised forming biofilm matrix, enclosing the components into a biological community (12).

The extracellular matrix in biofilms mainly consists of water and macromolecules derived from microbes. The matrix provides architectural structure and mechanical stability to attached bacteria. The matrix structure and integrity is severely influenced by the surrounding macro-environment. The biofilm matrix is constantly undergoing changes, regularly replacing exopolymers and resident cells. Hence, biofilm matrix is considered as a dynamic heterogeneous system (25, 26). Additional to work as a three-dimensional network it also has a protective role, by protecting resident cells against antimicrobial agents. The matrix works like a physical barrier, preventing entry of those agents into the microbial community.

Bacteria have their own communicating system called quorum sensing, which includes expression of large number of genes called autoinducers, according to density of the bacterial population. Quorum sensing allow bacteria to regulate a variety of physiological functions and it is an important strategy in bacterial communities to regulate biofilm formation, expression of virulence factors and antibiotic resistance to mention a few (27).

It is not surprising to know that commensal and potentially pathogenic bacteria might coexist in oral microbial communities, and their counts and relative proportion could determine the presence or absence of health and disease. Most of the oral bacteria are considered to be a part of the commensal flora, relatively harmless, providing benefits for the host. When changes in the environment occur, bacteria considered to be a part of the commensal flora could show opportunistic behaviour and, therefore, acting pathogenic. This behaviour often appears when the homeostasis, which exist between commensal flora, is interrupted, for example by inflammation or by the use of antimicrobials. Certainly, most of the people got periods in their life suffering from localized episodes of disease in the mouth caused by imbalances in their resident oral microflora (16). The most common diseases caused by oral bacteria are dental caries and periodontal diseases.

4. Use of personal protective equipment in dental practice

Genetic material such as genes encoding antibiotic resistance can be exchanged between bacterial populations colonizing in the same environment. As a dentist or dental student, you have to be aware of transmission routes for infecting strains when treating patients. You also need to obtain knowledge about how to get infection control, by prevention. In dental practice, interaction with the patients, the use of rotary instruments such as handpiece, and ultrasonic scalers create a risk of catching a visible or invisible droplet of e.g. saliva, blood, microbes or aerosols spread in the working surrounding area. These droplets could also settle in a short distance on nearby dental equipment, on the dentist cloths, other dental health care personnel or the patient. To avoid widely distribution of infectious agents that could carry resistance genes, dental students and dentists are obliged to work with good hygiene habits and always use personal protective equipment in the clinic to achieve optimal infection control conditions.

Before treating the patient, the dentist should perform a thorough hand wash with soap, followed by hand disinfection. When treating the patient, the dentist should always wear gloves, surgical mask, protecting eyewear and protective clothes as a standard of care during dental treatments. The gloves and the surgical mask should be changed between different patients or during the treatment procedure if the gloves tears, or if the mask becomes wet. Also, the eyewear should be gently cleaned with water and soap, and disinfected between patients. Occasionally, protective eyewear should also be offered to the patient. The scrubs should be changed daily, or when leaving the clinic. The personal protective equipment works as a barrier to protect the skin and mucosal surface of eyes, nose and mouth when exposed to potentially infectious microbes during dental treatment. This is of great importance to prevent contamination from saliva, blood, and any potential infectious agent between patient and dentist (28).

5. Saliva

5.1. Function

Human saliva consists mostly of water (over 99%), but also contain important substances both inorganic and organic, such as electrolytes, proteins, glycoproteins and enzymes (29). Saliva has many beneficial functions and plays an important role in the maintenance of oral health. Saliva is secreted by major and minor salivary glands and is produced by clusters of cells called acini. They produce the glycoprotein mucin, which is the main component in mucous that lubricates and helps with swallowing, mastication and speech (29). Saliva also allows us to taste by acting as a solvent for taste substances. Enzymes in saliva already start the digestion process in the mouth and brakes down starch. It also protects us from harmful components, by rinsing and removing microorganisms and food from the oral cavity (29). Immunoglobulins, mainly IgA, are produced by plasma cells in the salivary glands and provide a more specific bacterial defence. By buffering action, saliva neutralizes acids in the mouth (29).

5.2. "Dry mouth"

Xerostomia is defined as a subjective feeling of oral dryness and often just described as having a "dry mouth". Hyposalivation, on the other hand, is based on an objective measurement of the saliva flow. Salivary gland hypofunction is a term that can be used to cover both subjective symptoms and objective signs of dry mouth (29). Some of the major risk factors developing salivary gland hypofunction are the use of medication, Sjogren´s syndrome and radiation treatment associated with cancer therapy. More than 500 types of medication have xerostomia as a possible side effect and especially a combination of several drugs can cause difficulties with dry mouth (30, 31). According to studies, elderly people more often have a reduced saliva secretion (32). The reduced production of saliva impairs oral functions and also increases the risk of caries, oral candidiasis and other diseases. Dry mouth is often reported after a 50 % reduction in saliva secretion (29).

5.3. Saliva flow rate test

Saliva is produced by three major salivary glands submandibular, sublingual and parotid glands in addition to many minor salivary glands. Saliva secreted in association with food intake is produced mainly in the parotid glands. Saliva flow rate can be measured with a stimulated saliva test. While chewing a piece of paraffin, saliva is collected in a collection

tube for 5 minutes (33). According to the clinical reference values used by the dental education in Tromsø, normal secretion is in the range of 1,00 – 3,00 ml/min, whereas low variation is between 0,70 – 1,00 ml/min. Low secretion/hyposalivation is secretion of less than 0,70 ml/min of saliva (34). The saliva test is a diagnostic tool, but even though a low secretion is measured it does not necessarily mean the subject is experiencing having a dry mouth. Also, those experiencing xerostomia might secrete normal amounts of saliva (33). Thus, a careful oral examination is necessary supplement to identify people with reduced saliva secretion.

5.4. Saliva fluid as a diagnostic fluid

Saliva can easily be collected non-invasively using simple equipment. Compared with blood samples there is no need for trained technicians to do the collection and the procedure is pain-free (35). Whole saliva is most frequently collected for clinical analysis, but saliva can also be collected directly from a specific salivary gland. Whole saliva also contains microorganisms, gingival crevicular fluid, a mixture of substance from the airways and gut, food debris and systemic substances. This is why saliva has been stated to be a reflection of the body (36). Dentists can assess caries risk factors by salivary analysis determining the saliva rate, buffer capacity and by detecting the amount of *Lactobacilli* and *Streptococcus Mutants*. Other areas of use are the detection of biomarkers such as hormones and antibodies. Measurements of salivary cortisol levels are used in diagnosing Cushing's syndrome (37) and oral tests detecting antibodies to Human Immunodeficiency Virus (HIV) are currently on the marked.

6. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is a technique that allow us to generate large amounts of specific sequences of DNA, which otherwise would be too small to detect. The segment to be amplified is called the template and a great number of copies, called amplicons, can be generated through repeated thermal cycles of denaturation, annealing and extension. Denaturation at a temperature of about 94-98°C separates the DNA double helix in two complementary strands. During annealing, cooling to around 50-65°C degrees allows primers (short nucleotide sequences of about 20 base pairs) to hybridize to the single stranded DNA in each end marking the starting point of the replication. A probe is a signal molecule that binds between the primers and emits fluorescence when the replication is complete. During extension the enzyme DNA polymerase synthesizes a complementary DNA strand from the 3`-end to the 5`-end of the strand by adding free nucleotides. Repeated cycles gives an exponential growth of the PCR product.

7. The aim of our study

We know today that bacteria and resistance genes are being exchanged between different communities, individuals, and bacterial population. Despite good hygiene habits, dentists/dental students have an increased risk of contamination because they work closely with infected people and use equipment that can help microbe to spread in the working environment such as handpiece. Based upon this, our hypothesis is that 5th-year students have higher levels of resistant genes in their saliva compared to 1st-year students. The aim of the current study is to investigate the occurrence of antimicrobial resistance genes in saliva obtained from dental students and to assess the relationship between the prevalence of these genes and commitment to hygiene procedures and habits by dentists/dental students in the clinics. We also want to assess knowledge and attitudes among dental students related to the use of protective equipment to achieve optimal infection control measures. It is unknown how frequently resistant genes occur in the saliva among our study population and which factors could contribute to the spread of resistance. In other words, is the prevalence and levels of resistance genes found in saliva depending on how long the dentist/dental student has been working in the clinic?

8. Materials and methods

1st-year dental and medical students and 5th-year dental students attending their studies and clinical practice at the Department of Clinical Dentistry, UiT The Arctic University of Norway were invited to participate voluntary to the study. After getting a proper consent, participants that fulfil the inclusion criteria were asked to donate a saliva sample and to complete a questionnaire on their demographic data and, where applicable, questions on commitment to proper hygiene practices at the clinic were also included. The Regional Committee for Ethical approval of North-Norway approved the study protocol and procedures to obtain saliva samples prior to the study (Reference number: 2015/1048/REK-nord).

8.1. Collecting saliva samples

8.1.1. Study population

Whole saliva specimens were collected from dental and medical students, attending education programs at UiT - The Arctic University of Norway. Medical students were invited to attend, as they have the same curriculum as the dental students in the first year of their education, and thus considered to be similar to 1st -year dental students. The dental education is a 5-year

master programme. The first two years the students are in a preclinical phase and are taught in basic sciences such as biochemistry. Third year the dental students are introduced to the clinic and patient care. Samples were collected from 1st-year students, attending either dental or medical studies and who have no contact with patient clinics at this stage of their education. The 5th year students came from two different graduating classes (graduating in June 2015 and June 2016). Subjects in both classes had at least 18 months of experience in patient treatment at dental clinics. All study subjects presented with general good health prior to the sample collection. The exclusion criteria were any antibiotic therapy within the three last months or any systematic disease that could influence the composition of oral bacteria.

8.1.2. Consent

The participants were asked to sign a consent form prior to the collection of saliva (see Appendix I). The consent form contains information about the study aims and how saliva sample will be collected, used, stored and dealt with during and after the study.
The participants were informed that the collected data will be treated confidentially, and participants will remain anonymous in any form of report of study findings.

8.1.3. Saliva sample collection

Each study subject donated a whole saliva specimen using a test for stimulated saliva. The participants were informed about the procedure of collecting saliva beforehand in a separate session, and the information was also stressed prior to the sample collection. The subjects were informed not to eat, drink, or use any form of nicotine within the last hour prior to sampling. The specimen collection was done in a quiet room available for students at the UiT The Arctic University of Norway. The subjects were relaxed and calm during the procedure. The participants were sitting in an upright position with the head inclined forward. To stimulate saliva secretion, the students were given a piece of sterile paraffin wax to chew on for approximately 30 seconds before the collection of saliva. Then the participants were spitting saliva frequently into a sterile collecting tube for five minutes. Saliva sample was then stored in -80°C freezer for further analysis.

8.1.4. Questionnaire

The participants were asked to answer a questionnaire concerning their general health, use of antibiotics and other medications in the past, and use of tobacco. They were also asked questions about their dental health, oral hygiene practices and their personal hygiene. The questionnaire also investigated commitment to hygiene practices in the dental clinic and

attitudes and knowledge related to infection control measures. In addition, knowledge about how to clean and disinfect instruments and other equipment, and how to use protecting supplements to prevent infection at the clinic were also investigated. The questionnaire comprised of 34 questions for the 5th year dental students and only 22 questions for the 1st year students (See Appendix II).

8.2. Laboratory analysis

8.2.1. Saliva inspection

The graduated test tubes containing saliva samples were visually inspected. The amount of saliva was compared to the reference values represent normal amount of saliva secretion. According to the reference values, saliva samples containing less than 5,0 ml were considered as being in the low secretion range. Samples containing 5,0 ml or more were considered to be in the normal range.

