Susceptibility profile of bacteria obtained from human oro-dental plaques at the dental clinic of the University of Benin Teaching Hospital, Benin City, Nigeria.

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https://doi.org/10.60787/wapcp-v35i1-339

ABSTRACT

Background: The oral cavity harbours a diverse community of anaerobic bacterial; many of which have been implicated in life threatening oro-dental and systemic infections.

Objectives: This study evaluated the antimicrobial susceptibility profile of aerobic and anaerobic bacterial isolates obtained from plaque scrapings in patients who visited the study center for scaling and polishing.

Methods: Plaques were scraped from the tooth surface of each study participants with the aid of a sterile tweezer; into specimen collection bottles containing 5mL of sodium thioglycolate broth. This was transported within 2 hours to the laboratory and then subcultured into appropriate growth media for aerobic and anaerobic incubation. Anaerobiosis was achieved by chemical method (pyrrogalor crystals+ NaOH solution). Gram staining and biochemical tests were used to identify resultant bacterial colonies. Susceptibility profile of bacteria isolates were determined by agar disc diffusion assay.

Results: In order of distribution, *Streptococcus spp.,* had the highest frequency of occurrence (42%), followed by *Prevotella spp* which accounted for (34%). *Staphylococcus and Clostridium spps*. were least frequently encountered accounting for 15 and 9 % respectively. Susceptibility test result showed that the perfloxacin had high antimicrobial activity, while erythromycin, aminoglycosides and cefotaxim were moderately effective against bacterial isolates. However, amoxicillin and cotrimoxazole showed less activity.

Conclusion: Continuous surveillance studies on the antimicrobial susceptibility profiles of plaque associated bacteria is crucial in identifying emerging resistance patterns. This data can guide healthcare providers in adapting treatment strategies and developing new therapeutic approaches in the management of oro-dental infections.

Keywords: Plaques, susceptibility, perfloxacin, antimicrobial activity.

Profil de sensibilité des bactéries obtenues à partir de plaies bucco -dentaires humaines à la Clinique Dentaire du Centre Hospitalier Universitaire de Bénin, Benin City, Nigeria.

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RÉSUMÉ

Contexte: La cavité buccale abrite une communauté diversifiée de bactéries anaérobies dont beaucoup ont été impliquées dans des infections bucco-dentaires et systémiques menaçant le pronostic vital.

Objectifs: Cette étude a évalué le profil de sensibilité aux antimicrobiens d'isolats bactériens aérobies et anaérobies obtenus à partir de raclages de plaies chez des patients qui ont visité le centre d'étude pour un détartrage et un polissage.

Méthodes: : Les plaques ont été prélevées de la surface dentaire de chaque participant à l'étude à l'aide d'une pince stérile ; elles ont été placées dans des flacons de prélèvement d'échantillons contenant 5 ml de bouillon au thioglycolate de sodium. Ce bouillon a été transporté en moins de 2 heures au laboratoire, puis repiqué dans un milieu de croissance approprié pour une incubation aérobie et anaérobie. L'anaérobiose a été obtenue par une méthode chimique (cristaux de pyrrogalor + solution de NaOH). La coloration de Gram et des tests biochimiques ont été utilisés pour identifier les colonies bactériennes obtenues. Le profil de sensibilité des isolats de bactéries a été déterminé par un test de diffusion sur disque de gélose.

Résultats: Dans l'ordre de distribution, *Streptococcus spp* avait la fréquence d'apparition la plus élevée (42 %), suivie de *Prevotella spp* qui représentaient (34%). *Staphylococcus* et *Clostridium spp* ont été les moins fréquemment rencontrés, ce qui se traduit par 15 et 9 % respectivement. Les résultats des tests de sensibilité ont montré que la perfloxacine avait une forte activité antimicrobienne, tandis que l'érythromycine, les aminosides et le céfotaxime étaient modérément efficaces contre les isolats bactériens. Cependant, l'amoxicilline et le cotrimoxazole ont montré une activité moindre.

