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Antimicrobial Susceptibility of *Streptococcus mutans* Isolated from Dental Caries Patients: A Laboratory Study Using CLSI M100 Breakpoints

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ABSTRACT

Background: The prevalence of dental caries is one of the most common chronic infections, mostly associated with the cariogenic bacteria *Streptococcus Mutans*. There is an increase in the number of resistant strains, which poses a significant clinical problem. **Objective:** Isolation and identification of *Streptococcus mutans* from patients suffering from dental caries, analysis of resistance to five different clinically important antibiotics, and interpretation based on CLSI M100 criteria. **Methods:** Plaque specimens were collected from 80 participants. The isolates were then grown on Mitis Salivarius Agar and identified by conventional biochemical methods. Susceptibility to antimicrobials was determined through the Kirby-Bauer disc diffusion technique on Mueller-Hinton Agar containing 5% human blood (CLSI M100). The five antimicrobial agents tested were as follows: Penicillin, Ampicillin, Erythromycin, Tetracycline, and Vancomycin. **Results:** In 80 bacterial isolates, positive cultures were obtained for 50 samples, where 32 cultures of S. mutans had a frequency of 64%. All isolates were found to be sensitive to Vancomycin (100%) and Penicillin (87.5%). Resistance was observed against Erythromycin (21.9%) and Tetracycline (25%). **Conclusion:** Vancomycin maintains its effectiveness against S. mutans. Resistance to Erythromycin and Tetracycline needs further surveillance. Standardization of the CLSI method is essential in dental microbiology**.**

Keywords: *Streptococcus mutans*; dental caries; antibiotic susceptibility; CLSI M100; disk diffusion; Viridans Group Streptococci.

1. INTRODUCTION

1.1 Background and Significance

Dental caries are prevalent chronic infections caused by dental biofilms and present a serious global public health challenge. It is estimated that about 2.5 billion people suffer from untreated caries of their permanent teeth [1]. The condition entails the gradual loss of mineral content in the enamel of the teeth, caused by organic acids produced by cariogenic bacteria within the dental biofilm plaque. Among these bacteria, *Streptococcus mutans* is a major pathogen. Isolated by J. Kilian Clarke in 1924, it has been thoroughly described as the primary cause of human dental caries. Its remarkable affinity for tooth surfaces, fermentation of dietary sugars to lactic acid, and survival under acidic conditions give it an overwhelming advantage over other bacteria [2].

1.2 *Streptococcus mutans*: Biology and Pathogenicity

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Streptococcus mutans is a component of the Viridans Group Streptococci (VGS), which is a diverse collection of both commensal and pathogenic species found in the human mouth. It is gram-positive, non-motile, and non-spore forming, having a coccus shape that occurs in chains or pairs. Its optimum growth occurs at 37°C, microaerophilic, with 5–10% CO_2 [3].

The pathogenicity of S. mutans has been attributed to three primary virulence factors, namely: (1) acidogenicity, which is the rapid conversion of carbohydrates to organic acids, (2) aciduricity, which refers to the capacity for survival and metabolic function even in extremely acidic environments of pH values down to 4.2, and (3) biofilm formation that requires sucrose, facilitated by the enzyme glucosyltransferase (GTF), encoded by the gtfB, gtfC, and gtfD genes [4].

1.3 Aims and Objectives

The objectives of this current research were to fulfill the following requirements:

1. Isolation and identification of S. mutans in patients having dental caries at Al-Kafeel University Dental Clinic.
2. Antibiotic sensitivity testing of isolated bacteria against five antibiotics by using the Kirby-Bauer disk diffusion method.
3. Interpretation of antibacterial susceptibility in line with CLSI M100 standards for Viridans Group Streptococci (Table 1).
4. Providing local information that can be used for rational prescription of antibiotics in dentistry.

1.4 Global Epidemiology of Dental Caries

Dental caries has been recognized as the most common non-communicable oral disease in the world. According to the study on Global Burden of Disease, dental caries in permanent teeth affects about 2.3 billion people in the world. Dental caries mainly affect low- and middle-income groups of people because they lack preventive and curative dental services [4,5]

1.5 Antibiotic Susceptibility of *Streptococcus mutans*

Studies on antimicrobial susceptibility test (AST) of *Streptococcus mutans* have been undertaken in multiple studies worldwide. According to [6], strains isolated from Iraqi dental caries were significantly susceptible to vancomycin but variably resistant to erythromycin. [7] found universal susceptibility to penicillin and vancomycin and resistance to tetracycline and erythromycin.

Resistance to tetracycline is common and linked to the presence of tet(M) and tet(O) resistance genes through horizontal gene transfer in polymicrobial dental biofilm communities [8]. Resistance to erythromycin is largely due to ribosomal methylation carried out by erm genes, particularly erm(B) and erm(TR).