8.2.2. Bacterial DNA extraction

Bacterial DNA was extracted using QIACube and QIAamp ® DNA Mini Kit (QIAGEN). In brief, a total of 500-800 µl of saliva were transferred to sterile Eppendorf tubes and were diluted with equal amount of Dulbecco's Phosphate Buffered Saline (PBS)(Sigma® Life Science. The tubes were centrifuged for five minutes at 21100 G to pellet bacteria. After centrifugation, the excess fluid was discarded in each tube. Automated DNA extraction in QIACube was performed by using QIAamp ® DNA Mini Kit with a proper extraction protocol according to the manufacturer's instructions for isolation of bacterial DNA from body fluids. At the end of the extraction procedures, DNA samples were eluted in 50µl of TE buffer.

8.2.3. Agarose gel-electrophoresis

The yield of DNA extraction was checked with agarose gel-electrophoresis. Visualization of extraction bacterial DNA from saliva was done in 1 % agarose gel to confirm whether the extraction succeeded, or not. Agarose gel was prepared, by dissolving agarose powder (Amresco®, VWR) in TAE (Tris-acetate-EDTA) buffer. Nucleic acid stain GelRed™ Nucleic Acid (Biotium) was used to stain DNA in the agarose gel. GelRed™ is a fluorophore that binds DNA, and when excited with UV-light it will fluoresce. In brief, DNA samples were prepared as follows: A total of 10 µl of extracted DNA is mixed with 2 µl of 6x Gel Loading Dye Blue (New England BioLabs, UK). Then 10 µl of the mixture was loaded on the gel.

Also, 10 µl of 1kb DNA Ladder (New England BioLabs, UK) was also loaded in a separate lane in the gel to act as a molecular weight reference for DNA size. The 1kb DNA ladder contains bands ranging from 0,5 to 10 kilobases (kb). The agarose gel was run at 100V for approximately 50 minutes. The gel was then visualised with UV-trans-illuminator in the gel documentation system ChemiDoc™Touch Imaging System (Bio-Rad).

8.2.4. Measurement of extracted DNA concentration

The DNA concentration after DNA extraction was measured using Qubit Fluorometric (ThermoFisher Scientific). Quantitation method was done according to the manufacturer's protocol. In brief, Qubit® working solution was made by mixing 1µl Qubit® ds DNA HS reagent with 199 µl Qubit® ds DNA HS buffer. Approximately 10µl of extracted DNA and 10µl of the DNA standards from the kit (Qubit® ds DNA HS Standard #1and #2) were mixed with 190 µl Qubit working solution in small tubes and then vortexed and incubated for 2 minutes prior to the measurement of DNA concentration.

8.2.5. Droplet Digital PCR (ddPCR)

Extracted DNA samples were tested for the presence of antibiotic resistance genes by using Droplet Digital PCR (ddPCR) system (QX200 ddPCR system, Bio-Rad). The ddPCR mixture consists of 10 µl ddPCR™ Supermix for Probes (no dUTP), 1 µl of each DNA Probe (Table 1), 1 µl of diluted DNA sample and the mixture is adjusted to 20 µl reaction mix with molecular biology grade sterile water (Sigma ® Life Science). In ddPCR, each sample will be divided into approximately 20 000 small droplets. In brief, a total of 8 ddPCR mixtures representing 8 different samples were transferred to a DG8 cartridge and 70 µl of droplet generation oil for probes (Bio-Rad) was added for each ddPCR mixture in the same cartridge. The DG8 cartridge was then covered by a DG8-gasket and placed in Droplet Generator (Bio-Rad) to generate droplets (Figure 1).

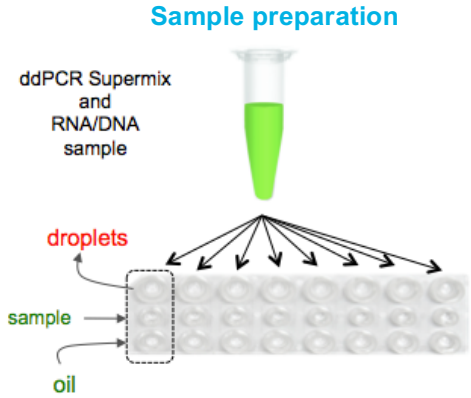

Figure 1. *Illustrates sample preparation. Picture adopted from Droplet Digital TM PCR Applications Guide (Bio-Rad)*

The droplet generator "use specially developed reagents and microfluids to partition each sample into 20 000 nanoliter-sized droplets, and the target and background DNA are distributed randomly into these droplets during the partitioning process" (38).When droplets were made, 40 µl of each PCR sample was then transferred into a 96-well microtiter plate and sealed with a perusable foil using a plate sealer (PX1™ PCR Plate Sealer, Bio-Rad) at 180°C. The PCR amplification is carried out within each droplet, using a Thermal Cycler (C1000 Touch ™ Bio-Rad). The DNA amplification protocol that was used started with preheating and enzyme activation at 95°C for 10 min, followed by amplification using the following conditions: denaturation at 95°C for 30 s, annealing and extension at 58°C for 1 min. In total 40 cycles were performed, followed by enzyme deactivation at 98°C for 10 min (39). Initially, the amplification procedure was optimized by running a temperature gradient PCR ranging from 55°C to 59°C. At 58 °C, the sample achieved satisfying separation between negative and positive droplets. Therefore, this temperature was selected when analysing the extracted DNA from saliva samples. When PCR amplification was complete, the plate containing the droplets was placed into a droplet reader (Droplet Reader QX200™ Bio-Rad), which analyse each well individually. The generated data were directly transferred into QuantaSoft™ software, where positive and negative droplets were counted and copy number of the target DNA is calculated statistically using Poisson distribution. Figure 2 illustrates the ddPCR workflow.

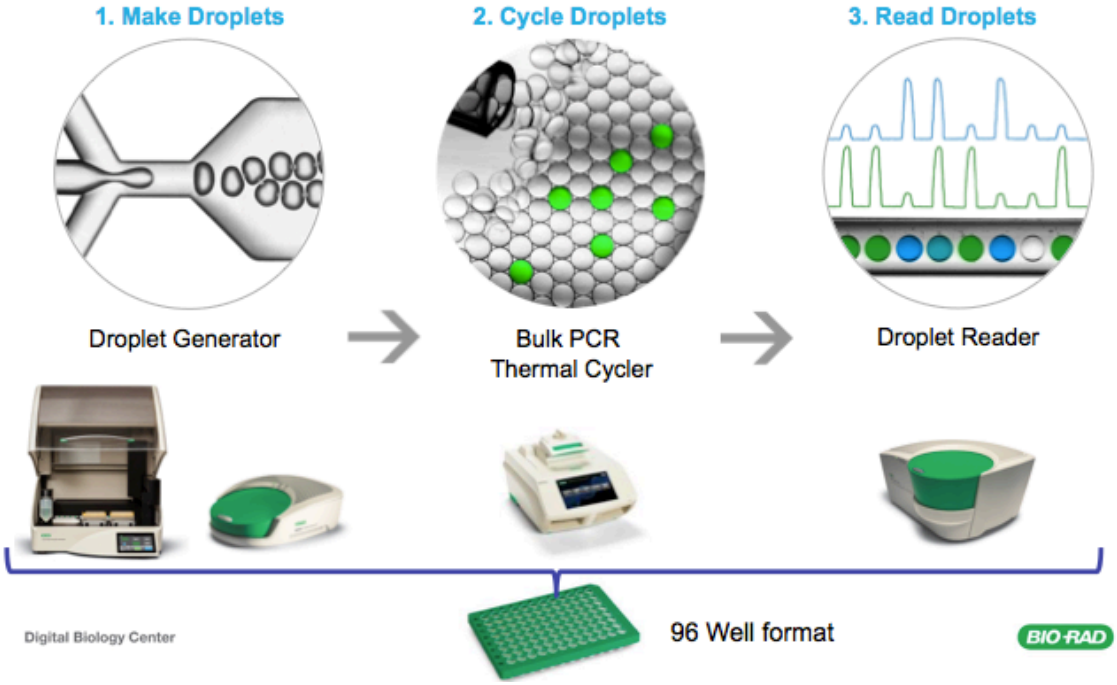

***Figure 2**. Illustrates the workflow when running ddPCR. Picture adopted from Dropet Digital TM PCR Applications guide (Bio-Rad)*

Table 1. *Presents the genetic targets used in the current study, their function, the PCR primers and probes used in these targets, and the amplicon size in PCR amplification.. The yellow sequence is the forward primer (F), the green sequence the reverse primer (R), and the blue is the probe (P).*

Gene	***DNA Sequence***	***Function***	***Remarks***
cfxA	tgcattttcatcttggtattttcattgttccataaatcagcgacaaaagatagcgcaaatcctccttta acaaatgttttgactgatagcatttctcaaattgtctcagcttgtcctggcgaaattggtgtggcgg ttattgttaataacagagatacggttaaggtcaataataagagtgtttatcctatgatgagtgtgttt aaggttcatcaggcattagctctttgtaatgactttgacaataaaggaatttcacttgataccttagt aaatataaaatagggataaacttgacccaaagacttggagtcctatgct gaaagattattcagggccagtcatatcattgacagtgagagatttgctgcgttatactcttactca gagtgacaacaatgcaagcaaccttatgtttaaggatatggttaatgtcgctcaaacagatagttt tatagccacactcattcctcgttcaagttttcagatagcttatacggaagaggaaatgtcggctga ccataacaaggcttactctaactatacatctcctcttggtgctgcaatgttgatgaatcgtttgttta ctgaaggtcttatcgatgatgagaaacaaagtttcattaagaatacgttaaaagaatgcaaaaca ggtgtagataggatagcagctccacttcttgataaagaaggggttgttatagcgcataagacag gttcaggttatgttaatgaaaatggtgttcttgcagctcacaatgatgttgcctatatatgtctgcct aataatatcagttatacctagcggtatttgttaaggatttcaagggaaataaatcacaagcgtca caatatgttgcgcatatatcagctgtagtatattctttattaatgcaaacttcagtaaaatcttaaact gcacttgctttgataattaatgataaacaatctaaaagcactctaatcgttatcggagtgcttttaga ttactaatcaaatt	Destruction of ß-lactam antibiotics	Amplicon size 81 bp
erm(B)	atgaacaaaaatataaaatattctcaaaacttttaacgagtgaaaaagtactcaaccaaataata aaacaattgaatttaaaagaaaccgataccgtttacgaaattggaacaggtaaagggcatttaa cgacgaaactggctaaaataagtaaacaggtaacgtctattgaattagacagtcatctattcaac ttatcgtcagaaaaattaaaactgaacattcgtgtcactttaattcaccaagatattctacagtttca attccctaacaaacagaggtataaaattgttgggagtattccttaccatttaagcacacaaattatt aaaaaagtggtttttgaaagccatgcgtctgacatctatctgattgttgaagaaggattctacaag cgtaccttggatattcaccgaacactagggttgctcttgcacactcaagtctcgattcagcaattg cttaagctgccagcggaatgctttcatcctaaaccaaaagtaaacagtgtcttaataaaacttacc cgccataccacagatgttccagataaatattggaagctatatacgtactttgtttcaaaatgggtc aatcgagaatatcgtcaactgtttactaaaaatcagtttcatcaagcaatgaaacacgccaaagt aaacaatttaagtaccgttacttatgagcaagtattgtctatttttaatagttatctattatttaacggg aggaaataa	Destruction of erythromycin.	Amplicon size 133 bp
16S rRNA	gttgtaaacctctttcagcagggaagaagcgaaagtgacggtacctgcagaagaagcgccgg ctaactacgtgccagcagccgcggtaatacgtagggcgcaagcgttgtccggaattattgggc gtaaagagctcgtaggcggcttgtcacgtcgggtgtgaaagcccggggcttaaccccgggtc tgcattcgatacgggctagctagagtgtgtggtaggggagatcgggaattcctgggtgtagc ggtgaaatgcgcagatatcagggaggaacaccgggtggcgaaggcggatctctgggcccat tactgacgctgaggagcgaaagcgtggggagcgaacaggattagataccctggtagtccac gccgtaaacggtgggaactaggtgttggcgacattccacgtcgtcggtgccgcagctaacgc attaagttccccgcctggggagtacggccgcaaggctaaaactcaaaggaattgacgggggc ccgcacaagcagcggagcatgtggcttaattcgacgcaacgcgaagaaccttaccaaggctt gacatacaccggaaacgtctggagacaggcgcccccttgtggtcggtgtacaggtggtgcat ggctgtcgtcagctcgtgtcgtgagatgttgggttaagtcccgcaacgagcgcaaccctgtcc tgtgttgccagcatgcccttcggggtgatggggactcacaggagaccgccggggtcaactcg gaggaaggtggggacgacgtcaagtcatcatgccccttatgtcttgggctgcacacgtgctac aatggccggtacaatgagctgcgaaaccgtgaggtggagcgaatctcaaaaagccggtctca gttcggattggggtctgcaactcgaccccatgaagtcggagttgctagtaatcgcagatcagca ttgctgcggtgaatacgttcccgggccttgtacacaccgcccgtcacgtcacgaaagttggtaa cacccgaagccggtggcccaaccccttgtgggagggagctgtcaaaggtgggactagcgat tgggacgaagtcgtaacaaggtagccgtaccggaaggtgcgg	Subunit of bacterial ribosome	Amplicon size 203 bp

