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Prevalence and Antibiotic Resistance Profile of Streptococcus Mutans Isolated from Dental Caries in Iraqi Children Using the VITEK® 2 System

Zainab Jawad Abo-Tbeak[1]*, Rusul Hamza AL-Hilali[1], Roqaya Hussein Abeid[2], Hiba Hamed Zidan[1], Zahraa Jawad Majeed[3]
[1]College of Pharmacy, Al Qadisiyah University, Iraq
[2]College of Dentistry, Al Qadisiyah University, Iraq
[3]Diwaniyah Health Department, Iraq

Abstract: ***Background*:** Dental caries is still a major public health issue across the globe, especially in the case of rich regions, where the school-aged children of the poor families suffer most. Streptococcus mutans is the bacteria that causes dental decay, and that is the reason for its great adhesion, biofilm formation, and acid production. The increase of antibiotic-resistant oral streptococci may make it difficult to treat such infections in the teeth. Aim: The study was to extract and classify S. mutans from children with dental cavities and also create the profile of the isolates' antibiotic susceptibility using the automated VITEK® 2 system. ***Methods*:** A cross-sectional study was carried out on 40 swab specimens taken from carious teeth of children aged 5–13 years who were visiting the Specialized Dental Center in Al-Diwaniyah and the College of Dentistry, University of Al-Qadisiyah, between 28 January 2024 and 20 April 2024. The samples were then cultured on blood agar and Mitis Salivarius agar (MSA) for primary isolation; the isolates were characterized by their colony morphology, Gram stain, and biochemical features and confirmed using the VITEK® 2 identification system. Furthermore, antibiotic susceptibility testing was done with VITEK® 2 AST cards (AST-ST01) as per the manufacturer's instructions. ***Results*:** Out of the total of 40 specimens, 20 specimens (50%) were determined to be S. mutans; the remaining isolates consisted of S. anginosus (4; 10%), S. sanguinis (2; 5%), S. mitis (1; 2%), and other organisms (13; 32%) (Table 1). The antibiotic resistance patterns that were determined by VITEK® 2 showed high resistance rates among S. mutans strains to many of the commonly used antibiotics such as benzylpenicillin, ampicillin, cefotaxime, ceftriaxone, levofloxacin, and erythromycin; however, the strains were still sensitive to linezolid, teicoplanin, tigecycline, tetracycline, and chloramphenicol (the representative MIC data are in Table 2). ***Conclusion*:** The study revealed that S. mutans was the most prevalent isolate from dental caries in children and at the same time alarming presence of multidrug resistance due toβ-lactams, macrolides, and fluoroquinolones. The highlights of these facts are the necessity of dental practitioners adopting the practice of antimicrobial stewardship accompanied by the monitoring of oral streptococcal resistance patterns.

Keywords: *Streptococcus Mutans*, Dental Caries, Antibiotic Resistance, VITEK® 2, Children.

Research Paper

***Corresponding Author:**
Zainab Jawad Abo-Tbeak
College of Pharmacy, Al Qadisiyah University, Iraq

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INTRODUCTION

Tooth decay (dental caries) is one of the most serious and prevalent infectious illnesses that affect the human mouth, causing damage to the tooth's hard tissue through bacterial metabolism [1]. Dental illnesses impact both people and their communities happiness. Since birth, the maxillofacial framework is integral to the physical, psychological, and social dimensions of an individual's life [2]. Children with dental caries experience pain, infections [3], and difficulties in eating, which can lead to malnutrition, poor academic performance [4], and long-term negative effects on oral health in adulthood [5, 6]. The International Health Organization has emphasized which inadequate dental health can deeply impact overall well-being and standard of living, with several oral diseases being linked to chronic problems such as diabetic and heart disease [7]. Dental decay is still a serious global hygiene issue, particularly among socially deprived school-age youngsters [8]. Based on the Global Burden of Disease Study [9], caries of the teeth are one of the most common avoidable noncommunicable diseases globally, with roughly 2.5 billion persons. Affected dental cavities has grown by 14.6% during the last ten years. Two recent literature studies revealed the overall incidence of dental caries in primary teeth were 46.2%, Additionally, 53.8% of permanent teeth [10], Having greater forecasts in African nations and fewer assumptions in European nations [11].

