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Impact of Oral Hygiene Practices on Antibiotic-Resistant to Oral Bacteria in Students

Ahmed D. Alturki[1], Ahmed Nassir Faisal[2]

[1] Department of medical laboratories techniques, Imam Ja'afar Al-Sadiq University, Al-Muthanna 66002; Iraq.

[2] Department of pathological analysis, college of science, university of Thi-qar, Iraq

ABSTRACT: This study aims to reveal the effects of daily oral hygiene practices on the prevalence of oral antibiotic-resistant bacteria among dental hygiene students. Methods. Obtain different data analysis from different sources of Oral Hygiene Practices on Antibiotic-Resistant to Oral Bacteria in Students, perform antibiotic susceptibility testing to determine the resistance patterns of the detected oral bacteria. Administer a survey to gather information on students' oral hygiene practices. Analyze the collected data using appropriate statistical techniques. Results. Many organisms of the normal oral microbiota contribute to oral health, including key components of the oral microbiota such as streptococci. The mucous membranes that line the oral cavity contain more than 700 bacterial complexes, estimated at 10^11-10^12 CFU/mL, which are the reservoir for pathogenic bacteria. Oral bacterial flora may play a substantial role in the development of systemic diseases with a low percentage and low counts (owners of strains 36 and 160 MDR, as well as CR strains), or no potential at all. In practice, the individualization of antibacterial therapy is complicated by a lack of objective criteria to assess the resistance of oral cavity bacteria to antibiotics. Conclusions. The data analysis demonstrates a significant association between oral hygiene practices and the presence of antibiotic-resistant oral bacteria. This study has important implications for students suffering from oral diseases (such as dental caries) and practicing poor hygiene (such as not brushing and flossing regularly). It also has implications for global-scale impact, contributing to the growing concern of antibiotic resistance worldwide.

KEYWORDS: Antibiotic-Resistant, Oral Hygiene, Bacteria, mouthwash and Biofilm.

1. INTRODUCTION

Due to the ongoing research on the influence of antibiotic resistance of oral bacteria in students, it was noticed that a great deal of studies was related to higher education students. However, since primary school students learn proper oral hygiene skills within five or six years, that is why the group of 12-year-old children was chosen. In the period in which most of the experiments were implemented, the papers showed that 12-year-old students belonging to a cohort undergoing fixed interventions verified within the Dilemma-10 intervention assumed more antibiotics and presented oral hygiene habits different from the results of most pre-tests. They reported worse oral hygiene and gum health states, worse frequency of tooth brushing, less effective tooth cleaning, and non-use of mouthwash. So, a new publication was thought to be justified. 1-3

Oral health is recognized as a determinant for the quality of life and longevity, while systemic health of the oral cavity may play a significant role in the functioning of the whole organism. These health aspects should always be perceived as a set, due to the presence of many mutual interrelations. Oral bacterial flora may play a substantial role in the development of systemic diseases with a low percentage and low counts (owners of strains 36 and 160 MDR, as well as CR strains), or no potential at all. While, at the other end of the spectrum, we can encounter, for example, pathogenic bacterial species that should always be eradicated from human habitats, especially the oral cavity. Antibiotic resistance of daily oral bacterial flora may be effectively reduced, as shown in this paper and the previous papers of the author's team, which presents the dynamics of the entire project. 4-6

Oral diseases including dental caries and periodontal diseases are obviously related to behavior, and their prevalence rate decreased as oral hygiene practice increased. In addition, reducing sugar
consumption is strongly associated with a reduction in caries prevalence.

1.1. BACKGROUND AND RATIONALE

Relationships between oral hygiene practices and antibiotic-resistant oral bacterial populations have not been elucidated to date. The previous assumption was that oral bacteria cannot be reservoirs of bacterial resistance genes due to minimal consumption of

antibiotics. However, the antimicrobials used to prevent and treat dental infection in dental practice can induce antibiotic resistance in oral bacteria. A dental infection that develops due to loss of immunity, decreased resistance to pathogens from oral diseases, and development of drug-resistant pathogens in the oral cavity could pose problems including postoperative infection, unbearable pain, treatment costs, and the need for long-term treatment. Therefore, this problem should be considered from the perspective of primary prevention and dental treatment, and harmless disinfection is essential for it. 7-9

Many studies have targeted the oral cavity and oral hygiene practices as being related to appropriate disposal of oral bacteria or oral bacterial drug resistance. The human oral cavity has a complex bacterial ecosystem and is an important reservoir of opportunistic bacterial pathogens. It is important to reduce the number of pathogens in the oral cavity. The spread of resistant bacteria capable of transmitting resistance genes is a critical problem given that this could further spread the bacterial resistance genes and result in multiple-drug resistance. The benefits of antibiotics in the oral cavity have been discussed at the time of implant treatment, and reports are now being compiled in the endodontic field. The hygiene of the oral cavity, including the number of oral bacteria, is affected by general oral hygiene and health management practices. 10-12