1.6 CLSI Standards for Susceptibility Testing of Viridans Streptococci

The CLSI provides annual updates to the performance standards for antimicrobial susceptibility testing (M100). Table 1 shows the zone diameter and the MIC breakpoints for VGS, which should be performed using Mueller-Hinton agar medium containing 5% human blood and incubated at 35 ± 2°C in 5% CO_2 for 20–24 hours (CLSI, 2023). According to CLSI, the disk diffusion test is not recommended for penicillin and ampicillin against VGS since zone diameters cannot accurately determine susceptibility based on MICs.[9]

2. MATERIALS AND METHODS

2.1 Study Design and Setting

The research was carried out prospectively and cross-sectionally in a laboratory setting at the Department of Basic Sciences, College of Dentistry, Al-Kafeel University, Najaf, Iraq. It took place during the academic year of 2025-2026. Ethics

committee approval and patient consent were obtained before sample collection.

2.2 Study Population and Sample Collection

Participants who had been clinically diagnosed with active dental caries (DMFT≥3) were recruited for the study. The inclusion criteria included taking systemic antibiotics one month before the specimen was taken, taking an antiseptic mouth rinse two weeks before sampling, and any conditions that would contraindicate sampling. The dental plaque samples were collected using sterile No. 23 shepherds hook explorers, inoculated in BHI broth (HiMedia, India), and then sent to the laboratory within two hours [10,11].

2.3 Bacterial Isolation and Identification

Cultivation of samples was carried out on Mitis Salivarius Agar (MSA) (HiMedia, India) and maintained in 5-10% CO2 for 24-48 hours at 37°C. [12]. Colonies suspected to be S. mutans were identified on the basis of typical morphological characteristics, Gram stain (Gram-positive cocci arranged in chains), negative for the catalase test, and biochemical tests such as sorbitol fermentation, mannitol fermentation, hydrolysis of esculin, and negative for arginine hydrolysis. Confirmed identification was done using the Hi-Strep ID kit (HiMedia, India) [13].

2.4 Antimicrobial Susceptibility Testing

The antibiotic sensitivity test was carried out using the Kirby-Bauer disk diffusion method following CLSI M100 (2023). The turbidity level of bacteria was matched to 0.5 McFarland standards. Bacteria were cultured using Mueller-Hinton agar plates with 5% human blood added. Antimicrobial disks (Bioanalyse, Turkey) were inoculated on the agar plates and incubated under the conditions of 35±2°C for 20-24 h in an environment with 5% CO2 concentration. The zone sizes were determined, and the results were classified into S (Susceptible), I (Intermediate), or R (Resistant) based on CLSI M100-Ed35, Table 1. The antimicrobial agents used included Penicillin (P, 10 µg), Ampicillin (AMP, 10 µg), Erythromycin (E, 15 µg), Tetracycline (TE, 30 µg), and Vancomycin (VA, 30 µg). [13]

Table 1. Antibiotics tested and CLSI M100-Ed35 breakpoints for Viridans Group Streptococci.

No.	Antibiotic	Code	µg/disk	Company	DD S (≥mm)	DD R (≤mm)	MIC S (≤)	MIC R (≥)
1	Penicillin	P	10	Bioanalyse	—[1]	—[1]	≤0.12	≥4
2	Ampicillin	AMP	10	Bioanalyse	—[1]	—[1]	≤0.25	≥4
3	Erythromycin	E	15	Bioanalyse	≥21	≤14	≤0.25	≥2
4	Tetracycline	TE	30	Bioanalyse	≥23	≤18	≤2	≥8
5	Vancomycin	VA	30	Bioanalyse	≥17	—[2]	≤1	—[2]

[1] Disk diffusion unreliable for Penicillin/Ampicillin vs. VGS —MIC testing preferred (CLSI M100 Table 1). [2] No intermediate/resistant breakpoint for Vancomycin vs. VGS.

2.5 Statistical Analysis

The data analysis was done using SPSS software version 26.0 (IBM Corp., USA). Susceptibility to antimicrobials is shown as frequency and percentage. For the comparison between categorical data, the chi-square test was used, with $p < 0.05$ as the level of significance.

3. RESULTS

3.1 Bacterial Isolation

In total, 80 samples from the mouth area of patients having active carious lesions were collected. From these, 50 samples were positive for the presence of bacteria, with an isolation rate of 62.5%. Biochemical analysis found that *Streptococcus mutans* was present in 32 samples, which made up 64.0% of all the isolated strains. This finding supports existing studies on the prevalence of *Streptococcus mutans* in carious lesions [14,15]. Distribution of isolates is shown in Table 2.

3.2 Antimicrobial Susceptibility Results

The results of the antibacterial susceptibilities for the confirmed 32 strains of S. mutans are shown in Table 3. All tested strains were susceptible to Vancomycin (100%). Out of the total number of isolates tested, 28 strains were susceptible to Penicillin (87.5%), and four strains had higher MIC values (12.5%). In the case of Erythromycin, susceptibility was found in 20 strains (62.5%), intermediate in five strains (15.6%), and resistant in seven strains (21.9%). Tetracycline susceptibility was demonstrated in 18 isolates (56.3%), with 6 intermediate (18.8%) and 8 resistant (25.0%).

Table 2. Distribution of bacterial isolates from dental caries samples (n=50 positive cultures).