Conclusion: Les études de surveillance continue des profils de sensibilité aux antimicrobiens des bactéries associées à la plaque dentaire sont cruciales pour identifier les schémas de résistance émergents. Ces données peuvent guider les prestataires de soins de santé dans l'adaptation des stratégies de traitement et le développement de nouvelles approches thérapeutiques dans la gestion des infections bucco-dentaires.

Mots clés: Plaques, sensibilité, perfloxacine, activité antimicrobienne

INTRODUCTION

Bacterial adhesion causes the formation of plaque biofilm matrices, which are mostly composed of extracellular DNA and bacterial extracellular polysaccharides.[1] These macromolecules aid in the colonization of bacteria and the development of multicellular clusters. Through a series of co-aggregation and co-adhesion events, late colonizers-both Gram-positive and Gram-negative species-build onto the original streptococcal foundation until a mature plaque is attained within any specific individual. Once established, many species within the community live there in a state of equilibrium.

Plaque associated bacteria have been linked to a number of oral and systemic diseases such as dental caries, periodontal disorders, and bad breath (halitosis). Life-threatening systemic diseases such as endocarditis, premature term birth have also been associated with anaerobic bacteria pathogens that found ways to the systemic circulation.[2]

Due to the development of microenvironments with lower oxygen levels, plaque becomes more supportive of the growth of facultative anaerobes as it ages thus providing substantial contributions to the diversity of microorganisms in the oral cavity.[3] Due to variations in oxygen, nutrition availability, and pH, dental plaque can form various layers with varying microbial compositions. The biofilm's layered structure aids in the spatial arrangement of the bacteria and also enhance the exchange of genetic information through quorum sensing and plasmid transfer[4] resulting in the exchange of resistant genes within the plaque bacterial community. Genetic interactions between bacteria community have been documented as the leading cause of bacterial resistance to many antimicrobial agents.[5]

Numerous variables may affect bacteria's susceptibility in plaque. First, anaerobic bacteria's location and species diversity within plaque are crucial factors in determining their vulnerability. According to studies, several species display differing degrees of resistance to antimicrobial substances. By acquiring these genes through horizontal gene transfer, the bacteria can create defenses against the effects of antibiotics. In addition, the plaque's chemical and physical characteristics, such as pH, oxygen tension, and nutrient availability, can also influence the susceptibility of these bacteria. The acidic environment in plaque can promote the growth of acid-tolerant bacteria, which can be more resistant to antimicrobial agents. Overall, understanding the resistance and/or susceptibility pattern of bacteria cells in plaque is crucial for developing local susceptibility/resistance break points for effective management of oral infections.

METHODS

Specimen collection

This study evaluated a total of 105 specimens obtained from oro-dental plaque of patients who presented for scaling and polishing at the study center. Data and specimen were collected in the months of April to August 2023. Following the receipt of ethical approval from the hospital's institutional review boards (Protocol number: ADME/E 22/A/VoL.VII/148301144). and informed consents from study participants, plaques were scraped from the tooth surface with a sterile tweezer and a mirror was used as visual aid. Scrapped plaques were immediately introduced into a sterile specimen collection bottle containing 5 ml thioglycolate broth. All specimens were transported within 2 hours to the Department of Pharmaceutical Microbiology laboratory for further microbiological investigations.

Characterization and Identification of Isolates

In the laboratory all transported specimens were subcultured in duplicate into 10% blood agar plate. One set was incubated aerobically and the second was incubated anaerobically at 37^{o}C for 24 and 48 hours respectively. To obtain a pure culture, resultant colonies were further subcultured based on colonial morphologies. In the instance, where two or more colonies on the blood agar plate appeared to be identical, only one colony was sub-cultured into fresh blood agar plate and incubated for 18-24 hours. where two colonies appeared to be different on the blood agar plate, both were sub-cultured into separate plates. Pure colonies, from incubated plates were /suspended in 0.5 McFarland to give an inoculum size of 10^{8}CFU/mL and adjusted inoculum were diluted 1:100 to give an inoculum size of approximately 10^{6}CFU/mL. Each of the pure isolates were identified based on colonial characteristics on the blood agar plates, standard biochemical test methods (such as catalase test, coagulase test, oxidase test, citrate test, indole test, urease test, lactose test, maltose test, sucrose test and D-glucose test) and Gram staining characteristics.[4,5]