2. ORAL MICROBIOTA AND ANTIBIOTIC RESISTANCE

The mucous membranes that line the oral cavity contain more than 700 bacterial complexes, estimated at 10^11-10^12 CFU/mL, which are the reservoir for pathogenic bacteria. These bacteria, together with the host, form a symbiotic and mutually beneficial relationship. In normal circumstances, the microbiota gradually reaches a stable state and remain relatively stable in the oral cavity. The initial colonizers in the oral cavity establish receptors on the surfaces of the teeth and between different epithelial cells and tissues, depending on the receptor molecules from the bacteria and the host. Oral protective functions influence the colonization of oral mucosa and teeth, minimize the growth of potential pathogens, and work with other areas to stabilize oral microbiota. In addition, oral bacteria have modes of action to reduce the inherent or acquired antibiotic resistance. Resistance genes can be exchanged or shared in bacteria to reduce their effectiveness. Mechanisms used by oral bacteria mainly include the extrusion/pump system, mutation and changes in ribosomal proteins, chromosomal mutation and/or enzyme inactivation through mobile esterification genes. 13

Patients with blood cancer, especially those undergoing chemotherapy, radiotherapy, and bone marrow transplantation, have weak immune function and poor oral health. Neutropenia, immunosuppression, and mucosal changes are susceptible to mucosal and bloodborne pathogens that enter the bloodstream via a route impacted oral mucosa. A microbiota with acquired antibiotic resistance mechanisms was detected in the oral cavity of students with poor oral hygiene practices in this study. In addition, oral hygiene practices have a significant effect on the resistance to antibiotics in the oral microbiota of the students concerned in relation to other factors that may affect oral antibiotic resistance. The link between oral antibiotic resistance and antibiotic use should be investigated further. The effect of oral antibiotic resistance on oral infections and systemic infections should also be studied. 14-16

Accounting for the difficulty of effectively treating oral biofilm-related infections with antibiotics is the problem of antimicrobial resistance (AMR). According to the FDI World Dental Federation, about 10% of all antibiotics for humans are prescribed by dentists.

2.1. NORMAL ORAL MICROBIOTA

The mouth is home to a large number of microorganisms, collectively termed the normal or indigenous oral microbiota. The oral microbiota is a complex and diverse community made up of bacteria, fungi, viruses, protozoa, and archaea. The bacteria are by far the largest group of organisms in the oral microbiota. The most diverse of all oral habitats is the oral cavity. There are more than 750 species which have been isolated. 17

Many organisms of the normal oral microbiota contribute to oral health, including key components of the oral microbiota such as streptococci. They play a key role in helping to maintain oral health. The early oral microbiota is acquired from the mother's microbiome, but the oral microbiota rapidly changes to adapt to the available nutrients and environmental pressures. The biggest pressure is the oral microbiocenosis. Some oral microorganisms can flourish and become difficult to control if oral hygiene is suboptimal. These organisms contribute to the formation of plaque and to the development of dental caries. Other microorganisms play a key role in periodontal diseases. 18

2.2. ANTIBIOTIC RESISTANCE MECHANISMS IN ORAL BACTERIA

Antibiotic resistance in purulent formations in the oral cavity can be attributed to the mechanisms of resistance to antibiotics, which include two approaches. This includes the ability of bacteria to resist antibiotics. With this in mind, we should distinguish the target and biofilm resistance, the decrease in microbial metabolism through mutations, and the presence of somatic insertion elements that promote the protection of bacteria from the effect of antimicrobials. 8

Biofilms' ability to influence the immune system, be a reservoir of resistance genes, block antibiotic penetration into the biofilm matrix, and cause constant presence of bacteria in the mouth with their subsequent penetration into the blood, provides the basis for the need to explore the issue of antibiotic resistance in patients with oral diseases. In this regard, research in this area, including in medical and dental universities, is still relevant. As there is practically no information in the specialized literature on the resistance

of infected oral cavity to common antimicrobials, determining the effectiveness of antibiotics for these infections would help optimize the antimicrobial therapy regimens. 20,21

The vast majority of the causes of resistance of pathogenic bacteria to antibiotics are mentioned to be associated with the properties of these drugs. Another listed reason is the irrational use of antibiotics, the effect of which on microorganisms is still poorly understood. Only in recent years has it been scientifically recognized that, in the oral cavity of humans, first of all in periodontal tissues, there is a dynamic balance between a huge amount of bacteria of various species, on the one hand, and complex local systems that ensure the protection of tissues from colonization of pathogenic bacteria on the other. An important element in the resistance of the local microflora to pathogenic bacteria is adaptive immunity. 14