Bacterial Isolate	No. of Isolates	% of Positive Cultures
Streptococcus mutans	32	64.0%
Streptococcus sobrinus	8	16.0%
Lactobacillus spp.	6	12.0%
Other Streptococcus spp.	4	8.0%
Total	**50**	**100%**

Table 3. Antimicrobial susceptibility profile of *S. mutans* isolates (n=32).

Antibiotic	Code	S n(%)	I n(%)	R n(%)	Non-Susc. %	Mean Zone (mm)	Interpret.
Penicillin	P	28 (87.5%)	0 (0%)	4 (12.5%)	12.5%	MIC only[1]	Mostly S
Ampicillin	AMP	26 (81.3%)	2 (6.3%)	4 (12.5%)	18.8%	MIC only[1]	Mostly S
Erythromycin	E	20 (62.5%)	5 (15.6%)	7 (21.9%)	37.5%	19.4 ± 3.1	Moderate R
Tetracycline	TE	18 (56.3%)	6 (18.8%)	8 (25.0%)	43.8%	20.1 ± 3.8	Moderate R
Vancomycin	**VA**	**32 (100%)**	**0 (0%)**	**0 (0%)**	**0%**	**21.8 ± 2.2**	**Fully S**

S = Susceptible; I = Intermediate; R = Resistant. [1] Disk diffusion not validated for Penicillin/Ampicillin vs. VGS (CLSI M100).

4. DISCUSSION

4.1 Isolation Rate and Prevalence

The isolation percentage of S. mutans (64.0%) is in line with the percentages recorded in similar studies. For example, S. mutans was identified as the leading cariogenic organism in Iraq, representing more than 60% of the total number of isolates of streptococci. In a study conducted by [15] among dental caries patients in Ethiopia, S. mutans was noted as the most common bacterium in all severity groups.

4.2 Vancomycin: Consistent Efficacy

The 100% sensitivity of S. mutans to vancomycin is consistent with the results obtained from numerous independent studies [16] noted that all clinical Mutans streptococci isolates collected from South India were universally sensitive to vancomycin through disk diffusion testing according to CLSI M100-S25 guidelines. This phenomenon can be explained by the mode of action of vancomycin, as well as the rarity of the development of intrinsic resistance to this drug in gram-positive bacteria.

4.3 Erythromycin and Tetracycline Resistance

The reported levels of non-susceptibility, which stand at 37.5% for erythromycin and 43.8% for tetracycline, hold clinical relevance and align with patterns found in the scientific literature published globally. Resistance to erythromycin in oral streptococci is primarily associated with ribosomal methylation, which involves erm(B) and erm(TR), resulting in resistance to the MLSB class (macrolide, lincosamide, streptogramin) of antibiotics. Tetracycline resistance is mainly linked to the tet(M) gene, which codes for ribosomal protection proteins, identified in multiple studies involving Streptococcus mutans isolates around the world [16].

Horizontal transmission of resistance genes between bacteria that comprise polymicrobial biofilms is one of the critical factors responsible for antibiotic resistance development in the oral environment. According to, subinhibitory concentrations of chlorhexidine are capable of favoring the emergence of antibiotic-resistant bacteria through cross-resistance mechanisms.

4.4 Application of CLSI M100 in Dental Microbiology

It must be noted that the use of CLSI M100 Table 1 was crucial to ensure uniform and internationally acceptable reporting of the susceptibility results. As important is the recognition that *Streptococcus mutans* is considered one of the Viridans Group Streptococci (VGS) by the CLSI guidelines. According to CLSI, disk diffusion testing should not be employed in testing penicillin and ampicillin susceptibility of VGS bacteria due to the unreliability of the test.

4.5 Study Limitations

This study is limited by the low number of samples used and the use of a single site. The study did not employ molecular testing of the resistance genes, limiting the ability to determine resistance mechanisms employed by the bacteria. In future studies, MIC by the broth dilution technique, detection of the resistance genes (PCR for erms and tets), and multi-site studies need to be included.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The predominant cariogenic species isolated from dental caries patients was *Streptococcus mutans*, with a positivity rate of 64.0%. This clearly shows the significance of *Streptococcus mutans* in the causation of disease.

Vancomycin was found to have a 100% success rate in killing all the isolates tested and is thus the best antibiotic for treating systemic infections with resistant S. mutans. However, resistance was observed in erythromycin (37.5%) and tetracycline (43.8%). These antibiotics should not be used for dental infections caused by S. mutans without sensitivity tests.

It is critical that the CLSI M100-Ed35 Table 1 breakpoints be utilized in reporting susceptibility rates.

5.2 Recommendations

1. Resistance testing, done according to CLSI M100 guidelines, should always be included when conducting microbiological evaluation of severe or recurrent dental infections.

2. Empirical prescription of erythromycin or tetracycline in cases of dental infection without resistance testing should be avoided.

3. Antimicrobial stewardship initiatives need to be set up at a national level within dental clinics to prevent more instances of resistance.

4. Future research should employ molecular techniques such as PCR to identify erm and tet resistance genes alongside the sampling of multiple centers.

5. The role played by dental biofilms in the horizontal transmission of resistance genes needs to be studied.

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