Method for isolation of anaerobes

Anaerobiosis was generated using chemical method as previously documented in literature.[6] At the bottom of

the anaerobic glass jar, 5.0 g of pyrogallol crystals were weighed and put in one side of the glass dish. A metal wire gauze with perforations was used to cover the dish. Over the perforated metal gauze on the glass dish, all of the inoculated culture plates were placed upside down. With the aid of a sterile pipette 10 mL of 4% NaOH solution was added to the pyrogallol crystals in the dish. The anaerobic glass jar was quickly sealed tightly and then rocked gently to mix the reagents there in (Pyrogallol+ sodium hydroxide). In the chemical reaction pyrogallor (an oxygen scavenger) absorbs oxygen rapidly in alkaline solution thus producing an environment that is oxygen-free or anaerobic for all the plates, which were incubated at 37^{o}C for 1-3 days.[7]

Antimicrobial susceptibility test

Isolates were subjected to antimicrobial susceptibility testing using the disc diffusion technique on Mueller-Hinton agar, as previously described.[8] Test antimicrobial agents used included perfloxacin (30 ug), erythromycin (30 ug), gentamycin (30 ug), cotrimoxazole (30 ug), amoxicillin (30 ug) and cefotaxim (10 ug) all were products of Optun Laboratories Nigeria Limited. Approximately 1 mL of overnight broth culture of Pure colonies of each isolate was adjusted to 0.5 McFarland standard to give an inoculum size of 10^{6} CFU/mL. This was then diluted 1:100 to give an inoculum size of approximately 10^{6} CFU/mL. For the disc diffusion test, a6 sterile swab stick was applied to each of the adjusted inocula and used to evenly streak the entire surface of a pre-prepared Mueller-Hinton agar plate. Antibiotics containing disc were placed onto the inoculated culture media and incubated at 37^{o}C for 24 and 48 hours for aerobic and anaerobic bacteria respectively in triplicates. The positive control Petri dishes contained individual bacterial isolate and no antibiotics. Inhibition zone diameters measured were appropriately recorded to the nearest millimeter. These were interpreted as susceptible or resistant by comparison with published guidelines for antimicrobial susceptibility testing for commonly occurring pathogens, as obtained from clinical isolates[9].

RESULTS

Based on distribution of *Streptococcus spp*., was more prevalent (42%), followed by *Prevotella spp*. (34%). *Staphylococcus spp* (15%) and *Clostridium spp* (9%) were less encountered as presented in Figure 1. Susceptibility test profile of bacterial isolates to the different antibiotics (Figure 2.) showed that over 80% of all isolates were highly susceptible to perfloxacin. While moderate susceptibility was observed for erythromycin, gentamycin and cefotaxim as seen in approximately 60% of all clinical isolate. However a low susceptibility profile to amoxicillin and cotrimoxazole was observed in all clinical isolate with about 40% susceptibility.