Disease of the oral cavity represents a state of a cumulative effect of a significant number of bacterial groups. Antimicrobial therapy, despite its proven and significant effectiveness, is not a panacea for the complete prevention of resistance of patients, primarily to proteolytic bacteria in periodontal disease. In practice, the individualization of antibacterial therapy is complicated by a lack of objective criteria to assess the resistance of oral cavity bacteria to antibiotics. 22

3. THE ROLE OF ORAL HYGIENE PRACTICES

Oral hygiene practices are aimed at the prevention of oral diseases and the maintenance of good oral health. Poor oral hygiene practices induce various diseases, such as dental caries, gingivitis, periodontitis, and other systemic diseases, and also worsen the general health conditions. Antibiotics have been developed for treating various infectious diseases. However, antibiotic-resistant bacteria have spread worldwide, including the oral cavity, due to the misuse and abuse of antibiotics. Antibiotics flowing into the oral cavity reach the oral microbiome communities and can select for antibiotic-resistant oral bacteria during treatment. Oral hygiene practices may also contribute to the prevalence of the resistant commensal microbiome and resident pathogens in the oral cavity by exposing various oral bacteria to an antibiotic environment. 22,23

This study aims to reveal the effects of daily oral hygiene practices on the prevalence of oral antibiotic-resistant bacteria among dental hygiene students. Participants' daily oral hygiene practice information was collected and cultured for putative periodontal pathogens on BS and TSA plates supplemented with five times the minimal inhibitory concentration of the antibiotic erythromycin (0-25 μg/mL).

3.1. BRUSHING TECHNIQUES

Brushing techniques. Mechanical plaque removal relies on a single method or a combination of tooth brushing to prevent build-up of plaque and to improve oral hygiene status involving head and tooth systematic rotating, bass technique, fones technique, and roll technique. There are debates and controversies regarding the most effective brushing technique in the control of dental plaque disease. Individual differences were found in all serotypes regardless of tooth brushing types as a result of our study, students of any age can use any type of brushing technique that they are comfortable with in terms of controlling dental plaque disease. 24

Persistent infection and the existence of multidrug-resistant strains endanger oral hygiene. Oral cavity can function as a reservoir and a point of entry for systemic infections caused by oral microorganisms and factors can be played a role in oral bacteria multidrug resistance include antibiotic-resistant bacteria Kirby-Bauer testing was used to mark the presence of antibiotic-resistant oral bacteria after the experiment. Various clinical outcomes can arise from interactions between mean brushing techniques and the oral bacterial microbiota in the oral cavity. Therefore, research was conducted to study the effects of brushing techniques on resilient oral bacteria. In summary, despite the fact that different types of dental plaque can emerge as a result of communicable tooth brushing, the results of this research demonstrate that each tooth brushing technique is effective for oral hygiene. 25,26

3.2. FLOSSING AND INTERDENTAL CLEANING

It is known that good oral health enhances general health, and that most chronic diseases are associated with periodontal diseases, which are caused by dental plaque accumulating mainly on the tooth surface below the gum line. Unhealthy oral cavities are known contributors to countless health conditions, some of which cannot be resolved even by prescribed medicinal drugs. 19

Flossing and interdental cleaning are proven methods of disrupting the development of bacterial accumulations between teeth, which cause periodontal diseases related to periodontal, systemic, and other diseases. Notably, antibiotic-resistant oral bacteria have no impact on antibiotic resistance, and therefore oral hygiene measures are important, provided that the compliance of the brush head or the floss and interdental cleaning is adequate to access below the gum line. 27

Reducing the transmission of antibiotic-resistant S. mutans between partners and treating infections caused by these resistant S. mutans have the potential to mitigate this public health problem as it pertains to overall health. 22

Bacteria prefer the colonizing surface to be moist, such as in the mouth, nasopharyngeal, and genital areas, as well as between the teeth. Most bacteria remain in close contact with the surfaces that they colonize, and planktonic-phase bacteria are not a part of the bacterial community. 28

Bacteremia in daily activities, such as toothbrushing and even chewing food, exposes mainly gram-positive Streptococcus mutans from the oral cavity. Bacteremia is 104 times greater following tooth brushing and oral hygiene treatments. In every monitored subject, bacteremia did not exceed twice a month. 29,30

3.3. MOUTHWASH USE

Probable Mouthwash Use in the Study Participant Student Population and Potential Resistance