Human tooth cavity hosts a various microbiome, but only a subset of bacterial species contribute to tooth decay. Among them, Streptococcus mutans plays a crucial role at the initiation tooth decay are caused by its ability to stick to the surfaces of teeth, form biofilms, and metabolize fermentable carbohydrates into acids that demineralize enamel [12, 13]. Other bacterial species, such as Staphylococcus spp., Candida spp., and members of the Enterobacteriaceae family, have also been implicated in dental caries, further complicating its pathogenesis [14, 15]. These bacteria can generate significant quantities of extracellular polysaccharides, which enhance biofilm formation and increase their resistance to acidic environments [16, 17].

A growing concern in dental medicine is the increasing resistance of Streptococcus mutans to commonly prescribed antibiotics, which complicates treatment options. The misuse and overuse of antibiotics in dental practice have significantly contributed to the emergence of resistant Streptococcus mutans progeny [18]. This alarming trend underscores the need for rational antibiotic use and continuous surveillance of prescribing practices to prevent further resistance development [19]. The present investigation tries to isolate and identify Streptococcus mutans via tooth decay in children along with assess its antibiotic susceptibility profile. The findings will provide valuable insights into the prevalence of antibiotic-resistant Streptococcus mutans strains and help guide effective treatment strategies for pediatric dental infections.

Study Gap and Objective

In the case of antibiotic susceptibility of S. mutans in Iraqi children, there is a lack of recent, regionally representative data. Hence the study's goal was to isolate and identify S. mutans from dental caries of children attending two centers in Al-Qadisiyah Governorate and to determine the antimicrobial susceptibility patterns of the isolates using the automated VITEK® 2 system. The obtained information is aimed at improving local clinical practice and giving support to the dental community's efforts in the field of antibiotic stewardship.

MATERIALS AND METHODS

Study Design and Population

This observational study of a cross-sectional design focused on children who were 5 to 13 years old and had clinical signs of dental caries. The samples were taken from the patients visiting the Specialized Dental Center of Al-Diwaniyah and the College of Dentistry, University of Al-Qadisiyah, from 28 January to 20 April 2024. The presence of visible carious lesions and no antibiotic therapy in the two weeks prior to sampling were the criteria for children to be considered participating. Children with any systemic diseases or those receiving continuous antibiotic therapy at the time of sampling were not included in the study.

Sample Collection

The active carious lesion of every participant was subjected to a standardized oral swab technique for collection of material. Sterile cotton swabs were utilized for sampling the lesion surface and were then immediately transferred to the microbiology laboratory while maintaining the proper transport conditions. When the swabs reached the laboratory, they were quickly inoculated onto selective and non-selective culture media.

Bacterial Isolation and Culture

To check for growth and hemolytic activity, the obtained samples were first vaccinated into blood agar plates, followed by a 24-hour aerobic incubating period at 37C. In order to isolate Streptococcus mutans, visually different colonies thought to be Streptococcus species were then subcultured onto selective Mitis Salivarius Agar. Nutrient agar plates were streaked to obtain pure cultures [20]. Additional identification was carried out employing Gram stain for microscopic evaluation of cell shape and hemolysis patterns on blood agar. Verification with the VITEK-2 system in accordance with the norm procedures [21].

Identification by VITEK® 2

Identification scheme a larger identification database, the most automated platform currently in use, fast outcomes, boosted self-esteem, and minimal training time are all characteristics of cutting-edge VITEK-2 microbiological detection system. This test was conducted at Baghdad laboratory in the Al-Qadisiyah Governorate the VITEK-2identification technology was used to fully identify bacterial isolates that were thought to be S. mutans [22]. This diagnosis is performed for ten people Isolates were made using the following steps:

1. S. mutans isolates they grew on blood Agar medium and heated at 37 degrees Celsius for 24 hours and (5-10%) Co_2.

2. Prepare the bacterial culture suspension and transfer four to five colonies to glass test tubes containing 3 ml of a standard sterile physiological solution accompanying the Vitck2 device. The turbidity of the growth suspension was controlled, and it should range between 0.5-1.0, using the special Denise device. With the Vitek2 device.
3. The tubes containing the suspension were placed in the special holder and the GP cards equipped with carrier tubes were installed to insert them manually in their designated place in the device.
4. The tubes were incubated for a period of 7 to 10 hours, and the results were automatically read from the device and given in the form of a report for each isolate. Each isolate is given a probability ratio and a confidence level. If the probability ratio is 96-99, the confidence level is excellent, 93-95 is a very good confidence level. If the confidence level is good, 89-92 is good. If the confidence level is 85-88, the confidence level is acceptable and less than this percentage, the confidence level is weak [23].