8.2.6. ddPCR Data Analysis

The software QuantaSoft™ is used to analyse the data obtained from the droplet reader. The software used a two-colour detection system, measuring the number of positive and negative droplets for each fluorophore in each sample. Two channels in the QX200 is used to detect fluorophores i.e. FAM and HEX. These two channels were used to detect the presence of the resistance genes in our study. The erythromycin resistance gene *erm(B)* was detected in the FAM channel while the β-lactamase resistance gene (*cfxA*) was detected in the HEX. Detection of the presence of fluorescence in the two channels is then performed for each droplet. The number and intensity of positive and negative droplets are shown in 1-D and 2-D plot. Poisson algorithm is used then to report the concentration of each genetic target as copies/μl of the final 1x ddPCR reaction (38).

8.3. Statistical analysis

The data obtained from the ddPCR as well as the data obtained from the questionnaire were analysed by SPSS Statistical software v22.0 for any significant difference between the study subjects using the nonparametric Mann-Whitney U Test.

8.3.1. Variables retrieved from the questionnaire

Variables for descriptive statistics were retrieved from the questionnaire. They are: gender, year of birth, year of study, general health state, oral health state, a total of antibiotic-courses taken, presence of a chronic disease, smoking status, smoking duration, snuff user status, tobacco use and duration, count of daily cigarettes/snuff portions, frequency of teeth brushing, frequency of interdental cleaning appliances use, hand washing after phone usage in dental clinic, hand washing after filling in ambulatory medical card in dental clinic, hand washing after performing X-ray examination in dental clinic, hand washing after each patient in dental clinic, hand washing before each patient in dental, propensity to minimize hand washing in dental clinic, age. Initially nominal variable retrieved from question number 8 in the questionnaire was converted into a categorical variable comprised of the following categories: 0 – none, 1 – antihistamines, 2 – oral contraceptives, 3 – drugs for treatment of colitis, 4 – thyroid hormones, 5 – immune-modulators, 6 – several drug entities.

The participants reported a commercially available mark of drug for open-ended question 8 in the questionnaire, if applicable. Among these answers were following pharmaceutical names: "Levaxin", "Mercilon", "Microgynon","Cerazette","Loette", "Colazide", "Grazax", "Aerius",

"Zyrtec", and "Zetirizine". In addition some of participants provided answers containing application of a therapeutic agent, e.g. antihistamines and oral contraceptives, without using their commercial names. Nominal variable were converted into category variable sorting the drugs according to pharmaceutical registry (40) with following categories: (I) thyroid hormones, (II) drugs for colitis treatment, (III) oral contraceptives, (IV) immune-modulators, and (V) antihistamines.

8.3.2. Variables retrieved from laboratory work

The resulting figures retrieved from the laboratory work were: (1) absolute number of resistance genes (either *cfxA* or *erm(B)*) copies in each sample, reported as gene copy number per 1 µL of sample analysed, (2) absolute number of *16S rRNA* gene in sample reported as gene copy numbers per 1 µL of sample analysed, (3) the concentration of DNA samples was reported as nanogram per microliter of the sample. The results were directly transferred from the QuantaSoft™ software output (readings in the column "CopiesPer20uLWell") to the SPSS file. The outcome variables to report were as follows: (4) relative copy number of resistance genes reported as number of genes per 10^6 copies of *16S rRNA* genes, and (5) a relative copy number of resistance genes reported as number of genes per 1 nanogram of total DNA analysed.

The laboratory results (1) and (2) were adjusted for dilution of the original sample of extracted DNA. Some of the initial DNA samples had 500-fold dilution prior to ddPCR. Therefore, (1) and (2) from QuantaSoft readings were multiplied by the dilution factor in order to refer to the initial DNA sample concentration.

To compute the outcome variable (4) the following formula was used:

$$\text{relative copy number of resistance genes}^{1} = \frac{\text{absolute number of resitant gene copies}^{2}}{\text{absolute number of } 16S\ rRNA \text{ gene}^{3}} \times 10^{6}$$

The reason for computing the new (4) variable is that the outcome will represent the number of copies of *cfxA* and *erm(B)* genes attributed to the bacterial community in the mouth. In contrast, the outcome variable (5) shows number of copies of resistance genes attributed to all

[1] number of *cfxA or erm(B)* gene per 10^6 copies of *16S rRNA* gene
[2] *cfxA or erm(B)* gene copy number per 1 µL of sample analysed
[3] *16S rRNA* gene copy numbers per 1 µL of sample analysed

DNA extracted from saliva. The latter may include DNA, which originates from bacteria, viruses, fungi etc.

To compute the outcome variable (5) the following formula was used:

$$\text{relative copy number of resistant gene}^{4} = \frac{\text{absolute number of resitant gene copies}^{5}}{\text{DNA concentration}^{6}}$$

In order to perform the parametric statistical tests without violating the assumptions, the outcome variables (4) and (5) were undergone log10 transformation for further statistical analysis. Accordingly, any differences in copy numbers of *cfxA* and *erm(B)* genes between the 1st- and the 5th-year dental students were compared using the Mann-Whitney U test.

[4] Number of the gene per 1 ng of total DNA analysed
[5] Resistance gene copy number per 1 µL of sample analysed
[6] Nanograms of DNA per 1 µL of sample analysed

9. Results

9.1. Saliva sample collection

Whole saliva specimens were collected from 97 subjects. 14 subjects were excluded because of recent use of antibiotics within the last three months. A total of 83 subjects fulfilled the inclusion criteria, of which, 41 samples were collected from 1st-year students, both dental and medical students. A total of 42 samples were collected from 5th-year students (dental students). The 5th-year students came from two different graduating classes (graduating in June 2015 and June 2016). The volume of the saliva samples was visually evaluated by using the gradient marks on the test tubes and compared to the reference values. A total of 96 out of 97 samples had volumes above the threshold (5,0 ml) and were considered to have a normal secretion in the range of 1,00 – 3,00 ml/min. Only 1 sample had a value beneath the lower threshold (5,0 ml). These values were as expected, the participants in this study are relatively young and in good general health, with a low consumption of medications, which might influence the secretion.

9.2. Questionnaire

All the subjects presented with general good health. All the subjects were in the age group of 19-35 years old. The mean age of the subjects was 22 years in the group of 1st-year students and 26-years old in the group of 5th-year students. In total 27 % boys and 73 % girls were recruited from the 1st-year students. The correspondent figures for the 5th year students were 21 % and 79 %. The demographic characteristics and answers to the different questions in the questionnaire of the study subjects are shown in Table 2.

When we investigated the infection control practices in the clinic it revealed that the 5th-year students have a general good knowledge and practices in these issues. Figures 3-5 (see below) are illustrating a selection of the data set from the questionnaire, which reflects the 5th-year students' knowledge regarding infection control and hygiene in the clinic.

Significantly more 1st-year students (19.5%) reported that the status of their dental health "neither good nor bad, (statistically significant, Pearson Chi-Square test, p-value=0.010). The majority of respondents reported no use of any medications on a regular basis (62.7%). Use of oral contraceptives was as twice as high in the 5th-year females (33.3%) compared to females

in the 1st-year group (14.6%) (statistically significant, Pearson Chi-Square test, p-value=0.041).

Table 2. *Comparison of 1st- and 5th-year students regarding their answers to the different questions included in the questionnaire.*

		1st-year students (N=41)	***5th-year students (N=42)***	***Total (N=83)***
Gender	Females	30 (73.2%)	33 (78.6%)	63 (75.9%)
	Males	11 (26.8%)	9 (21.4%)	20 (24.1%)
Dental health	Very good	5 (12,2%)	17 (40,5%)	22 (26,5%)
	Good	27 (65.9%)	23 (54.8%)	50 (60.2%)
	Neither good nor bad	8 (19.5%)	2 (4.8%)	10 (12%)
	Not entirely good	1 (2.4%)	0 (0%)	1 (1.2%)
General health	Very good	22 (53.7%)	24 (57.1%)	46 (55.4%)
	Good	17 (41.5%)	17 (40.5%)	34 (41.0%)
	Neither good nor bad	2 (4.9%)	1 (2.4%)	3 (3.6%)
Frequency of sickness last 2 years	Never	3(7.3%)	5(11.9%)	8(9.6%)
	Seldom	22 (53.7%)	25 (59.5%)	47(56.6%)
	Occasionally	12 (29.3%)	12 (28.6%)	24 (28.9%)
	Often	4 (9.8%)	0 (0%)	4 (4.8%)
Satisfaction with teeth appearance	Very satisfied	17 (41.5%)	17 (40.5%)	34 (41.0%)
	Fairly satisfied	22 (53.7%)	24 (57.1%)	46 (55.4%)
	Rather dissatisfied	2 (4.9%)	1 (2.4%)	3 (3.6 %)
Medicines used daily	None	31 (75.6%)	21 (50.0%)	52 (62.7%)
	Oral contraceptives	6 (14.6 %)	14 (33.3%)	20 (24.1%)
	Antihistamines	3 (7.3%)	1 (2.4%)	4 (4.8%)
	Immune-modulators	1 (2.4%)	0 (0%)	1 (1.2%)
	Thyroid hormones	0 (0%)	2 (4.8%)	2 (2.4%)
	Adrenomimetics	0 (0%)	1 (2.4%)	1 (1.2%)
	More than 1 drug	0 (0%)	3 (7.1%)	3 (3.6%)
Total amount of antibiotic courses in life	Never	10 (24.4%)	1 (2.4%)	11 (13.3%)
	1-2 courses	13 (31.7%)	20 (47.6%)	33 (39.8%)
	3-10 courses	15 (36.6%)	15 (35.7%)	30 (36.1%)
	More than 10 courses	3 (7.3%)	6 (14.3%)	9 (10.8%)

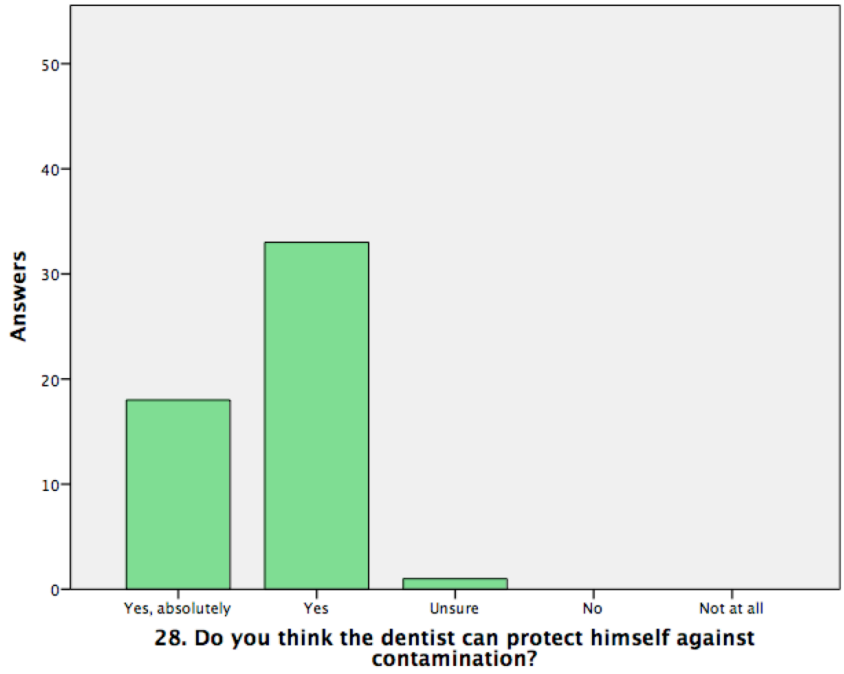

Figure 3. *Reflects attitudes among the students regarding the ability to protect himself/herself as a dentinst against contamination*