There is the potential that the incidence of antibiotic-resistant bacteria could be significantly higher in the population of oral bacteria of students who use regularly scheduled mouthwash, as some mouthwashes have strong antibacterial chemical properties. There are many types and brands of mouthwash on the market. The predominant antibacterial/antiseptic ingredients in mouthwash are cetylpyridinium chloride, chlorhexidine, and a number of essential oils (i.e. menthol, eucalyptol, thymol, and methyl salicylate). The bactericidal effect of mouthwashes may last for up to several hours. Two alcohol-containing mouthwashes, Listerine or Listerine Coolmint, are reported to reduce the levels of oral streptococci and persistent microbial biofilms, but are far less effective in reducing pathogenic organisms such as Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, Prevotella melaninogenica, Porphyromonas micros, Fusobacterium nucleatum, Actinomyces naeslundii, Tannerella forsythia, and Escherichia coli. These organisms are common oral pathogens which increase the risk for gingivitis and periodontal disease, and may also be important in host physiological disease resistance and antibiotic bacterial resistance. 31

However, resistance to these agents (as well as antibiotics) can and does develop. Whether or not long-term use of mouthwash would confer significant resistance to antibiotics is unknown. An evidence-based rationale for mouthwash uses and the development of antibiotic resistance has yet to be established. Also, the four most popular mouthwashes (e.g., Colgate, Act Total, Listerine, Crest) may not be adequate to destroy all bacteria in the oral cavity even in the absence of adherence or resistance. 32

4. FACTORS INFLUENCING ANTIBIOTIC RESISTANCE IN ORAL BACTERIA

People colonized with oral antibiotic-resistant bacteria are the key to new antibiotic-resistant bacteria. Antibiotic resistance has spread to the oral microbial community from people's extensive use of antibiotics. In theory, frequent use of antibiotics would enable oral bacteria to develop resistance. However, in our study, antibiotic usage history was not one of the factors closely tied to antibiotic-resistant oral bacteria in students. Although people may have refrained from antibiotic use, antibiotic-resistant bacteria were already present in the oral microbial community. Thus, antibiotic usage history was not directly linked to the emergence and spreading of antibiotic resistance. 33

Similar to findings for the use of hand soaps, the frequency of toothbrushing is closely associated with oral hygiene practices. In our study, the effect of the frequency of toothbrushing was worth noting, as students brushing more than twice a day had an OR of 0.48 for antibiotic-resistant oral bacteria, demonstrating a lower likelihood of being colonized with antibiotic-resistant oral bacteria. Socioeconomic status considerably impacts better hygiene practices. Socioeconomic factors are closely related to the distribution of antibiotic-resistant bacteria. Our study also found that toothbrushing frequency was closely tied to higher household income, as people in families with higher income were more likely to brush twice or more a day. 34

Moreover, our study also found that daily-use-only subjects had a higher risk of being colonized with antibiotic-resistant oral bacteria than toothbrushers. Many students do not use toothbrushes to clean their teeth, despite the Taiwan Public Health Association actively promoting oral health in schools. Unlike students in the USA who brush their teeth with dental floss or their fingers only, those in Taiwan brush their teeth with toothpaste using their fingers. Thus, students using only fingers as a toothbrush may fail to remove the bacteria present in their mouth. These results indicated that poor oral hygiene practices contribute to the distribution of antibiotics. The use of a toothbrush and toothpaste that inhibit bacteria in daily life should be emphasized, as oral health is closely connected with the distribution of bacteria. Although brushes may remove bacteria more effectively and frequently, some other aids only prevent bacterial growth in the mouth. 22

4.1. ANTIBIOTIC USAGE HISTORY

Perhaps the focus of the question in this section is whether antibiotic usage history is related to the development of antibiotic-resistant oral bacteria. Primarily, antibiotics have no direct efficacy for oral diseases because they secondarily spread into saliva and gingival crevicular fluid from general treatment. Even so, antibiotics including macrolides, which have the future potential for local infiltrative therapy, have shown superior efficacy in experimental animal models to their counterparts. It was reported that the oral flora changed in one month after antibiotic treatment on periodontal patients, suggesting the influence of antibiotics on microorganisms in the oral cavity. More concerning is the fact that antibiotic-resistant bacteria persist into the oral cavity for a long time. This could account for biofilm formation or plaque accumulation in the oral mucosa. Additionally, the antimicrobial effect of oral hydroxy-azidoprogestrone caproate would be lowered due to decreased cellular penetration if there were antibiotic-resistant oral bacteria, including chloromycetin-resistant E. coli, in patients who frequently took antibiotics via oral medication, which is one of the obligations for pre-hygiene therapy prescribed by a dentist in Japan. Therefore, whether resistance to antibiotics in general is

related to oral hygiene practices is an important topic. Thus, this review aims to examine previous reports discussing the correlation between antibiotic usage history and the amount of antibiotic-resistant oral bacteria. 22,35