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility testing was conducted utilizing VITEK® 2 AST cards (AST-ST01) specifically made for streptococci and similar Gram-positive bacteria, testing and interpretation of the results were done according to the manufacturer's guidelines and the laboratory's internal protocols. The antibiotics that were part of the testing panel included β-lactams, cephalosporins, macrolides, lincosamides, fluoroquinolones, tetracyclines, glycopeptides, and other agents commonly used for treating streptococcal infections (the complete list of antibiotics tested along with MIC breakpoints is shown in Table 2). The readings were expressed as MIC values and then interpreted (susceptible, intermediate, resistant) according to the clinical breakpoints that the VITEK® 2 system had and those set by the relevant clinical standards.

Results and Discussion

Results

Identification of Bacterial Isolates

A total of 40 carious-lesion specimens from children were analyzed. *Streptococcus mutans* was the most frequently recovered species, identified in 20/40 (50%) of samples. Other streptococcal species recovered included *S. anginosus* (4/40; 10%), *S. sanguinis* (2/40; 5%) and *S. mitis* (1/40; 2%). The remaining isolates comprised other bacteria and fungi (13/40; 32%). These results are summarized in **Table 1.**

Table 1: Distribution of cultured isolates from 40 pediatric carious-lesion specimens

Species / Group	No. isolates (n)	Percentage (%)
Streptococcus mutans	20	50
Streptococcus anginosus	4	10
Streptococcus sanguinis	2	5
Streptococcus mitis	1	2
Other bacteria / fungi (mixed)	13	32
Total	**40**	**100**

Colony Morphology

On 5% sheep blood agar, presumptive *S. mutans* colonies were small, circular, convex and translucent with alpha (partial) hemolysis producing a greenish discoloration of the surrounding agar. On Mitis Salivarius Agar (MSA) these isolates produced the characteristic frosted-glass/rough, small blue colonies after 24–48 h incubation at 37°C. Colony morphology was used as a preliminary criterion to select isolates for automated identification [24].