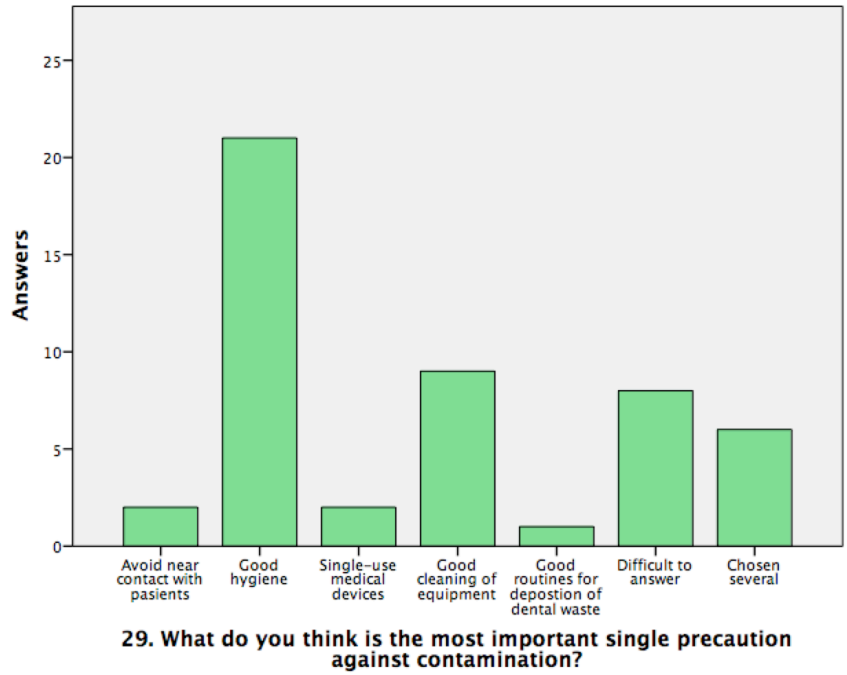

Figure 4. *Displays knowledge and attitudes regarding single precautions in dental clinic*

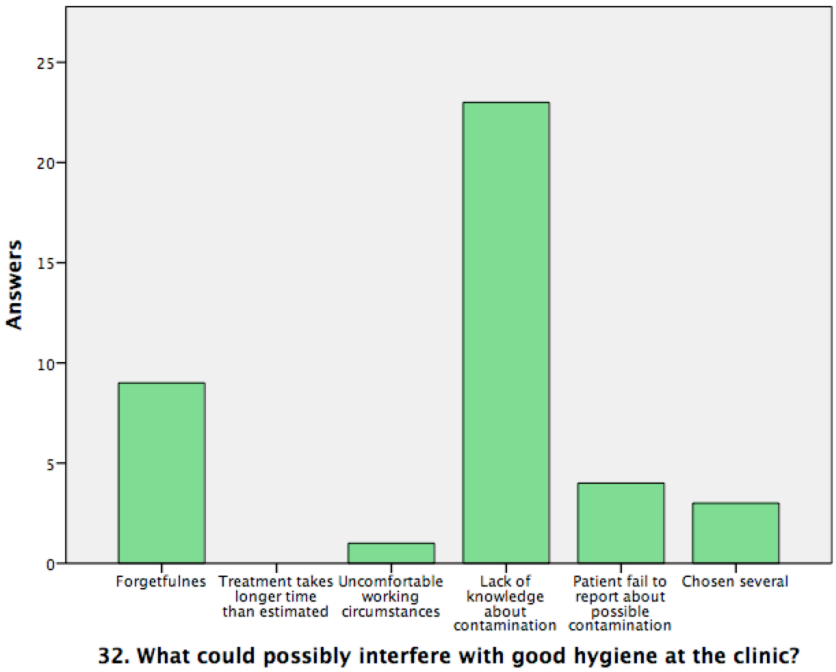

Figure 5. *Presenting attitudes and knowledge among students concerning impairment of good hygiene in the clinic*

9.3. Bacterial DNA extraction

Initially, the volume of saliva used to extract DNA was 500 µl. This was later adjusted to 800 µl after evaluation of the extraction product to ensure a sufficient amount of DNA.

9.4. Gel-electrophoresis

The presence of DNA and its molecular size were verified by gel electrophoresis. One sample was re-extracted after detecting low fluorescence intensity. After the second extraction, the amount of DNA obtained from the sample was adequate.

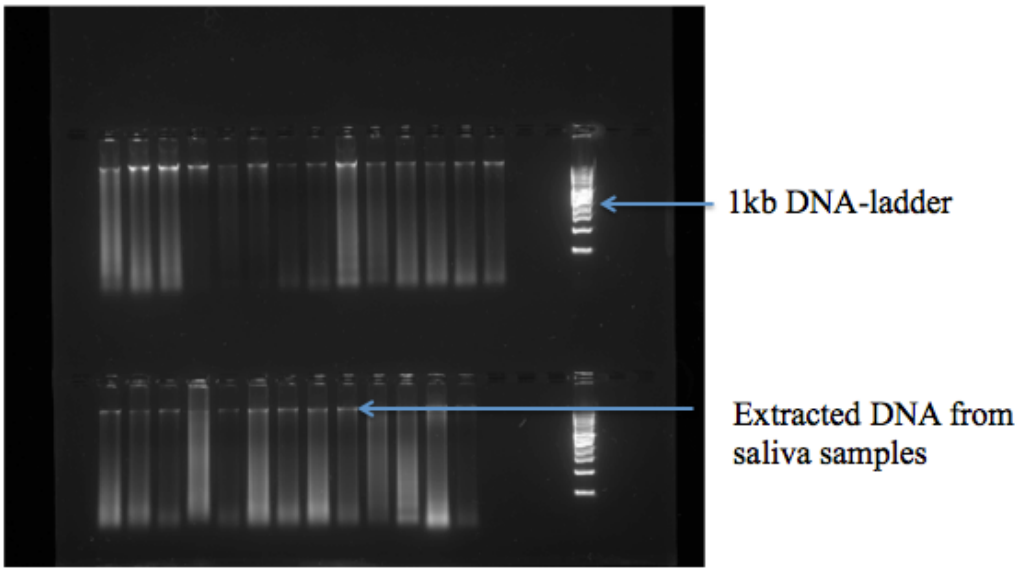

***Figure 6**. Agarose gel that shows DNA yield after DNA extraction*

9.5. Measurement of DNA-concentration

The concentration of DNA after extraction was measured using Qubit Fluorometric (ThermoFisher Scientific). The DNA concentrations obtained of all the samples are presented in Table 3.

***Table 3**. Concentrations of extracted DNA in ng/μL obtained from all saliva samples*

Sample ID	***Concentration ng/μL***	***Sample ID***	***Concentration ng/μL***	***Sample ID***	***Concentration ng/μL***
A1	5,1	**B13**	44,2	**B45**	40,6
A2	8,2	**B14**	33,8	**B46**	26,2
A3	7,0	**B15**	11,4	**B47**	40,0
A4	11,8	**B16**	8,0	**C1**	7,8
A5	2,7	**B17**	7,3	**C3**	10,0
A6	8,9	**B18**	5,2	**C4**	9,6
A7	40,8	**B20**	8,8	**C5**	59,6
A8	46,9	**B21**	9,0	**C6**	10,2
A9	16,0	**B22**	6,6	**C7**	10,4
A10	5,1	**B23**	9,6	**C8**	12,0
A11	18,9	**B24**	10,6	**C9**	48,2
A15	1,2	**B25**	6,8	**C10**	30,0
A16	1,4	**B26**	9,4	**C11**	10,4
A17	6,0	**B27**	9,9	**C12**	11,0
A18	4,2	**B29**	7,4	**C13**	10,2
A19	6,1	**B30**	39,4	**C14**	10,2
A21	2,1	**B31**	14,8	**C15**	37,4
A22	4,1	**B32**	19,6	**C16**	11,6
B1	11,1	**B33**	17,8	**C17**	18,7
B2	13,0	**B34**	12,0	**C18**	77,0
B3	14,8	**B35**	10,1	**C19**	29,6
B4	11,7	**B36**	34,8	**C21**	33,4
B5	13,9	**B37**	9,2	**C23**	12,1
B6	8,0	**B38**	6,9	**C24**	20,0
B7	4,8	**B39**	11,8	**C25**	14,5
B8	5,3	**B40**	4,3	**C27**	32,8
B9	4,0	**B41**	36,4	**C28**	22,0
B12	17,7	**B42**	10,6		

(Sample A12, A13, A14, A20, B10, B11, B19, B28, B43, B44, C2, C20, C22 and C26 were excluded from the analysis because of recent use of antibiotics within the last three months)

9.6. Droplet Digital PCR (ddPCR)

In the ddPCR, droplets are made in Droplet Generator (BioRad), then amplified before reading them in the Droplet Reader (BioRad). The Quantasoft Software is used to analyse data obtained from the droplet reader. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence while negative droplets do not. Figure 7 and 8 represent 1-D plot of positive and negative droplet separation of *erm(B)* and *cfxA*, respectively, of 8 DNA samples. Droplets that are located above the purple threshold line are designated as positive ones and all droplets located below the threshold line are designated as negative droplets. The threshold was set at 5000 amplitude intensity for *erm(B)* and *cfxA*, respectively, as droplets showed satisfying separation between negative and positive droplets above and under this chosen threshold.