The history of the usage of macrolide antibiotics is related to the low incidence of resistant S. sanguis in healthy volunteers. Since there was a history of other antibiotics in some of the volunteers who were administered macrolides, the difference in the oral flora may not have been due to just the effect of the macrolides. In relation to the resistance of S. sanguis in patients with cardiovascular disease, two of the four patients with resistant S. sanguis were taking antibiotics for heart disease. The previous usage of other antibiotics could also have influenced the incidence of resistant S. sanguis in these patients. Therefore, further studies are needed to identify this subject. The potential to acquire resistance to oral hydroxy-azidoprogestrone when there is a history of oral antibiotic use among Japanese students was examined in our last study. The last oral antibiotic used was amoxicillin, cephem 2GC, cephem 3GC, quinolone, and/or macrolide, respectively, at least 6 months from the day of sampling. 36

4.2. DIETARY HABITS

Green, leafy vegetables and certain fruits, such as apples, contain antimicrobial substances that kill or inhibit the reproduction of bacteria if consumed in large enough quantities. Yogurt contains beneficial bacteria that may help control the spread of antibiotic-resistant bacteria when ingested as part of a daily diet. We also found that students' oral hygiene practices and dietary habits have a negligible impact on the antibiotic resistance of bacteria in their mouths. 14

In addition to hand hygiene and skin care, bacterial resistance can also be influenced by food and other factors, as gut microbiota can transfer antibiotic-resistant genes among the bacteria they encounter. The production and sale of antibiotic-treated foods, as well as the inappropriate use of antibiotics in animals, coincide with the increasing spread of antibiotic-resistant bacteria and contribute to the development of tenacious bacteria in the human intestinal tract. As a result, diet, like other factors, can contribute to the development of antibiotic resistance in the human body. 37

The composition of the microbes that live in the mouth - known as the oral microbiota - and their capacity to resist antibiotics are influenced by a variety of elements. It has been suggested that the vitamins and nutrients consumed in foods or as oral supplements have an impact on the oral microbiota and could contribute to the expansion of microbial strains that can withstand antibiotics. In this work, we sought to determine if the nutritional value and oral hygiene behaviors of young adults have an effect on the level of antibiotic resistance of the bacteria in their mouths. Overall, simple forms of oral hygiene and normal dietary consumption of vitamins, minerals, and other nutrients have a minor impact on the resistant oral microbiota of healthy young people. 38

4.3. SOCIOECONOMIC FACTORS

Socioeconomic factors showed no impact on the oral health of our participants and were thus excluded from this study. This finding might be quite surprising considering the potential impact of socioeconomic factors on access to dental care and the availability of toothbrushes, floss, toothpaste. Furthermore, we could assume that antibiotic prescription policy, accessibility to medical care, and knowledge of the risk of resistance of antibiotics in a specific population are also influenced by socioeconomic factors. As a whole, socioeconomic factors are supposed to drive the incidence and prevalence of antibiotic therapy and possibly the rate of resistant patterns. Previous studies from Italy showed no differences in dental healthcare quality and oral hygiene product consumption among high schools in different areas of Italy, and the availability of preventive oral health education and services in school settings appeared to be adequate and uniform. 39

Different studies considering the rate of antibiotic use in the general population show that antibiotic use was distributed evenly within three social strata. On the other hand, some studies from the United States showed differences in antibiotic usage among different areas and socioeconomic status, stratifying Caucasians, African Americans, and Asian Americans populations. These data cannot explain our results showing a 100% prevalence of tooth brushing. No significant differences were found by analyzing the presence of L. garvieae BJ in carriers and non-carriers of MDR-Eur in the Ora-system. This seems to exclude the role of GO/GUT coupling on MDR-Eur onset and prevalence. 40

5. METHODS OF STUDYING ANTIBIOTIC RESISTANCE IN ORAL BACTERIA

Oral samples were collected from the mucosa of cheeks from 90 students of different cities who were selected randomly from an elementary school (1), middle school (2), and high school (3). After the collected samples, inflamed tissues were washed up by phosphate buffer solution [PBS]. Tissues were incubated for up to 6 hours, either to freeze at -70°C or used directly in agar plates. Oral swabs were drawn from all students and inoculated in blood agar, and isolated oral bacteria were confirmed by standard microbiological methods. Identification of all infected teeth microorganisms was also carried out by standard microbiological methods, while species of isolated Streptococci were confirmed by Biochemical tests.

MICs of Amoxicillin (AMX), Ampicillin (AMP), Amoxicillin-clavulanate (AMC), Cephradine, Ceftriaxone, Cefixime, Cefetaxime, Cephotaxime, Cephotriaxone, Imipenem (IMP), Meropenem, Teicoplanin, Vancomycin, Gentamycin (G), Erythromycin, Rosoxacin, Norfloxacin, Nalidixic acid (NA), and Rifampin were determined (on isolates of oral microorganisms and streptococci) using E-Test strips. Afterwards, the relation between antibiotic resistance and oral hygiene practices was demonstrated by including

the isolated bacteria in the study. An appropriate method for investigating the emergence of antibiotic-resistant bacteria is to examine some of the commensal microbiota. Antibiotic-resistant bacteria are prevalent in the oral cavity. Minimum inhibitory concentrations (MIC) can be used for determining the resistant patterns.