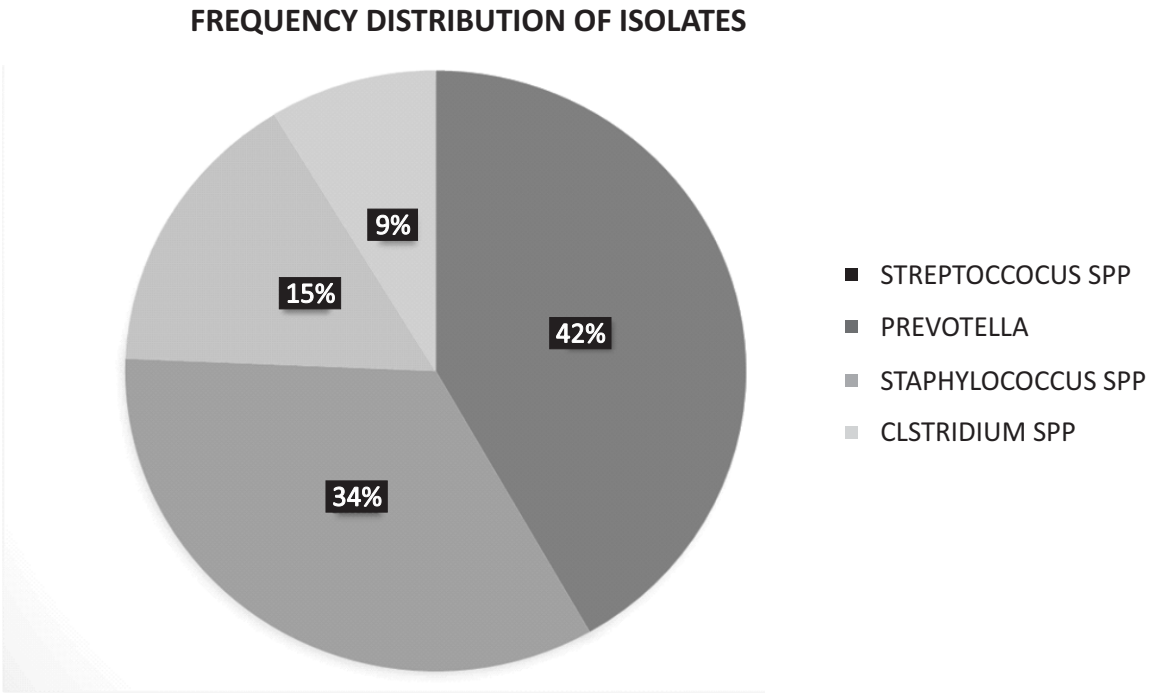

FIGURE 1: Frequency distribution of anaerobes

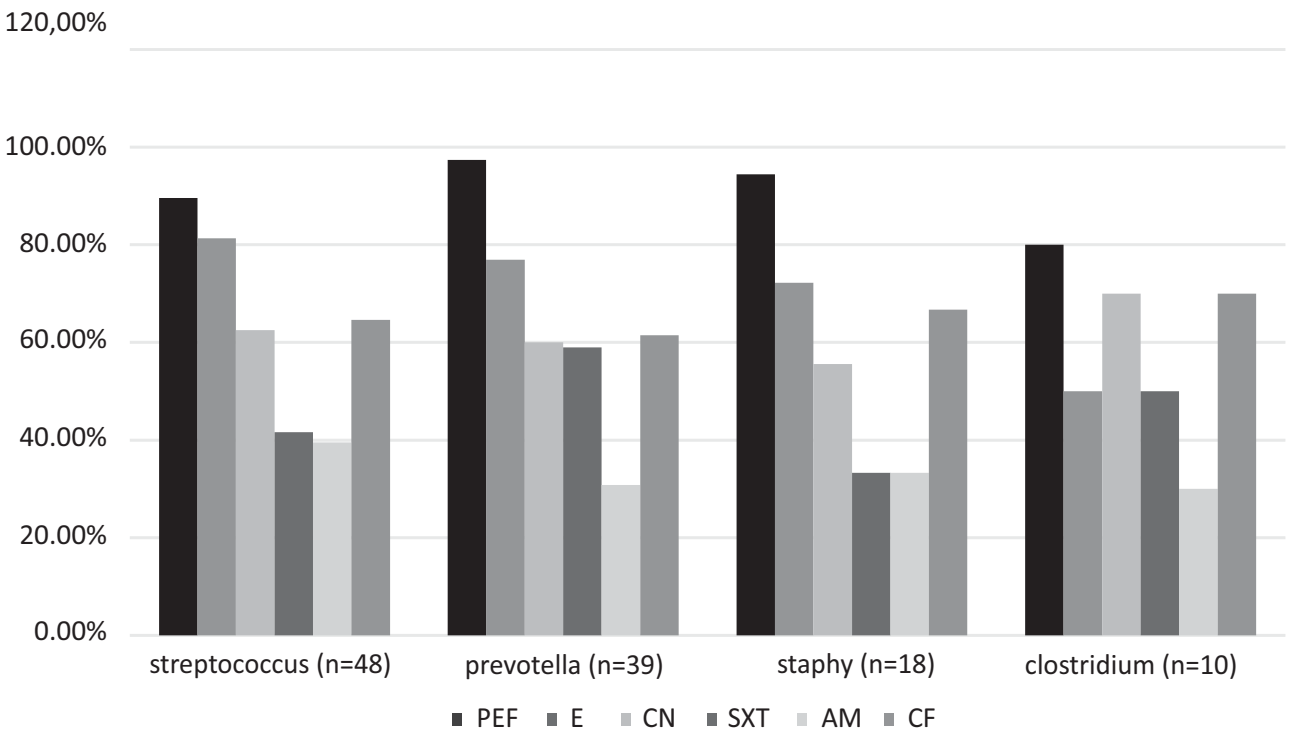

FIGURE 2: Susceptibility results of anaerobic bacterial isolates

KEY: PEF = perfloxacin, E= erythromycin, CN= gentamycin, SXT = antifolates, AM= amoxicillin, CF= cefotaxim

DISCUSSION

The results revealed that *Streptococcus spp*. had the highest frequency of occurrence at 42%, which buttress the fact that *Streptococcus spp* are significant oral primary colonizers, with a variety of high-affinity adhesins that facilitate initial attachment to tooth surfaces through interactions with albumin, proline-rich proteins, glycoproteins, mucins, and sialic acid found in salivary pellicle.[10] *Prevotella* species was the second most abundant isolate (34%) in the study population. This Gram negative anaerobic rods have been previously documented to cohabit with *Streptococcus spp*., in the oral cavity. Where the later breaks down complex sugars into simpler ones, which *Prevotella spp* can use as energy source for growth and replication. Additionally, the acidogenic nature of *Prevotella spp* lowers the pH in their immediate environment, making it more conducive for itself and other anaerobic organisms to thrive[11]. Isolation of *Staphylococcus spp* from dental plaque at a frequency of 15% is an interesting finding, as *Staphylococcus spp* is not typically considered a primary component of the oral microbiota. *Staphylococcus spp,* are more commonly associated with skin and mucus membrane of the gastrointestinal tract, rather than oral cavity colonization[12]. However, the observed result may be due to a possibility that contaminants from the skin or the environment can occasionally find their way into the mouth making *Staphylococcus spp* a transient colonizer of the oral cavity. *Clostridium spp* had the lowest frequency of 5% in oro-dental plaque, which may indicate that, *clostridium spp* does not play a significant role in the development of oral dental infections due to its low frequency as observed from this study. In clinical settings, the majority of patients with dental illness are often treated empirically with antibiotics, primarily beta lactam antibiotics,[13] due to their broad spectrum of activity against aerobic and anaerobic bacteria. The excessive use of this class of antibiotics in particular and other classes of potent antimicrobial agent in general has resulted to the growing problem of antimicrobial resistance in both the community and hospital settings.[14]

From the antimicrobial susceptibility test results, over 80% of *Streptococcus* isolates was seen to be susceptible to the perfloxacin, this may be due to disruption of DNA replication and consequent bacterial cell death. *Streptococcus spp* typically have limited efflux pump activity compared to other bacteria like *Pseudomonas aeruginosa* and *Escherichia coli*.[15] This means they are less likely to actively pump out perfloxacin, erythromycin,