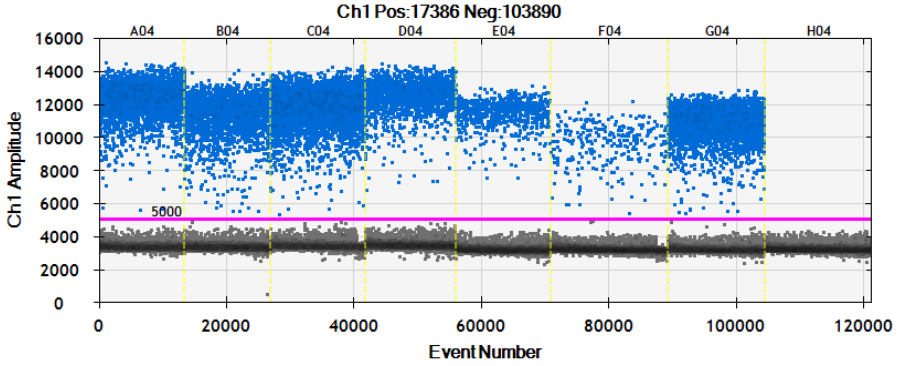

***Figure 7**. 1-D plot of 8 samples tested for the presence and level of erm(B) by the use of FAM-tagged probe. The plot illustrating positive droplets located above the purple threshold line, and negative droplets below that line.*

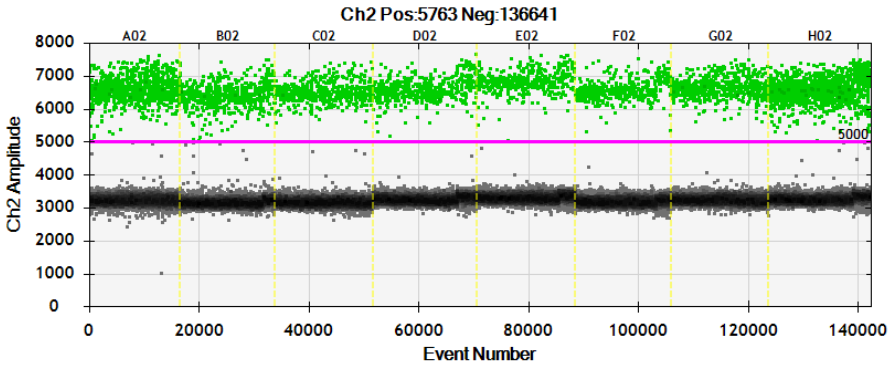

***Figure 8**. 1-D plot of 8 samples tested for the presence and level of cfxA by the use of HEX-tagged probe. The plot illustrating positive droplets located above the purple threshold line, and negative droplets below that line.*

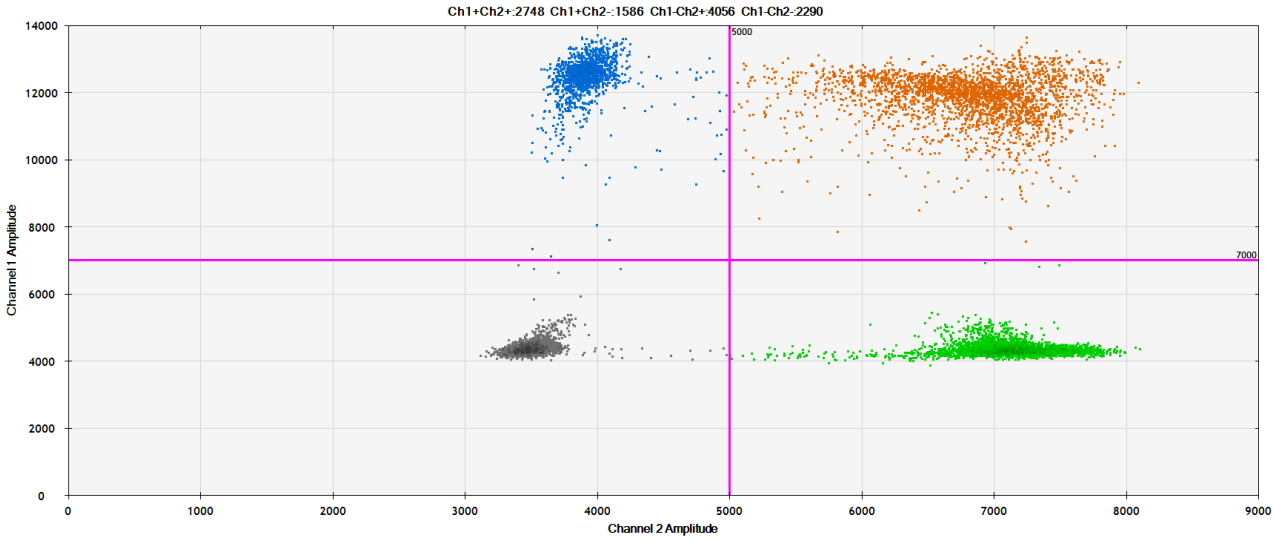

***Figure 9**. 2-D plot with droplets clustered at 4 different groups representing FAM negative, HEX negative (double-negative droplets, lower lift cluster), FAM positive, HEX negative (upper lift cluster), FAM negative, HEX positive (lower right cluster), and FAM positive, HEX positive (double-positive droplets, upper right cluster)*

The data obtained form the ddPCR from an experiment can be also llustrated in a 2-D plot. Figure 9 is a 2-D plot of *cfxA* and *erm(B)* detected in one sample. Droplets were plotted in 4 clusters. These are FAM negative and HEX negative (double-negative droplets), FAM positive HEX negative FAM negative and HEX positive, and finally FAM positive and HEX positive (double-positive droplets).

9.7. Data analyses

Data obtained from the ddPCR experiments for the 83 subjects are presented in Table 4. The data is presented as the level of *cfxA* and *erm(B)* genes detected per 1 ng of DNA obtained from saliva. We also report the level of these resistance genes among the tested samples in relation to the total bacterial population using *16S rRNA* gene as a measurement unit for counting bacterial species present in saliva. The statistical analysis reveals no significant difference between the 1st- and 5th-year dental students in the presence of the two resistance genes (P >0,05). More specifically, there was no significant difference in copy number of *cfxA* per ng DNA between the two groups (p=0,655). The difference in the copy number of *erm(B)* per ng DNA between the groups is not statistically significant and gave a p-value of 0,927. Comparing the copy numbers of resistance genes *cfxA* and *erm(B)* per 10^6 bacteria between the two study groups was found not statistically significant as well.

The level of resistant genes in saliva per 10^6 copies of *16S rRNA* and per one nanogram (ng) of DNA for *cfxA* and *erm(B)* is shown in Table 4. In addition, *Figures 10 to 13* illustrate the distributions of the log transformed data of the total amount of *cfxA* and *erm(B)* found in the 1st-year and 5th-year students per 10^6 copies of *16S rRNA* and per 1 ng of DNA.

***Table 4**. Median values of resistance genes detected in the study populations expressed as either resistance gene per one nanogram of DNA or resistance genes per 10^6 copies of 16S rRNA*

Year of study	***1st-year students (N=41)***	***5th-year students (N=42)***
cfxA/ng[1] DNA	7075.472	7658.936
(max[2], min[3], SD[4])	48250.0, 126.244, 13331. 756	58750.0, 176.351, 12563.946
erm(B)/ng DNA	434.650	395.876
(max[2], min[3], SD[4])	19458.333, 0.0, 3329.684	22403.846, 0.0, 3511.783
cfxA/10[6] 16S rRNA	11209.068	14492.107
(max[2], min[3], SD[4])	48326.180, 516.189, 12512.3021	47768.595, 640.491,12844.965
erm(B)/ 10[6] 16S rRNA	644.022	810.459
(max[2], min[3], SD[1])	12424.850, 0.0, 2888.647	13617.767, 0.0, 2575.799

1: nanogram; 2:Maximum; 3: Minimum; 4: standard deviation

We detected the *cfxA* and *erm(B)* in almost all the participants, with *cfxA* detected in 100% of the samples and *erm(B)* detected in 94%. The values of copy number of resistance genes detected in saliva samples per 10^6 copies of *16S rRNA* gene were categorizing as low, medium or high presence of resistance genes. Samples with copy number between 1 and 9999 were classified as low. On the other hand, the copy number between 10000 and 29999 was categorised as medium while samples with a copy number of 30000 or above were classified as high. According to this classification, the distribution of the *cfxA* gene in the low, medium and high group, were 32 (39%), 36 (43%) and 15 (18%), respectively. For *erm(B)* resistance gene, 75 of the samples (90%) with *erm(B)* were classified as low, and 3 samples (4%) as medium. None of the samples had *erm(B)* copy number higher than 30000 per 10^6 copies of *16S rRNA* gene.

***Table 5.** Percentage of samples categorised as low, medium and high presence of resistance gene per 10^6 copies of 16s rRNA.*

Gene	***Low (1-9999 copies)****	***Medium (10000-29999 copies)****	***High (≥30000 copies)****
cfxA	32 (39%)	36 (43%)	15 (18%)
erm(B)	75 (90%)	3 (4%)	-

* copy number of resistance genes per 10^6 copies of *16S rRNA*

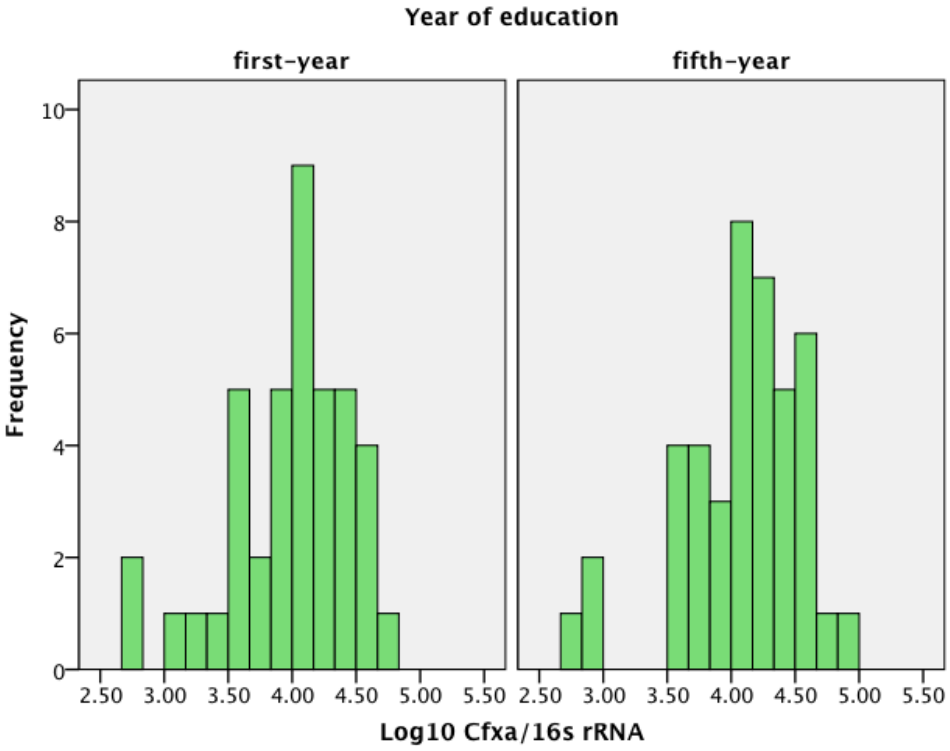

Figure 10. *Histograms of logtransformated relative copy numbers of cfxA resistance genes/ 16S rRNA in 1st year students and 5th-year students.*

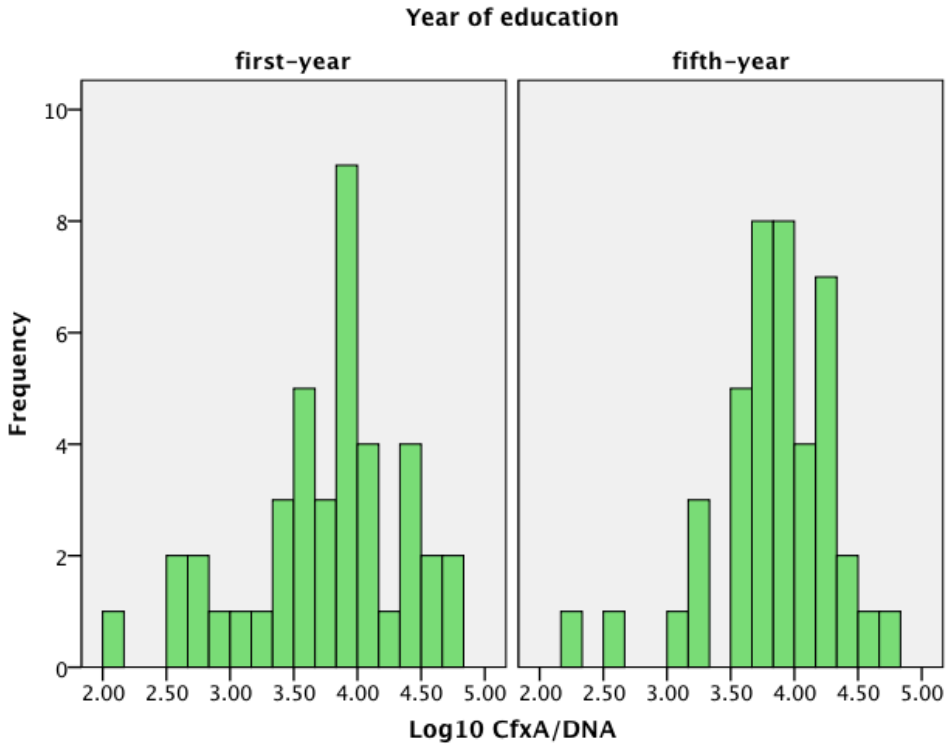

Figure 11*. Histograms of logtransformated relative copy numbers of cfxA resistance genes/ nanogram DNA in 1st year students and 5th year students.*

