Prevalence and antimicrobial resistance of bacterial agents isolated from the cases of dental caries

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ABSTRACT

Dental caries are mainly occur owing to the presence and activity of bacterial agents. The present study was done to assess the prevalence and antibiotic resistance of bacterial strains isolated from the cases of dental caries. Fifty patients with approved dental caries were included in the study. Sampling from the site of dental caries was done using the sterile swab. Swabs were transferred to laboratory and subjected to microbial culture. Species identification of bacteria was done using biochemical test. Bacterial isolates were subjected to disk diffusion to assess their antimicrobial resistance. S. aureus (40%) harboured the highest rate of contamination, while S. oralis (16%) and E. aerogenes (10%) harbored the lowest. S. aureus and S. mutans (6%) harbored the highest distribution amongst the cases of mix infections, while S. aureus and S. oralis (2%) harbored the lowest. S. aureus strains harbored the highest rate of resistance toward tetracycline (90%), penicillin (75%), ampicillin (75%), amoxicillin (60%), and erythromycin (60%). E. coli strains harbored the highest rate of resistance toward tetracycline (90%), gentamicin (80%), ampicillin (70%), and erythromycin (70%). S. mutans strains harbored the highest rate of resistance toward tetracycline (93.33%), ampicillin (86.66%), penicillin (80%), amoxicillin (80%), and erythromycin (80%). S. oralis strains harbored the highest rate of resistance toward tetracycline (100%), ampicillin (75%), penicillin (62.50%), and amoxicillin (62.50%). E. aerogenes strains harbored the highest rate of resistance toward tetracycline (80%), gentamicin (80%), and ampicillin (80%). S. aureus bacteria isolated from dental caries harbored the highest rate of MDR. Distribution of resistance against more than 3 antimicrobial agents amongst the S. aureus, E. coli, S. mutans, S. oralis, and E. aerogenes bacteria isolated from the cases of dental caries was 90%, 60%, 80%, 62.50%, and 80%, respectively. Application of disk diffusion can help practitioners to reduce the rate of resistance in bacteria responsible for dental caries.

Keywords: Dental caries, Bacteria, Antibiotic resistance, Prevalence.

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INTRODUCTION

Dental caries (also recognized as dental cavities or tooth decay) is the most mutual non-communicable disease of the oral cavity globally. Severe dental caries affects human health and frequently causes pain and infection, which may bring about tooth extraction. It is an expensive disease to treat, overriding 5–10% of healthcare budgets in developed countries, and is among the chief reasons for children hospitalization in some high-income countries. As a result, it is essential to determine all epidemiological aspects, etiological agents, and routine ways to treat subsequent infections.

High amounts of nutritional materials, epithelial debris, and secretions caused the mouth to be a favorable environment for growth and proliferation of bacteria. In this regard, Staphylococcus, Streptococcus, Escherichia, and Enterobacter species are the most significant and frequent species isolated from the cases of dental caries. Staphylococcus aureus, Enterobacter aerogenes, and Escherichia coli are considered to be the most common causes of bacterial infections in the oral cavity. These bacteria mainly harbored high rate of resistance against commonly-used antimicrobials, especially penicillin, aminoglycosides, macrolides, cephalosporin, quinolones, and tetracyclines. This issue increased the importance of oral infections caused by these bacteria, prolongs the hospitalized persons, and increased the costs of treatment.

Due to the lack of epidemiological, dental and microbiological studies in this field, the present study was conducted to evaluate the frequency of bacterial agents effective in causing dental caries and assess their antimicrobial resistance pattern.

MATERIALS AND METHODS

Media and chemical reagents

All culture medica and chemical reagents were purchased from Merck Company (Merck, Germany). Antimicrobial disks were purchased from the Oxoid company (Oxoid, UK).

Samples

The current cross sectional and descriptive survey was done on summer of 2023. A total of 50 patients who were referred to private dentistry clinics owing to the dental caries were included in the research. Disposable cotton swabs with standard protocol were applied for sampling from the site of dental caries. No cross contamination was occurred during the sampling. All samples were transferred to laboratory within 2 hr after collection using sterile refrigerator (4 ± 1°C).