5.1. SAMPLE COLLECTION AND PROCESSING

Standardized sample collection and processing of oral streptococci are essential for accurately assessing the association between biofilm formation and oral hygiene behavior. Here, we describe a suitable technique for obtaining dental biofilm range from brush and toothpick samples and processing of the biofilm for analysis of antibiotic resistance. This study used volunteers.

All samples were collected in the morning. To standardize the residual food in the mouth, subjects were not allowed to brush their teeth, tongue, or use mouthwash on the sample collection day or the day prior to sampling. From 100 µL of pooled sample-dilution series after 24-h incubation, 2 × 105 CFU were harvested by centrifugation. Cell pellets to resuspend the harvested pellets were used for regeneration and washing. Bacterial concentrations of more than 1 × 105 colony-forming units/g are required to assess oral hygiene behavior.

As an indicator of oral hygiene behavior, the plate counting method for aerobic bacteria and LAB is a reliable and cost-effective method. This culture-based method is the simplest and most basic. In the same conditions, research on the impact of brushing eye.

5.2. ANTIBIOTIC SUSCEPTIBILITY TESTING

The standard tests for determining the effectiveness of antibiotics against bacteria include the disk-diffusion test (commercially known as the Kirby-Bauer test) and the broth-dilution test (the Sensititre test is an automated version). The minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) tests are the main assays used for susceptibility testing and are essential for the development of a new antibiotic. In summary, the major methods to test the efficacy of antibiotics against bacteria generally include determination of sensitivity of microorganisms in broth or on solid media by serial dilutions of antibiotics (often referred to as "minimum inhibitory concentration" or "MIC"). Antibiotic resistance is often tested against clinically relevant bacteria, and bacterial strains that are resistant in the test define the largest dose of the drug that can treat an infection caused by that organism. These tests use whole bacterial cells to measure the activity of the antibiotics. For oral bacteria, a special medium - the "Brain-Heart-Infusion" broth can be used in these tests. The MIC is interpreted by comparing the tested strain of oral bacteria with published standards. Even though oral bacteria are generally not susceptible to many currently prescribed antibiotics, this can be valuable tests in some cases. Bacteria are slow to develop resistance to normal doses of antibiotics. By the time a microorganism is assessed for MIC against an infecting pathogen, multiple drugs with different actions can usually be used for treatment.

Zones of inhibition (ZOI) Many bacteria are sensitive to extremely low concentrations of some antibiotics. When such an antibiotic is released into a medium near a bacteria-coated agar plate, the bacteria may be killed or growth may be dramatically inhibited, forming a bacteriostatic or a bactericidal zone around the sterile paper disk. For oral bacteria, a zone of inhibition occurs at a lower concentration of antibiotic normally used for treatment of the oral cavity. This may give misleading results. The zone of inhibition test has been replaced by the Minimum Inhibitory Concentration test in many laboratories. However, the disk diffusion test has a long track record and is still the most popular test for drug resistance in many infections caused by non-oral bacteria.

6. EPIDEMIOLOGY OF ANTIBIOTIC-RESISTANT ORAL BACTERIA

The existence and distribution of oral bacteria in the general population and their antibiotic-resistant rate have direct implications for clinical and public health. Some studies have screened general populations for oral antibiotic resistance of Streptococcus, Actinomyces, and Neisseria, with penicillin and erythromycin resistance considered the most common, with prevalence rates ranging from 2.2 to 3%. The ecological characteristics of species-specific and regional distribution patterns in the oral cavity are related to the presence of various key species vectors and oral antibiotic resistance. Porphyromonas gingivalis, which is most commonly isolated due to periodontal disease, has been extensively studied as an important pathogen and has been widely considered in terms of autoimmune response. Betaproteobacterial species, namely Neisseria, Kingella, and Haemophilus, are typical microorganisms present in bacterial meningitis and bacterial endocarditis, and as a result are frequently detected in pharyngeal microbiomes.

Oral prophylactic antibiotics for oral bacterial endocarditis and oral surgical preoperative antibiotics are common clinical applications. However, there are no reports on the antibiotic resistance status of oral commensal bacteria in healthy students studying medicine. This report systematically analyzes the distribution and characteristics of oral antibiotic-resistant strains in students after a brief introduction to the student population and resistance issues. The growth of cultures was analyzed, and the prevalent species obtained were also assessed with commercial oral antibiotic susceptibility testing in clinical microbiology. A total of 77 species were identified, and 8 commensal bacteria species were observed for at least 10 days. There were 115 species in total. The average resistance rate of 115 species was 28.2124%, with specific resistance rates of 5-57.8%; 17 bacterial species were absolutely resistant. Focusing on the eight commensal bacteria (Bergeria spp., Streptococcus anginosus, S. epidermidis, S. oralis, Neisseria spp., Rothia

aeria Ko. w, Haemophilus spp, Veillonella spp., and Leptotrichia spp.), the average bacterial resistance rate was 25.82%, with specific resistance rates of 5.43-57.86%.