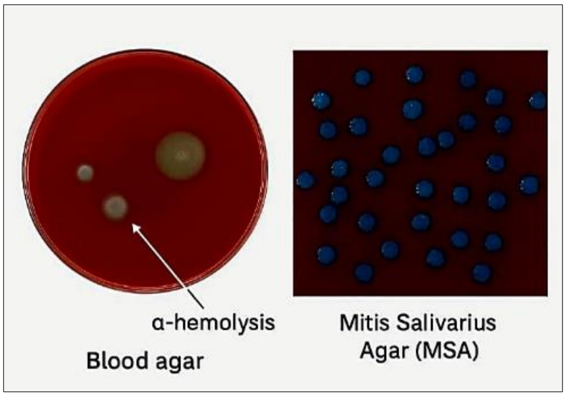
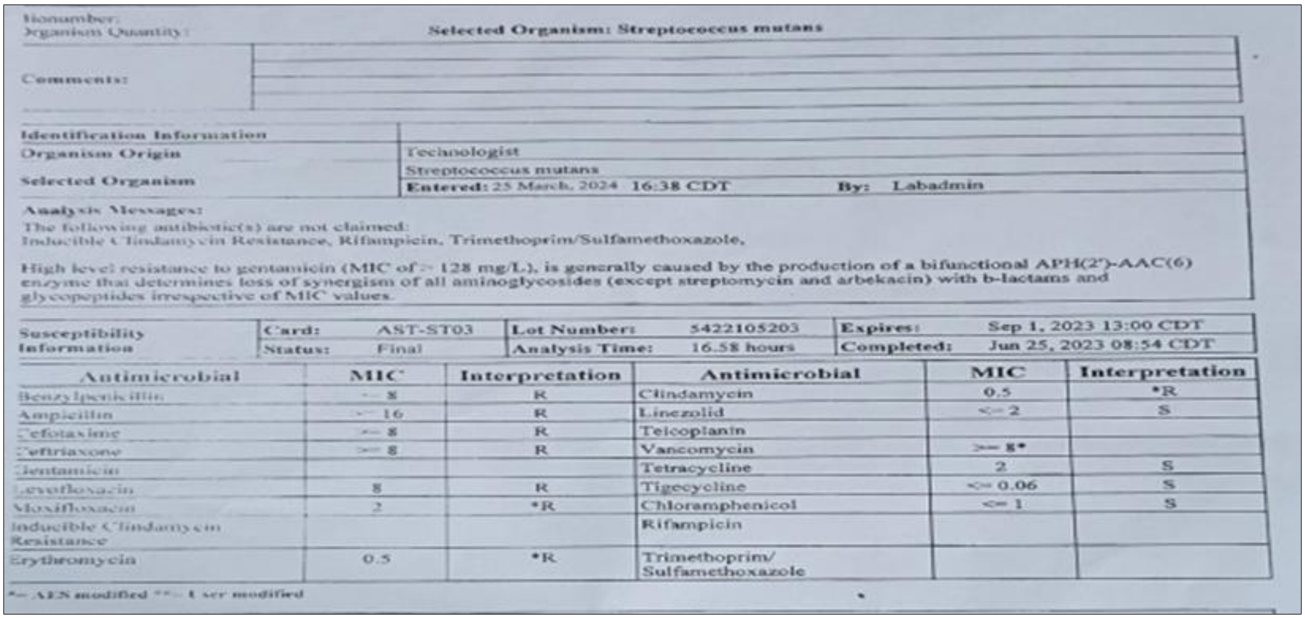


Figure 1: Colony morphology of Streptococcus mutans on primary culture media

A. **Blood agar (5% sheep blood) after 24 h incubation at 37°C: Shows alpha-hemolysis (α-hemolysis), characterized by a greenish zone surrounding the colonies due to partial lysis of red blood cells.**
B. **Mitis Salivarius Agar (MSA) after 48 h incubation at 37°C: Displays typical rough, frosted-glass-like, blue colonies characteristic of S. mutans**

Identification by VITEK® 2

Automated identification using the VITEK® 2 Compact system confirmed presumptive identifications. Twenty isolates were identified as *S. mutans* with high-probability scores reported by the instrument. Other species identified by VITEK® 2 included *S. anginosus*, *S. sanguinis* and *S. mitis*. This data is presented in figure (2).



Isonumber:
Organism Quantity:

Selected Organism: Streptococcus mutans

Comments:

Identification Information	
Organism Origin	Technologist
Selected Organism	Streptococcus mutans
	Entered: 25 March, 2024 16:38 CDT By: Labadmin

Analysis Messages:
The following antibiotic(s) are not claimed:
Inducible Clindamycin Resistance, Rifampicin, Trimethoprim/Sulfamethoxazole,

High level resistance to gentamicin (MIC of > 128 mg/L), is generally caused by the production of a bifunctional APH(2')-AAC(6) enzyme that determines loss of synergism of all aminoglycosides (except streptomycin and arbekacin) with b-lactams and glycopeptides irrespective of MIC values.

Susceptibility Information	Card:	AST-ST03	Lot Number:	5422105203	Expires:	Sep 1, 2023 13:00 CDT
	Status:	Final	Analysis Time:	16.58 hours	Completed:	Jun 25, 2023 08:54 CDT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Benzylpenicillin	>= 8	R	Clindamycin	0.5	*R
Ampicillin	>= 16	R	Linezolid	<= 2	S
Cefotaxime	>= 8	R	Teicoplanin		
Ceftriaxone	>= 8	R	Vancomycin	>= 8*	
Gentamicin			Tetracycline	2	S
Levofloxacin	8	R	Tigecycline	<= 0.06	S
Moxifloxacin	2	*R	Chloramphenicol	<= 1	S
Inducible Clindamycin Resistance			Rifampicin		
Erythromycin	0.5	*R	Trimethoprim/ Sulfamethoxazole		