gentamycin and cefotaxim, allowing the antibiotics to remain effective. Antibiotics like erythromycin had activity against over 66.6% of the bacterial isolate. *Streptococci* are susceptible to erythromicin because the binding site on their ribosomes is accessible and sensitive to these antibiotics.[16] *Streptococcus spp* also lack intrinsic resistance mechanisms that are commonly found in other bacteria. For example, they typically lack beta-lactamases, enzymes that can inactivate beta-lactam antibiotics like penicillin's. This makes *Streptococcus spp*. susceptible to a broader range of antibiotics, including perfloxacin and erythromycin. While resistance to these class of antimicrobial agents can occur in *Streptococcus spp* over time due to mutations in target genes or acquisition of resistance genes, the development of resistance tends to be slower compared to other bacteria. This slower resistance development can be attributed to the combination of factors mentioned above, including limited efflux pump activity and fewer intrinsic resistance mechanisms. *Prevotella spp* showed susceptibility to pefloxacin (97.4%) an antimicrobial agent known for it broad-spectrum activity against a wide range of bacteria, including both Gram-negative and Gram-positive species. This broad spectrum of activity extends to many anaerobic bacteria like *Prevotella spp.* The specific bacterial enzymes targeted by pefloxacin, including DNA gyrase, are critical for DNA supercoiling and maintaining DNA integrity during replication and repair. Prevotella spp relies on these enzymes for its genetic processes, making it vulnerable to the disruption caused by this type of fluoroquinolones. Prevotella also lack the ability to pump out macrolides, floroquinolones, aminoglycosides and cephalosporins from their cells, these contributes to the observed high level of Prevotella susceptible to many classes of antibiotics.[17]

Staphylococcus spp which is a gram-positive coccus showed susceptibility to pefloxacin (94.4%,). *Staphylococcus spp*. are known to exhibit varying levels of susceptibility to fluoroquinolones. While many strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) remain susceptible to some fluoroquinolones, the rise of methicillin-resistant *Staphylococcus aureus* (MRSA) has posed a significant challenge. *Staphylococcus spp* often display susceptibility to fluoroquinolones due to increase activity of the drugs to the quinolone resistance-determining regions (QRDRs) of their DNA gyrase and topoisomerase IV genes. Due to the low antimicrobial activity of macrolide against *Staphylococcus spp*.,, particularly MRSA, macrolides are generally not recommended as first-line agents for treating these infections. They are often reserved for cases where the bacteria are susceptible, or when alternative antibiotics are contraindicated.[18] The aminoglycosides (gentamicin=55.6%) displayed a moderate amount of effectiveness against the isolated *staphylococcus spp* in this study, and this could be attributed to the fact that aminoglycosides primarily target the bacterial ribosome, specifically the 30S ribosomal subunit. By binding to the ribosome, they interfere with the process of protein synthesis in bacteria. This interference disrupts the reading of the genetic code, leading to the production of faulty proteins or truncated polypeptide chains. Additionally, aminoglycosides also possess a post-antibiotic effect, which means that their antibacterial activity persists even after the antibiotic has been cleared from the body.[19] This prolonged effect further contributes to their efficacy against *Staphylococcus spp*. As a result, the bacteria are unable to synthesize essential proteins needed for their growth and survival. Amoxicillin (22.2%), was less active against Prevotella, Clostridium bacterial isolates compared to other test antibiotics. This may be due to the anaerobic nature of these bacteria and the need for antibiotics with better tissue penetration.

CONCLUSION

It has been shown that dental plaque is a reservoir of diverse aerobic and anaerobic bacteria; many of which showed high susceptibility to perfloxacin, and a moderate susceptibility to erythromycin, gentamycin and cefotaxim. However, this plague associated bacteria were resistant to amoxicillin; which is the drug of choice used at the study centre. This study has shown, Perfloxacin, erythromycin and /or cefotaxime as the most active antimicrobial agents against oro dental pathogens and they are therefore recommended as drug of choice in the management of infection associated with the oral cavity at the study centre.

ACKNOWLEDGEMENT

The authors are grateful to the Staff of the Dental Clinic, University of Benin Teaching Hospital for their assistance. We are also thankful to the members of staff of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, for their technical support.

The authors report that there are no conflicts of interest with regards to this research work.

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