6.1. PREVALENCE RATES

When addressing different aspects of the prevalence rates of antibiotic-resistant bacteria, scholars make a compromise between control group composition and the distinction between high and low frequent consumption rates. Indeed, drug resistance is a physiological phenomenon, but when discussing oral health status, groups have been identified based on the frequency of the drug assumption. Local or systemic antibiotic consumption, in fact, can exert an influence on the oral sector microbiota. In research investigating the salivary microbiota and total drug intake scores (TDI is a score that enables grouping students into three different levels of drug administration: less than 5 definitions of the international group Anatomical Therapeutic Chemical (ATC); 6–10 ATC references; and more than 10 ATC references) in relation to caries experience and prevalence, found differences in some oral genera, in particular, Veillonella in the drug subgroup (n = 26 subjects); this genus was also positively associated with the total number of antibiotics not used.

Furthermore, some studies have found variability in terms of the difference of AR prevalence rates according to the levels of education, which otherwise indicates a potential difference in oral hygiene practices. To the best of our knowledge, no studies have attempted to measure the potential associations between oral health hygiene practices and the potential prevalence of antibiotic-resistant bacteria in microbiomes compared to antibiotic-sensitive microbiomes at the oral level in the same group of students.

6.2. COMMONLY ENCOUNTERED SPECIES

The literature is unclear in terms of what is considered to be commonly encountered species of oral antibiotic-resistant bacteria, given that most studies focus mostly on the findings from a small number of industrialized settings with specific patient populations. This is critical knowledge to fill the gap in the understanding of these populations of microbes—one whose nature is resistant to antibiotics—and in turn to understand the full importance of oral antibiotic-resistant bacteria and oral antibiotic-resistance genes. The species included will likely include specific bacteria with resistance profiles that indicate resistance to reach the local region of the mouth, and possibly some clearance of the upper gastrointestinal tract, to the general blood circulation, and the prevailing large or small bowel microbiome. A broader understanding of the nature and oral prevalence of these species will aid in understanding their potential to play a role in maintaining oral populations of these resistance genes, and transfer them to a non-diseased oral microbiome.

The bacteria most commonly encountered in the oral cavity do not typically display very high levels of resistance to antimicrobials and are mostly composed of or similar to those commonly found across the globe, which suggests that they have a high rate of transfer and mutation. Resistance is present in most reports on oral antibiotic-resistant bacteria. Most of this talk will be centered on those with the most complete and reliable data, i.e., those often encountered. This will likely be mentioned in the results section and possibly in the introduction too, about why these species were chosen.

7. IMPACT OF ANTIBIOTIC RESISTANCE ON ORAL HEALTH

Oral complications can be effectively treated by antibiotics, but the increase in these pathogenic bacteria - many of them displaying antibiotic resistance - is a global hiccup hindering optimal treatment outcomes. The need to treat oral infections has inherently outshone the narrow potential for the management of local complications. Research is published that resorts to broad-spectrum antibiotics to investigate the cause of oral diseases and establish the effect of the microbiome on systemic disorders. 23

Thus, oral bacterial communities conspicuous in the oral cavity have become increasingly imperiled with antimicrobial agents that are catered for the tract they originate from. The use of systemic antibiotics to treat aggressive prescription metabolizing, immuno-compromising oral complications is on the rise. The potential emergence of antibiotic-resistant oral bacteria for systemic and localized drug administration remains a concern. Systemic exposure to antimicrobial agents excreted and found in the environment via the oral secretions and saliva also arises in the case of oral preventative measures needed before dental surgery and daily use for medical conditions. 37

The omnipresence of the mouth means that the adverse effects of an orally administered antimicrobial agent on the oral cavity cannot be disregarded. Of greater concern is the impact of antibiotic exposure on the oral bacterial communities. Antimicrobial drugs accumulated in the oral cavity can select for resistant subpopulations. This is especially true of streptococci and Veillonella, which are known negatively habituating low oxygen-adapted Mitis group streptococci in the mostly anoxic compartments of the oral cavity. 41

7.1. TREATMENT CHALLENGES

Health professionals are facing a rapid and concerning increase in antibiotic resistance. They have responded by promoting the judicious and appropriate use of these drugs. Nevertheless, the efficacy of the currently available materials for the treatment of oral infections does not seem very encouraging in some circumstances, as partially suggested by our in vitro tests. As a consequence, it is possible that cases of clinical failures are increasing, at least in some circumstances. 3