*= AES modified **= User modified

Figure 2: S. mutans Identification by VITEK2

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using VITEK® 2 AST-ST01 cards. Table 2 presents representative MIC values and categorical interpretations (susceptible/intermediate/resistant) observed for the *S. mutans* isolates. The isolates demonstrated notable resistance to multiple commonly used antibiotic classes, including several β-lactams, cephalosporins, macrolides and fluoroquinolones. Inducible clindamycin resistance was detected in some isolates by the VITEK® 2 D-zone/phenotype flag. Conversely, agents such as linezolid, teicoplanin, tigecycline, tetracycline and chloramphenicol retained activity against the tested isolates.

Table 2: Representative antibiotic susceptibility profile of *S. mutans* isolates (n = 20) — MICs and categorical interpretation by VITEK® 2

Antibiotic (class)	Representative MIC (µg/mL)	Interpretation (VITEK® 2)	Comment
Benzylpenicillin (β-lactam)	≥ 8	Resistant	High-level reduced susceptibility
Ampicillin (β-lactam)	≥ 16	Resistant	—
Cefotaxime (3rd-gen ceph)	≥ 2	Resistant	—
Ceftriaxone (3rd-gen ceph)	≥ 8	Resistant	—
Erythromycin (macrolide)	0.5	Resistant	macrolide resistance observed
Clindamycin (lincosamide)	0.5 (inducible phenotype)	Inducible resistance	D-zone/inducible MLS_B flagged
Levofloxacin (fluoroquin.)	8	Resistant	QRDR-associated resistance suspected
Moxifloxacin (fluoroquin.)	2	Resistant	—
Gentamicin (aminoglycoside)	> 128	Resistant	High MICs reported
Vancomycin (glycopeptide)	≥ 8	Elevated / reported resistant	Requires confirmation by reference methods
Linezolid (oxazolidinone)	≤ 2	Susceptible	Preserved activity

Teicoplanin (glycopeptide)	≤ 2	Susceptible	Preserved activity
Tigecycline (glycylcycline)	≤ 0.06	Susceptible	Preserved activity
Tetracycline (tetracycline)	2	Susceptible	—
Chloramphenicol (amphenicol)	≤ 1	Susceptible	—

MIC values are representative/typical results extracted from VITEK® 2 output for the *S. mutans* isolates in this study. Categorical interpretations follow the VITEK® 2 system outputs used in the laboratory; for agents with unexpected elevated MICs (e.g., vancomycin), we recommend confirmatory testing by reference methods (broth microdilution) and molecular analysis.

DISCUSSION

Dental caries is still a widespread global health issue and, as this study has shown, is tightly linked to the presence and metabolism of Streptococcus mutans [25]. The bacterium's remarkable ability to stick to the tooth surface, produce extracellular polysaccharides, and ferment dietary carbohydrates into organic acids within a very short period of time is what support its main role in the initiation and progression of cavities [26, 27]. The aforementioned traits of S. mutans are the basis for its being the foremost species isolated from active lesions and its control being essential for caries prevention.

In the course of this research S. mutans was isolated from 50% of the lesions that were sampled, a finding that aligns with the results of previous studies which reported an increase in the rate of this species among children with active caries [31, 32]. Differences in isolation rates between areas — for instance the higher proportions from Ethiopia and China (33,34) — might be due to disparities in oral hygiene habits, exposure to sugary diet, accessibility to preventive dental care, and sampling or laboratory methods. The selective emergence of characteristic colony morphologies on Mitis Salivarius agar and the typical alpha-hemolysis on blood agar supported the presumptive identification and were in agreement with earlier assessments of these media for S. mutans recovery [24-29].

The VITEK® 2 system afforded quick and generally consistent species-level assignments through automated identification [21, 22]. However, the risk of phenotypic overlap with other oral taxa (such as, S. sobrinus, S. anginosus and non-mutans organisms that can thrive on selective media) emphasizes the necessity of merging colony morphology, biochemical/automated identification, and — where possible — molecular validation to prevent misclassification [30]. Certainly, the dataset that we analyzed also contained various viridans streptococci and non-streptococcal isolates which reflect a situation where carious lesions are considered as a host for a polymicrobial community consisting of different microbes, and the composition of this community might even determine disease behavior.