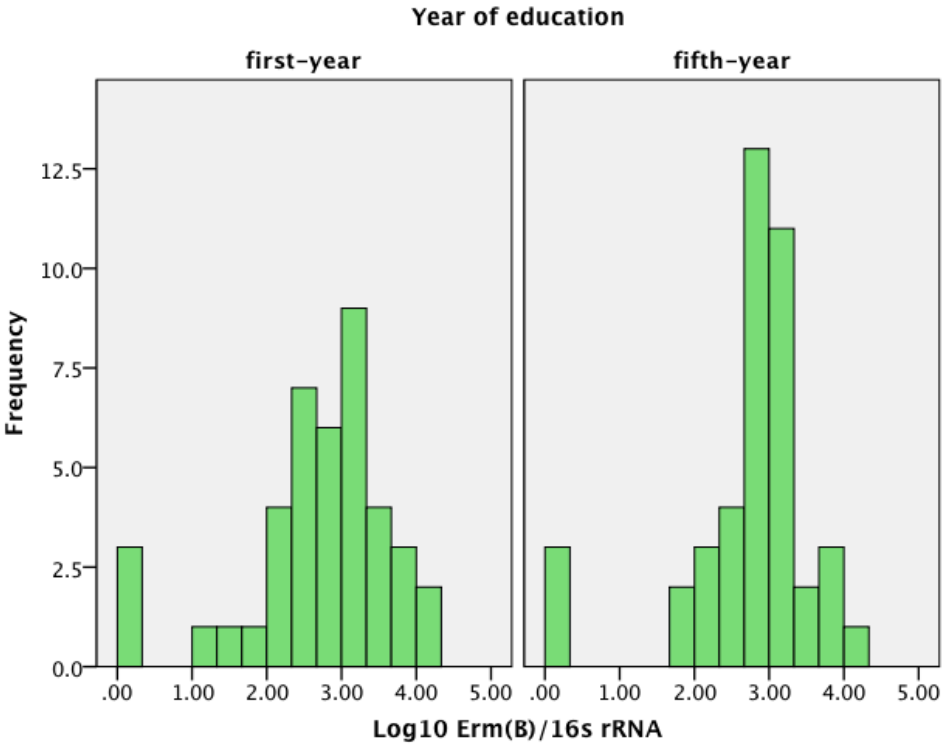

Figure 12. *Histograms of logtransformated relative copy numbers of erm(B) resistance genes/ 16S rRNA in 1st- and 5th-year students.*

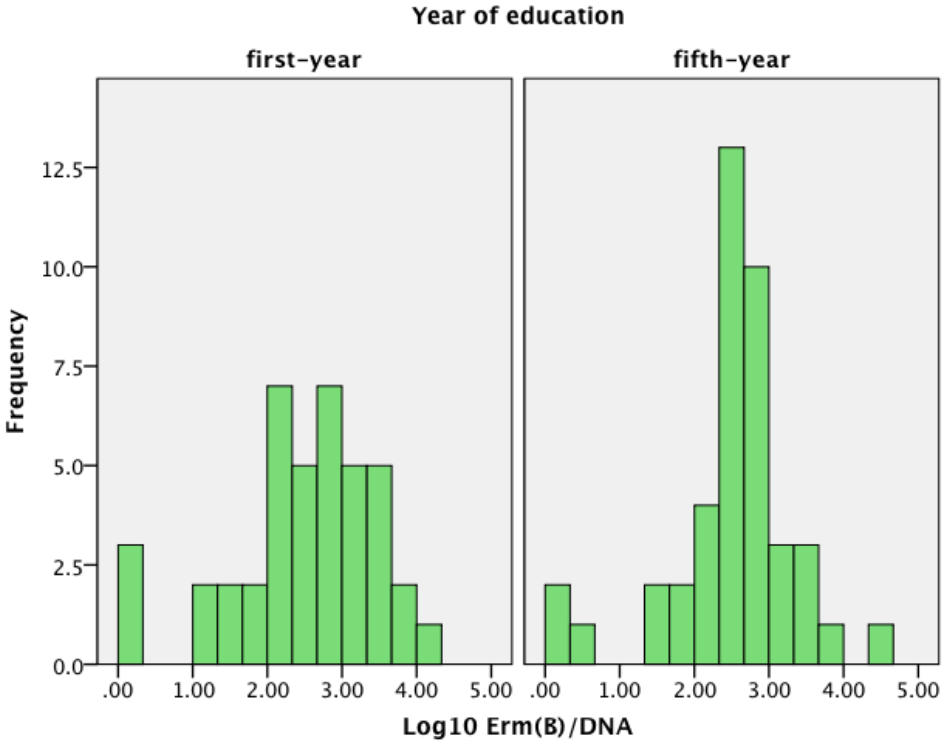

Figure 13. *Histograms of logtransformated realative copy numbers of erm(B) resistance genes/ nanogram DNA in 1st year students and 5th year students.*

Using regression analysis, we found an association between the numbers of resistance genes of *erm(B)* per ng/DNA and the use of 3-10 courses of antibiotics throughout life (p=0,035).

The use of more than 10 courses of antibiotics throughout life showed an even more significant association with the detected *erm(B)* gene copies in the saliva (p=0,005). Furthermore, we also found a significant association between the use of more than 10 courses of antibiotics and the level of resistance genes *erm(B)* per *16S rRNA* (p=0,022).

10. Discussion

The most commonly prescribed antibiotic in dental practice in Norway is phenoxymethylpenicillin (72% of total prescriptions)(7). Dentists in Norway also prescribe other antibiotics. These include, but not limited to, erythromycin, metronidazole and tetracycline (8).

The aim of the study was to investigate the occurrence of selected antimicrobial resistance genes to some of the mostly prescribed antibiotics in dental practice in Norway. In the current study, saliva samples were obtained from dental students to investigate the presence of *cfxA* and *erm(B)* genes that are responsible for phenoxymethylpenicillin and erythromycin resistance, respectively. In addition, we aimed to assess if there is any relationship between the prevalence of these genes and their levels (counts) in the saliva and being a working dentist, hygiene and infection control practices at the clinic, and any other related parameters revealed by the participants in the questionnaire, for example, history and frequency of the use of antibiotics in the past.

Although, antibiotic prescription in dentistry is much lower than that in medical practice, but resistant oral bacteria have been reported to be an increasing problem. Antibiotic resistance genes found in oral bacteria include these encode for multi-drug efflux pumps and resistance genes to aminoglycosides, β-lactams, bacitracin, and macrolides and tetracycline (41-45). Resistance to β-lactams antibiotics in oral bacteria is mainly mediated by the production of β-lactamases enzymes by bacteria. Several genes have been implicated for β-lactamase production among oral bacteria. The most reported β-lactamase-producing genes among oral isolates are *cfxA* genes (*cfxA1, cfxA2 and cfxA3*) that produce broad-spectrum β-lactamases (46, 47). On the other hand, erythromycin resistance genes that have been reported in oral bacteria include *erm* and *mef* genes (44, 48).

In our study, we compared the prevalence and levels of *cfxA* and *erm(B)* resistance genes in saliva samples obtained from 41 and 42 individuals recruited from 1st- and 5th-year dental

students, respectively. In our study, we use *16S rRNA* gene copy number as a representative of total bacterial counts in saliva samples. However, the *16S rRNA* copy number per genome varies from one bacterial species to another. Therefore, the absolute counts of resistances genes were reported per 10^6 copies of *16S rRNA* gene rather than bacterial cell numbers. Although our original thought was that there might be significant differences between the two groups based on exposure to patients during clinical practice, however, our result suggests otherwise. It might be that our sample size is small to detect differences in the level of resistant genes between the two groups. If any real difference exists between the two groups it would probably be revealed with a greater number of participants in each group. If dental practice could be considered as a risk factor for getting resistance bacteria from the working environment, then another factor that might mask this effect in our study, if any, is the time needed for the resistance genes to establish themselves permanently in the oral microbiome. Therefore, a total of 18 months of work in the dental clinic may be insufficient for resistance genes to be stable in the oral microbiome. Thus, it would be interesting to conduct a study with a population of dentist with many years of experience in treating patients.

When comparing our results with other studies in the literature, a challenge has been the lack studies with similar methodology. In fact, our study is the first, to the best of our knowledge, which report the copy number of resistance genes in saliva samples. The ddPCR is a relatively new method of gene quantification but it will certainly become more frequently used by other researchers in the coming years.

When comparing presence of resistance genes in saliva samples between 1st- and 5th-year students, we found no significant difference in the level of resistance genes between the two groups. Resistance genes of both types *cfxA* and *erm(B)* were present in, 100% and 94%, respectively, of the samples. In a previous study to investigate the prevalence of erythromycin resistant oral bacteria, 7% of cultivable oral bacteria were found to be resistant (48). In another study from Norway the overall proportion of ampicillin and metronidazole resistance among 18 identified oral species were 7.9% and 11.3%, respectively (49).

Our results show that the level of *cfxA* in the saliva samples is approximately 17 times higher compared to the level of the erythromycin resistance genes *erm(B)*. This could be explained, at least in part, to the human high proportion of use of β-lactam antibiotics in most society. In Norway, penicillins are the first choice for treatment when getting bacterial infectious

diseases that required antibiotic prescription (8). It should be logical to assume that factors that influence the accumulation of more resistance genes in oral bacteria is not limited to the systematic use of antibiotics for the treatment of oral infections but also when we use these drugs for the treatment of other infections in the body. Hence, people who had used 3-10 courses of antibiotics throughout life for any purpose showed higher copy numbers of *erm(B)* per one ng of DNA.

10.1. Conclusion

This is the first study in Norway that investigated the presence and levels of antibiotic resistance genes, namely *cfxA* and *erm(B)* in saliva samples. Although higher levels of resistance genes were found in 5th-year students compared to that in the 1st-year students, this was not found to be statistically significant. Therefore, we could not conclude that working in a dental clinic can be considered a risk factor for getting more antibiotic resistance genes. However, it seems that the use of saliva as a biological sample companied with the sensitivity of the ddPCR could be used as a quick microbiological assay in the future to reveal the presence and levels of resistance genes in a given individual. It also seems that the high levels of *cfxA* we found in saliva compared to that of *erm(B)* reflect the community use of β-lactam antibiotics where β-lactam antibiotics are the mostly prescribed antibiotics for human use. Dentists together with doctors are responsible for antibiotic prescription, and thereby both contribute to the total national consumption of these drugs. As health professionals, dentists are playing an important role by restricting the wide distribution and preventing inappropriate use of antibiotics.

A future follow up of this study is to determine the threshold level of a particular resistance gene in the saliva that would predict a failure in antibiotic treatment. As we know, the presence of resistant genes could threaten the effectiveness of antibiotic therapy. Therefore, it would be tempting to design a future study to link failure of empirical antibiotic therapy to the levels of antibiotic resistance genes that could be detected in the saliva.