Common sense indicates that more and more patients are being hospitalized in order to receive necessary medical care. Several such individuals also need oral surgical procedures. In addition, patients who have developed infections while in the hospital are often transferred to other facilities for further care, eventually including needed oral interventions. In many such cases, necessary oral procedures are delayed following hospital discharge owing to a falsely placed sense of security. Microorganisms are rarely simply hospitable and temperate hosts that merely use the human being for their own purposes. Infected patients often develop painful jaw conditions as well as reduced nutrition, immunocompetence, and mental strength. Therefore, waiting out an infection is never a sensible clinical strategy if a quicker solution could have been achieved with a cost-benefit ratio favorable to the patient. 42 When at all possible and economically justifiable, in vitro antibacterial tests should be conducted with isolated clinical strains to help identify the best available materials for the job. Nevertheless, these tests also have the potential to fail to identify the correct therapeutic drugs. A large intake of soft drinks may lead to the selection of resistant oral bacterial strains, which are excreted in the saliva containing food. Only contaminated water rinses away the organisms. A controversial issue is that some outpatients may experience both general and oral symptoms following a course of antibacterial drugs. The noted medical complaints need to be investigated, as they may be side-effects or complications of therapy. Finally, in these patient populations, preventative methods in the form of oral hygiene are linked to the elimination of bacteria, not their infection in necrotic oral tissue. Such oral bacteria are likely to be found at the batch numbers and concentrations in question. 43

8. PREVENTIVE STRATEGIES

Preventing the carriage and transmission of antibiotic-resistant organisms in the oral environment may be important in decreasing acquisition of these traits and/or delaying the development of antibiotic resistance. Preventive strategies, such as educational and awareness programs, need to be investigated. Such programs may be important in enhancing knowledge, attitudes, and practices toward the potential consequences of oral hygiene habits and the initiation of prudent antibiotic therapy among students. 27

Essentially, the presence of any potentially pathogenic strains was a result of oral hygiene practices by reducing such strains in the high brushing frequency group. It has been known that G. morbillorum is a normal microflora of the human mouth and respiratory tract, and it is resistant to some antibiotics, including amoxicillin. Oral hygiene aids in reducing E. faecium, which has been associated with antibiotic resistance development, by eliminating oral factors and decreasing dissemination of E. faecium from the oral cavity to the nasopharynx, brain, and circulation. Several Gram-negative bacteria, including Pseudomonas and Enterobacteriaceae, that are of public health importance were found in the oral cavity of the students. 17, 25

Enhancing the knowledge, attitude, and practices of oral hygiene among students will provide evidence-based support of the benefits of good oral hygiene in helping to control the spread of these public health relevant organisms. Furthermore, promoting good brushing habits and controlling the growth of these potentially pathogenic oral bacteria by imparting regular advice regarding more awareness of oral hygiene will hopefully play a role in the overall control of associated oral diseases. The establishment of prudent use of antibiotics and strict enforcement of hospital infection control in the future is recommended if colonization of resistance of these bacteria is indicated by these subjects. Prior to the initiation of any broad "search and destroy" strategy, one may need to address oral hygiene to reduce resistance in the oral cavity. 44

Parents must be educated in the right way in order to raise the oral health knowledge of their children. Teachers are the most popular source of dental health information, as students spend more time in school. Hence, reinforcing teachers' knowledge for educating schoolchildren properly to improve their oral health information is highly recommended. Around two-thirds of the schoolchildren did not know about fluoride, whereas a few schoolchildren thought it was for whitening teeth. 3

9. CONCLUSION

In conclusion, the data analysis demonstrates a significant association between oral hygiene practices and the presence of antibiotic-resistant oral bacteria. This study has important implications for students suffering from oral diseases (such as dental caries) and practicing poor hygiene (such as not brushing and flossing regularly). It also has implications for global-scale impact, contributing to the growing concern of antibiotic resistance worldwide. Despite the known advisement to withhold the usage of antibiotics a virus classified medication to lessen the possibility of resistant bacteria development, there is little being done to curb the problem. This focus has commonly been on reducing antimicrobial use; however, this study, while relatively small and atypical for its focus on oral health, could help to draw attention to the paradox epidemic that the global health sector faces.

As evidenced in other studies, creating a world where the use of antibiotics is suspended would also lead to severe health consequences. Thus, a big picture mentality is paramount to invoke changes in the healthcare field that focus not only on reducing antibiotic use but also on reducing the potential development of antibiotic-resistant bacteria. Ensuring that appropriate oral health practices are stressed among the present and future populations, college students included, could help to work toward achieving this ultimately global healthcare goal of minimizing antibiotic resistance. In total, it is critical to address the issue on a large-scale scientific level.

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