Bacterial isolation and identification

The dental caries samples which were taken using sterile cotton swab was cultured into different tubes containing chocolate agar, 5% sheep blood agar, and a selective medium. All media were then transferred to the private microbiology laboratory. All media were incubated at 37°C and 42°C for 24 to 48 h. After Gram staining and microscopy, different biochemical tests were performed to identify bacterial strains. Different biochemical examinations, including oxidase, catalase, urease, indole, Methyl Red, Voges Proskauer, Simon Citrate, Coagulase, and starch tests were applied. Analytical Profile Index (API 20E) (BioMerieuxVitek, Inc., MO, USA) system was used to identify bacteria.

Antimicrobial resistance

CLSI procedures were applied to assess the antimicrobial resistance of isolates. Mueller–Hinton agar (Merck, Germany) was applied. Diverse antimicrobial disks, including penicillin (10 μg/disk), ampicillin (10 μg/disk), amoxicillin (25 μg/disk), ceftriaxone (30 μg/disk), vancomycin (30 μg/disk), azithromycin (15 μg/disk), erythromycin (15 μg/disk), metronidazole (5 μg/disk), gentamicin (10 μg/disk), rifampin (30 μg/disk), and tetracycline (30 μg/disk), were placed on media. Microbial media with placed disks were incubated (35°C for 24 h). Aerobic and anaerobic conditions were applied according to the targeted bacteria. Guidelines of the CLSI were applied for susceptibility analysis.

Data analysis

Data were subjected to Microsoft Office Excel (version 15; Microsoft Corp., Redmond, WA, USA). The statistical analysis was performed employing the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher’s exact two-tailed test were applied to measure any significant relationship. P-value <0.05 was considered as a significant numerical level.

RESULTS

Distribution of bacterial strains

Table 1 shows the bacterial agents isolated from the total of 50 cases of dental caries. S. aureus (40%) harbored the highest rate of contamination, while S. oralis (16%) and E. aerogenes (10%) harbored the lowest (P <0.05).

Distribution of mix infections

Figure 1 shows the mix infections distribution amongst the examined samples. Rendering obtained findings,
S. aureus and S. mutans (6%) harbored the highest distribution amongst the cases of mix infections, while S. aureus and S. oralis (2%) harbored the lowest ($P < 0.05$).

**Antimicrobial resistance**

Figure 2 shows the antimicrobial resistance of examined bacterial agents against commonly-used antimicrobial agents in dentistry. S. aureus strains harbored the highest rate of resistance toward tetracycline (90%), penicillin (75%), ampicillin (75%), amoxicillin (60%), and erythromycin (60%). E. coli strains harbored the highest rate of resistance toward tetracycline (90%), gentamicin (80%), ampicillin (70%), and erythromycin (70%). S. mutans strains harbored the highest rate of resistance toward tetracycline (93.33%), ampicillin (86.66%), penicillin (80%), amoxicillin (80%), and erythromycin (80%). S. oralis strains harbored the highest rate of resistance toward tetracycline (100%), ampicillin (75%), penicillin (62.50%), and amoxicillin (62.50%). E. aerogenes strains harbored the highest rate of resistance toward tetracycline (80%), gentamicin (80%), and ampicillin (80%). The lowest rate of resistance in S. aureus, E. coli, S. mutans, S. oralis, and E. aerogenes strains were obtained against azithromycin (20%), azithromycin and vancomycin (20% each), azithromycin and vancomycin (26.66% each), azithromycin and vancomycin (12.50% each), and finally ceftriaxone, vancomycin, and rifampin (20% each). Statistically significant difference was obtained between type of bacteria and prevalence of antibiotic resistance ($P < 0.05$).