11. References

1. Powers JH. Antimicrobial drug development--the past, the present, and the future. Clin Microbiol Infect. 2004;10 Suppl 4:23-31.
2. Goering RV, Dockrell, H. M., Zuckerman, M., Roitt, I. M., Chiodini, P. L. Mims´ Medical Microbiology: Saunders; 2012. 580 p.
3. World Health Organization. Antimicrobial resistance 2015 [updated Apr 2015. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/.
4. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007;128(6):1037-50.
5. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794(5):808-16.
6. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control. 2006;34(5 Suppl 1):S3-10; discussion S64-73.
7. NORM/NORM-VET. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø/ Oslo: 2014 ISSN: 1502-2307 (print) / 1890-9965 (electronic).
8. Al-Haroni M, Skaug N. Incidence of antibiotic prescribing in dental practice in Norway and its contribution to national consumption. J Antimicrob Chemother. 2007;59(6):1161-6.
9. Bush K, Jacoby GA. Updated Functional Classification of beta-Lactamases. Antimicrob Agents Ch. 2010;54(3):969-76.
10. Okitsu N, Kaieda S, Yano H, Nakano R, Hosaka Y, Okamoto R, et al. Characterization of ermB gene transposition by Tn1545 and Tn917 in macrolide-resistant Streptococcus pneumoniae isolates. J Clin Microbiol. 2005;43(1):168-73.
11. Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013;69(1):137-43.
12. Marsh PD, Moter A, Devine DA. Dental plaque biofilms: communities, conflict and control. Periodontol 2000. 2011;55(1):16-35.
13. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. J Bacteriol. 2010;192(19):5002-17.
14. Olsen I, Tribble GD, Fiehn NE, Wang BY. Bacterial sex in dental plaque. J Oral Microbiol. 2013;5.
15. Uppsala University. Transfer of resistance genes between bacteria 2015 [Available from: http://www.reactgroup.org/toolbox/category/understand/the-rise-and-spread-of-antibiotic-resistance/transfer-of-resistance-genes/.
16. Marsh P, Martin M. Oral microbiology. Edinburgh: Churchill Livingstone; 2009.
17. Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. Annu Rev Microbiol. 2002;56:187-209.
18. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. Trends Microbiol. 2005;13(12):589-95.
19. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11):5721-32.
20. Papaioannou W, Gizani S, Haffajee AD, Quirynen M, Mamai-Homata E, Papagiannoulis L. The microbiota on different oral surfaces in healthy children. Oral Microbiol Immunol. 2009;24(3):183-9.
21. Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol. 2003;30(7):644-54.

22. Li J, Helmerhorst EJ, Leone CW, Troxler RF, Yaskell T, Haffajee AD, et al. Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol. 2004;97(6):1311-8.
23. Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect. 2000;2(13):1599-607.
24. Hannig C, Hannig M, Attin T. Enzymes in the acquired enamel pellicle. Eur J Oral Sci. 2005;113(1):2-13.
25. Allison DG. The biofilm matrix. Biofouling. 2003;19(2):139-50.
26. Sutherland IW. The biofilm matrix--an immobilized but dynamic microbial environment. Trends Microbiol. 2001;9(5):222-7.
27. K Bhardwaj A, Vinothkumar K, Rajpara N. Bacterial quorum sensing inhibitors: attractive alternatives for control of infectious pathogens showing multiple drug resistance. Recent patents on anti-infective drug discovery. 2013;8(1):68-83.
28. Centers for Disease Control and Prevention. Infection Control 2013 [Available from: http://www.cdc.gov/oralhealth/infectioncontrol/faq/protective_equipment.htm.
29. Bardow A, Lagerloef, B., Nauntofte, B., Tenovuo, J. The role of Saliva. In: Fejerskov O, Kidd, E., editor. Dental Caries: The Disease and Its Clinical Management. 2nd edition: Wiley-Blackwell; 2008. p. 189-208.
30. Villa A, Wolff A, Narayana N, Dawes C, Aframian DJ, Lynge Pedersen AM, et al. World Workshop on Oral Medicine VI: A systematic review of medication-induced salivary gland dysfunction. Oral Dis. 2015.
31. Porter SR, Scully C, Hegarty AM. An update of the etiology and management of xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(1):28-46.
32. Affoo RH, Foley N, Garrick R, Siqueira WL, Martin RE. Meta-Analysis of Salivary Flow Rates in Young and Older Adults. Journal of the American Geriatrics Society. 2015;63(10):2142-51.
33. Jonsson MV, Reksten TR, Delaleu N, Marthinussen MC. Diagnostikk av munntørrhet og bruk av saliva som diagnostisk verktøy2011. S. 908-13 : ill. p.
34. Tveit AB. Kliniske rutiner- Kariologi. Saliva sekresjonshastighet UiO 2006 [cited 2016 10 May]. Available from: http://www.odont.uio.no/studier/ressurser/kariologi/Kariesutredning/Salivatester/saliva_sekresjonshastighet.html.
35. Hofman LF. Human saliva as a diagnostic specimen. J Nutr. 2001;131(5):1621S-5S.
36. Lima DP, Diniz DG, Moimaz SA, Sumida DH, Okamoto AC. Saliva: reflection of the body. Int J Infect Dis. 2010;14(3):e184-8.
37. Nieman LK. Establishing the diagnosis of Cushing´s syndrome. 2015 [cited 2016 3 Feb]. UpToDate, Inc, [cited 2016 3 Feb]. Available from: http://www.uptodate.com/contents/establishing-the-diagnosis-of-cushings-syndrome?source=machineLearning&search=cushings&selectedTitle=1~150&sectionRank=2&anchor=H6 - H6.
38. Droplet DigitalTM PCR Applications Guide. Bio-Rad.
39. Prime PCRTM ddPCRTM Copy Number Variation (CNV) Assay. User Manual: Bio Rad. p. 2.
40. Felleskatalogen over farmasøytiske spesialpreparater markedsført i Norge 2016. Oslo: Felleskatalogen; 2016.
41. Ciric L, Brouwer MS, Mullany P, Roberts AP. Minocycline resistance in an oral Streptococcus infantis isolate is encoded by tet(S) on a novel small, low copy number plasmid. FEMS Microbiol Lett. 2014;353(2):106-15.
42. Al-Haroni M, Skaug N, Bakken V, Cash P. Proteomic analysis of ampicillin-resistant oral Fusobacterium nucleatum. Oral Microbiol Immunol. 2008;23(1):36-42.

43. Ciric L, Ellatif M, Sharma P, Patel R, Song X, Mullany P, et al. Tn916-like elements from human, oral, commensal streptococci possess a variety of antibiotic and antiseptic resistance genes. Int J Antimicrob Agents. 2012;39(4):360-1.
44. Seville LA, Patterson AJ, Scott KP, Mullany P, Quail MA, Parkhill J, et al. Distribution of tetracycline and erythromycin resistance genes among human oral and fecal metagenomic DNA. Microbial drug resistance (Larchmont, NY). 2009;15(3):159-66.
45. Olsvik B, Olsen I, Tenover FC. The tet(Q) gene in bacteria isolated from patients with refractory periodontal disease. Oral Microbiol Immunol. 1994;9(4):251-5.
46. Montagner F, Jacinto RC, Correa Signoretti FG, Scheffer de Mattos V, Grecca FS, Gomes BP. Beta-lactamic resistance profiles in Porphyromonas, Prevotella, and Parvimonas species isolated from acute endodontic infections. Journal of endodontics. 2014;40(3):339-44.
47. Iwahara K, Kuriyama T, Shimura S, Williams DW, Yanagisawa M, Nakagawa K, et al. Detection of cfxA and cfxA2, the beta-lactamase genes of Prevotella spp., in clinical samples from dentoalveolar infection by real-time PCR. J Clin Microbiol. 2006;44(1):172-6.
48. Villedieu A, Diaz-Torres ML, Roberts AP, Hunt N, McNab R, Spratt DA, et al. Genetic basis of erythromycin resistance in oral bacteria. Antimicrob Agents Chemother. 2004;48(6):2298-301.
49. Al-Haroni MH, Skaug N, Al-Hebshi NN. Prevalence of subgingival bacteria resistant to aminopenicillins and metronidazole in dental patients from Yemen and Norway. Int J Antimicrob Agents. 2006;27(3):217-23.

Appendix I

FORESPØRSEL OM DELTAKELSE I FORSKNINGSPROSJEKTET

Utbredelse av antimikrobielle resistensgener i spytt blant tannlegestudenter og tannleger

Dette er et spørsmål til deg om å delta i et forskningsprosjekt for å studere forekomst av antimikrobielle resistensgener i spytt, samt å vurdere sammenhengen mellom utbredelsen av disse genene og hygienevaner og tannleger/tannlegestudenters holdninger til bruk av smittevernutstyr. Bakterier og resistens-gener utveksles mellom ulike miljøer. Det er ukjent hvor hyppig resistente mikrober forekommer i befolkningen generelt og hvilke faktorer som bidrar til å utvikle resistens. Til tross for gode hygienevaner, kan tannleger/ tannlege-studenter ha en økt smitterisiko fordi de arbeider tett på mennesker og bruker utstyr som kan spre mikrober – for eksempel air-rotor. En problemstilling i studien er: Er utbredelsen av resistensgener avhengig av hvor lenge tannlegen/tannlegestudenten har vært i klinisk praksis?

Personene som blir inviterte til å delta i studien må ha generelt god helse og ikke ha tatt antibiotika de siste 3 månedene. Din medvirkning vil ta omtrent 20 min (informasjon om studien, spyttprøve og spørreskjema).

Forskningsansvarlig skal være instituttleder ved Institutt for klinisk odontologi (IKO) UiT, Claes-Göran Crossner. Prosjektleder er Mohammed Al-Haroni, tannlege og førsteamanuensis ved IKO, UiT.

HVA INNEBÆRER PROSJEKTET?

Vi vil samle inn spytt fra 1. års tannlegestudenter som enda ikke har behandlet pasienter og fra 5. års studenter/ instruktørtannleger ved IKO med klinisk erfaring. Du plasseres i et lyst rom i en stol med rett rygg. Du skal ikke ha spist, drukket, røykt eller hatt noe som helst i munnen den siste timen før undersøkelsen, og du skal ikke ha tatt andre medikamenter enn de «vanlige» kvelden før eller samme dag som undersøkelsen finner sted. Vi vil notere alle medikamentene som du vanligvis bruker. Du vil sitte stille i ca. 5 min. før prøven tas. I denne tiden skal du fortrinnsvis ikke snakke, men konsentrere deg om å roe ned kroppen. Eventuelle tannproteser beholdes i munnen. Parafinvoks (smakløs «kloss») tygges i 30 sek. slik at den blir myk. Spyttet svelges før testen begynner. Deretter må du IKKE svelge mens du samler spytt. Du skal tygge under hele testen (5 min), som om du spiser mat, litt på hver side. Du skal spytte regelmessig i et oppsamlingsbeger med glasstrakt. Deltakeren vil få sitt resultat umiddelbart. Dersom verdien er utenfor normalområdet, vil du bli oppmuntret til å ta kontakt med tannlege.

Referanseverdier for stimulert helsaliva	
0,70 – 0,99 ml/minutt	Lav sekresjon
1,00 – 3,00 ml/minutt	Normal sekresjon

Denne måten å samle inn spytt fra en pasient er den normale måten å samle inn spytt i en klinisk situasjon.

I prosjektet vil vi innhente og registrere opplysninger om deg. Kun informasjon som du selv gir til oss gjennom spørreskjema om personalia, tannhelseerfaring, holdninger og kunnskap, vil bli benyttet i studien.

MULIGE FORDELER OG ULEMPER

Deltakerne vil få individuell tilbakemelding om sin spyttproduksjon og informasjon om antimikrobiell resistens status. Det er så og si ingen ubehag, risiko eller kjente bivirkninger knyttet til å avgi en spyttprøve.

Appendix I

FRIVILLIG DELTAKELSE OG MULIGHET FOR Å TREKKE SITT SAMTYKKE

Det er frivillig å delta i prosjektet. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke. Dersom du trekker deg fra prosjektet, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til prosjektet, kan du kontakte Førsteamanuensis Mohammed Al-Haroni, tlf. 77649151, e-mail: mohammed.al-haroni@uit.no

HVA SKJER MED INFORMASJONEN OM DEG?

Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert.

Kun deltakere fra 1. kull og instruktørtannleger ved IKO sine navn vil bli noterte, for at vi skal kunne følge opp informasjon fra disse personene etter 5 år. Alle deltakere vil tildeles en ID-kode. Denne koden knytter deg til dine opplysninger, og eventuelt til ditt navn. Koblingslisten mellom navn og kode oppbevares separat. I analysefasen vil alle opplysningene bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger.

Prosjektleder har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. All informasjon om deg vil bli anonymisert og slettet senest fem år etter prosjektslutt. Studien avsluttes i november/desember 2020.

HVA SKJER MED PRØVER SOM BLIR TATT AV DEG?

Spyttprøven som tas av deg skal oppbevares i en forskningsbiobank under navnet *Saliva-Oral Ecology-IKO*. Biobank-ansvarlig skal være Instituttleder ved Institutt for klinisk odontologi (IKO), UiT; Claes-Göran Crossner. Vi vil kun studere DNA fra de bakteriene vi finner i spyttet ditt. Humant DNA fra dine celler vil hverken bli studert eller lagret i Biobanken.

Biobanken opphører etter prosjektslutt. Studien avsluttes etter at vi har samlet inn og analysert spytt for 2. gang; i november/desember 2020.

HVA SLAGS INFORMASJON KAN DE GENETISKE UNDERSØKELSENE I PROSJEKTET GI?

Formålet med Biobanken er å oppbevare innsamlede spytt-prøver og DNA fra de bakteriene vi finner i spyttet ditt, for å studere hvorvidt spytt-bakteriene dine er resistente mot antibiotika og for at vi skal kunne sammenligne resultatet med prøver som blir tatt fra tilsvarende grupper av tannlegestudenter og instruktørtannleger om 5 år.

- Tilbakemelding til deltager
  Dersom du ønske det vil Prosjektleder gi deg en individuell, muntlig tilbakemelding om hvorvidt bakteriene vi finner i spyttet ditt er resistente mot antibiotika, etter at de genetiske analysene er gjennomført.
- Tilfeldige funn
  Prosjektet skal ikke studere DNA i de andre cellene dine.

FORSIKRING

Som for all behandling av helse- og tannhelsepersonell i Norge er deltakerne i studien dekket av Norsk Pasientskadeerstatning (NPE).

Appendix I

OPPFØLGINGSPROSJEKT

Etter 5 år vil vi igjen samle inn DNA fra spytt fra grupper av tannlegestudenter og tannleger. Om du i dag er 1. kull student vil du igjen bli invitert til å delta i studien, sammen med nye personer fra de andre gruppene. For at vi skal kunne følge personer på individnivå, vil vi be om navn på deltakere fra 1. kull og fra de ansatte ved IKO

GODKJENNING

Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk, (2015/1048/REK nord).

SAMTYKKE TIL DELTAKELSE I PROSJEKTET

JEG ER VILLIG TIL Å DELTA I PROSJEKTET

Sted og dato

Deltakers signatur

Deltakers navn med trykte bokstaver

Appendix II

SPØRRESKJEMA

UTBREDELSE AV ANTIMIKROBIELLE RESISTENSGENER I SPYTT I EN TANNLEGESTUDENT POPULASJON

GENERELL INFORMASJON

1. Kjønn?

1 ☐ Kvinne
2 ☐ Mann

2. Når er du født?

Årstall:

3. Hvilket studieår/arbeidsfunksjon er du i?

1 ☐ 1. studieår (odontologi)
2 ☐ 5. studieår (odontologi)
3 ☐ Klinisk veileder (studentklinikken, IKO)

4. Hvordan er din generelle helsetilstand nå?

1 ☐ Svært god
2 ☐ God
3 ☐ Verken god eller dårlig
4 ☐ Ikke helt god
5 ☐ Dårlig

5. De siste to årene – har du ofte vært syk?

1 ☐ Aldri syk
2 ☐ En sjelden gang
3 ☐ En gang i mellom
4 ☐ Ofte syk
5 ☐ Flere ganger alvorlig syk

6. Hvordan er din tannhelse nå?

1 ☐ Svært god
2 ☐ God
3 ☐ Verken god eller dårlig
4 ☐ Ikke helt god
5 ☐ Dårlig

7. Er du fornøyd med utseende til tennene dine?

1 ☐ Svært fornøyd
2 ☐ Noen lunde fornøyd
3 ☐ Temmelig misfornøyd
4 ☐ Svært misfornøyd

8. Bruker du medikamenter/medisiner daglig?
I tilfelle ja, hvilke?

9. Har du tatt antibiotika i løpet av de siste 3 månedene?

1 ☐ Ja
2 ☐ Nei
3 ☐ Usikker

10. Hvor mange ganger gjennom livet har du tatt en antibiotika-kur?

1 ☐ Aldri
2 ☐ 1-2 ganger
3 ☐ 3-10 ganger
4 ☐ Mer enn 10 ganger

11. I hvilken grad er du plaget med:

(*1:aldri* ---------- *4:svært mye*)

	(1)	(2)	(3)	(4)
Føler du at du ofte er tørr i munnen?	☐	☐	☐	☐
Føler du deg tørr i munnen når du spiser?	☐	☐	☐	☐
Har du ofte problemer med kjeveleddet?	☐	☐	☐	☐
Smaker ofte maten lite?	☐	☐	☐	☐

Appendix II

*12. De 5 påstandene nedenfor refererer til hvordan du har følt deg i løpet av **de siste 2 ukene**.*
(Sett en ring ved hver påstand – rundt det tallet som passer best for deg)

		Hele tiden	Ofte	Mer enn halve tiden	Mindre enn halve tiden	Sjelden	Aldri
A	Jeg føler meg trist og nedfor	1	2	3	4	5	6
B	Jeg føler meg rolig og avslappet	1	2	3	4	5	6
C	Jeg føler meg energisk, sprek og aktiv	1	2	3	4	5	6
D	Jeg våkner opp og føler jeg meg frisk og uthvilt	1	2	3	4	5	6
E	Hverdagen min er full av ting som interesserer meg	1	2	3	4	5	6

15. Har du en kronisk sykdom som innebærer at du jevnlig må ha medikamentell behandling?
(Antibiotika eller annen medisin)

1 ☐ Ja
2 ☐ Nei
3 ☐ Usikker

16. Røyker du? Om ja, hvor ofte?

1 ☐ Røyker hver dag
2 ☐ Røyker av og til
3 ☐ Røyker aldri

17. Hvor lenge har du røykt?

1 ☐ Jeg røyker ikke
2 ☐ Mindre enn i 3 år
3 ☐ I 3 eller flere år

18. Snuser du? Om ja, hvor mye?

1 ☐ Snuser hver dag
2 ☐ Snuser av og til
3 ☐ Snuser aldri

19. Hvor lenge har du snust?

1 ☐ Jeg snuser ikke
2 ☐ Mindre enn i 3 år
3 ☐ I 3 eller flere år

20. Hvor mange sigaretter/snus porsjoner per dag?

Antall sigaretter:

Antall snusporsjoner:

PERSONLIG HYGIENE

21. Hvor ofte pusser du tennene dine?

1 ☐ Morgen og kveld
2 ☐ En gang per dag
3 ☐ En gang i blant

22. Hvor ofte bruker du tanntråd/tannstikker?

1 ☐ Etter hver tannpuss
2 ☐ En gang per dag
3 ☐ En gang per uke
4 ☐ Sjeldnere
5 ☐ Aldri

23. Hvor ofte vasker du hendene dine (i klinikken)?
(Merk: her kan du gi flere svar)

1 ☐ Etter telefonbruk
2 ☐ Etter å ha skrevet journal
3 ☐ Etter å ha tatt røntgenbilde
4 ☐ Etter hver pasient
5 ☐ Før hver pasient
6 ☐ Prøver å begrense antall håndvask

Appendix II

HOLDNINGER

24. Anser du at tannleger er under større risiko for smittespredning enn «folk flest»?

1 ☐ Ja, mer enn de fleste
2 ☐ Som «folk flest»
3 ☐ Nei, mindre enn de fleste
4 ☐ Vanskelig å besvare

25. Hvem er mest utsatt for smitte på et tannlegekontor?

1 ☐ Tannlegen
2 ☐ Pasienten
3 ☐ Tannhelsesekretæren
4 ☐ Vanskelig å besvare

26. Kjenner du til/har hørt om tilfeller der en pasient har blitt smittet etter et tannlegebesøk?

1 ☐ Ja
2 ☐ Nei

27. Kjenner du til/har hørt om tilfeller der tannlegen har blitt smittet på tannlegekontoret?

1 ☐ Ja
2 ☐ Nei

28. Tenker du at tannlegen kan beskytte seg mot smitte?

1 ☐ I svært stor grad
2 ☐ I stor grad
3 ☐ Usikker
4 ☐ I liten grad
5 ☐ Overhode ikke

29. Hva er det viktigste enkelt-tiltaket mot smitte?

1 ☐ Unngå nærkontakt med pasient (dråpesmitte)
2 ☐ God håndhygiene
3 ☐ Engangsartiklene
4 ☐ God rengjøring av utstyr
5 ☐ Sikre rutinger for å deponere klinisk avfall
6 ☐ Vanskelig å besvare

30. På hvilken måte kan tannlegen beskytte seg når han/hun har en «vanlig» pasient i stolen? (Merk: her kan du gi flere svar)

1 ☐ Sprite alle arbeidsflater mellom hver pasient
2 ☐ Godt såpe-håndvask mellom hver pasient
3 ☐ Godt håndvask med sprit mellom hver pasient
4 ☐ Ved å bruke engangsartikler som munnbind, hansker, kofferdam, plastfolie etc.
5 ☐ Ved å avstå fra å bruke f.eks. «air-rotor»
6 ☐ Ved å bruke spesielt egnet arbeidstøy
7 ☐ Ved å bruke øyebeskyttelse/ visir
8 ☐ Ved å bruke papirservietter og plast-hetter
9 ☐ Ved å alltid bruke assistent ved stolen
10 ☐ Ved å alltid spyle igjennom vann i treveis-sprøyten og drikkevannslangen før neste pasient
11 ☐ Ved å alltid la pasienten skylle munnen i ca. 1 minutt med munnskyllevæske
12 ☐ Ved å ikke berøre pasienten uten verneutstyr
13 ☐ På annen måte
14 ☐ Tannlegen kan ikke beskytte seg mot smitte
15 ☐ Vanskelig å besvare

31. Kan tannlegen eliminere smitterisiko ved å følge «hygieneveilederen»?

1 ☐ Ja
2 ☐ Nei
3 ☐ Vanskelig å besvare

32. Hva er viktige barrierer for god hygiene på tannklinikken?

1 ☐ Glemsomhet
2 ☐ Behandlingen tar lengre tid
3 ☐ Ubekvemme arbeidsforhold
4 ☐ Manglende kunnskap om smittevern
5 ☐ Pasienten forteller ikke om mulige smitte

Appendix II

33. Hvilken metode ville du benyttet for å reingjøre de forskjellige redskapene?
(Sett <u>en</u> ring rundt det tallet som passer best for deg)

		Varme-des-infisering	Kjemisk-des-infisering	Damp-auto-klavering	Vakum-auto-klavering	Tørr-sterilisering	Hydrogen-peroksid	Sprit
A	Ekstraksjons-tenger	1	2	3	4	5	6	7
B	Rotkanalinstrumenter	1	2	3	4	5	6	7
C	Håndstykker (bor)	1	2	3	4	5	6	7
D	Avtrykk	1	2	3	4	5	6	7
E	Undersøkelses brett	1	2	3	4	5	6	7
F	Skarpe instrumenter	1	2	3	4	5	6	7
G	Kirurgisk utstyr	1	2	3	4	5	6	7

34. I hvilke situasjoner ville du brukt de nedenfor nevnte hjelpemidlene?
(Sett <u>en</u> ring rundt det tallet som passer best for deg)

		For alle prosedyrer	For noen prosedyrer	For infektiøse pasienter	For noen prosedyrer og infektiøse pasienter	Aldri	Vanskelig å besvare
A	Hansker	1	2	3	4	5	6
B	To par hansker	1	2	3	4	5	6
C	Munnbind	1	2	3	4	5	6
D	Øyebeskyttelse	1	2	3	4	5	6
E	Visir	1	2	3	4	5	6
F	Engangsartikler	1	2	3	4	5	6
G	Plast over hele stolen	1	2	3	4	5	6
H	Plast også på tastatur, lampe, blyant, etc	1	2	3	4	5	6

TAKK FOR DINE SVAR!