**Multi-drug resistance distribution**

Figure 2 shows the distribution of MRD strains amongst the examined samples. MDR strains were determined as those which had simultaneous resistance to at least 3 antimicrobial agents. S. aureus bacteria isolated

![Figure 1: Mix infections distribution amongst the examined samples.](image1)

![Figure 2: Distribution of MRD strains amongst the examined samples.](image2)
from dental caries harbored the highest rate of MDR. Distribution of resistance against more than 3 antimicrobial agents amongst the S. aureus, E. coli, S. mutans, S. oralis, and E. aerogenes bacteria isolated from the cases of dental caries was 90%, 60%, 80%, 62.50%, and 80%, respectively.

**DISCUSSION**

In despite of all advances occurred in dentistry27-30, dental caries remain an important destroying issue of teeth and is a major problem for all people, especially in old age.

The present study showed that S. aureus, S. mutans, S. oralis, E. aerogenes, and E. coli were isolated from the cases of dental caries, with the highest distribution of S. aureus and S. mutans. Similar to tis research, S. aureus and S. mutans were also predominant bacterial agents responsible for dental caries, dental plaques, and other related infections of the oral cavity in diverse researches31-38. However, some surveys highlighted the role of other streptococcal species and E. coli, and E. aerogenes in the occurrence of dental caries, dental plaques, and other related infections of the oral cavity39-41. Daniyan and Abalaka (2011)42 stated that the distribution of S. aureus and S. mutans amongst the dental caries samples were 53.40% and 39.70%, respectively. In dental plaques, total distribution of S. aureus, S. mutans, and E. coli was 15%, 19%, and 10%, respectively43. Similar to this, their high distribution in the cases of maxillofacial surgery were also reported44.

Isolates harbored high resistance toward tetracycline, penicillin, amoxicillin, and erythromycin. Gram-negative isolates also harbored the high rate of resistance toward gentamicin. High antimicrobial resistance of Gram-negative and Gram-positive bacteria isolated from the dental caries against tetracycline, penicillin, amoxicillin, and erythromycin was also reported by 45-48.

Jassam et al. (2022)49 described that S. mutans isolates were resistant to penicillin (82.2%) and highly sensitive to amoxicillin (86.6%) and ciprofloxacin (71.1%). They showed that S. oralis strains were resistant to imperium (100%), and highly sensitive to gentamycin, ciprofloxacin, cefotaxime, and amoxicillin (100%). S. epidermidis was highly resistant to tetracycline at a rate of (88.40%) and highly sensitive to amoxicillin at the same rate (88.40% each). E. coli was highly sensitive to gentamycin, imperium, amoxicillin and vancomycin (91.40%). Yadav et al. (2015)49 stated that the prevalence of resistance of S. mutans bacteria isolated from dental plaque samples against ampicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, erythromycin, gentamicin, tetracycline, penicillin, and impanel was 26.92% 41.56%, 22.30%, 20%, 0%, 0%, 60.76%, 60.15, and 0%, respectively. Resistance rate of S. aureus against the above-mentioned agents was 61.70%, 2021%, 50%, 46.80%, 58.51%, 86.17%, 91.48% and 0%, respectively, which was similar to our findings.

Totally, this survey is one of the first reports of identification of antimicrobial resistance of pathogenic bacteria responsible for dental caries. Findings are limited to the low number of isolated bacteria, lack of demographical characters of the studied population and also absence of the determination of the history of gastrointestinal disorders among patients.

**CONCLUSION**

Totally, S. aureus, S. mutans, S. oralis, E. coli, and E. aerogenes were isolated from the cases of dental caries. Among them, S. aureus and S. mutans had the highest distribution. Most of bacterial isolates were resistant against tetracycline, ampicillin, amoxicillin, and gentamicin, erythromycin, and penicillin antimicrobials. This matter may show the low efficacy of these antimicrobrial agents for treatment and control of infections after dental caries. Application of disk diffuin can reduce the risk of the occurrence of antibiotic resistance amongst the dental bacteria. Supplementary researches can help to learn more about the role of dental caries bacteria and their antibiotic resistance in the oral cavity.

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