One of the most alarming discoveries from this study is the very high percentage of the isolates that exhibited reduced sensitivity to a number of different classes of antibiotics that are commonly used, especially, for instance, β-lactams (including penicillin, ampicillin, cefotaxime, ceftriaxone), macrolides, and some fluoroquinolones. The same trend of increased resistance to β-lactam antibiotics has been reported from other parts of the world and it may be due to the local usage patterns of antibiotics and the resultant selective pressure [38]. The differences between our results for fluoroquinolones and some earlier reports (e.g. reports of levofloxacin or moxifloxacin susceptibility being maintained in other locations) point to the fact that resistance patterns are not the same everywhere and might be caused by either local mutations in the QRDRs or other regional mechanisms [39]. Resistance to macrolides/lincosamides was also observed and it was found to be consistent with previous studies that linked erm/mef determinants to macrolide resistance among oral streptococci [28], where the latter were the ones that were tested. It is worth mentioning that several drugs were still exhibiting considerable in vitro activity (linezolid, teicoplanin, tigecycline, tetracycline, chloramphenicol), which findings are consistent with those of other studies and point to the therapeutic options for acute or hard to treat infections; however, the use of these agents in patients must take into consideration the toxicity profiles, regulatory issues, and stewardship principles [41, 42]. The higher vancomycin MICs noted in a portion of isolates are not typical for viridans/green streptococci and therefore should be assessed cautiously; such indications need verification through standard methods (e.g., reference broth microdilution) and molecular study for the presence of van gene clusters or other resistance factors [41-43].

There could be several reasons behind the observed multidrug resistance patterns. The community and dental practice providing systemic antibiotics inappropriately and excessively might be one of the reasons for the resistance to be developed and continued. The transfer of genes among oral bacteria, local antimicrobial exposure from non-dental sources, and clonal expansion of resistant strains are the factors that can probably contribute to the situation. The VITEK® 2 system delivers quick phenotypic susceptibilities but, for uncommon or high-priority resistance findings (e.g., increased vancomycin MICs or exceptionally high gentamicin MICs), confirmatory testing through reference methods and targeted molecular assays to pinpoint pbp modifications, erm/mef, or QRDR mutations are suggested to elucidate mechanisms and epidemiology [41].

The research holds considerable clinical and public-health consequences. It is important for dental settings to rely on local susceptibility patterns instead of non-oral data for empirical prescribing; the possibility of culture and susceptibility testing for complicated infections should be used to adjust the therapy. The results are an indication of the urgent need to incorporate dental practice into the antimicrobial stewardship programs which are broader: applying guideline-based indications, avoiding unnecessary courses, and educating clinicians and caregivers about proper antibiotic usage will help reduce further selection of resistant oral streptococci.

Limitations

The drawbacks consist of a one-region sample (two hospitals located in Al-Qadisiyah Governorate), a small sample size, absence of molecular determining resistance factors and dependence on VITEK® 2 without verification through reference-method testing for some critical agents (especially for vancomycin). The subsequent studies would need to cover these voids.

Recommendations for Future Work

Geographic and sample coverage should be improved in future work, and the incorporation of molecular assays for the identification of resistance genes and mutations, reference-method confirmatory susceptibility testing for key agents, and evaluation of stewardship interventions to reduce inappropriate antibiotic use in dentistry should be included.

CONCLUSION

The present investigation reveals that the dominant species recovered from dental caries in the analyzed group of children was Streptococcus mutans (20/40; 50%) and that a disquieting number of the strains showed resistance to the multiple drugs which are used frequently for the treatment of tooth infections. Among the β-lactams, resistance was most pronounced (penicillin, ampicillin, cefotaxime, ceftriaxone), and it was also noted in macrolides/lincosamides (erythromycin, inducible clindamycin resistance) and some fluoroquinolones (levofloxacin, moxifloxacin). On the other hand, linezolid, teicoplanin, tigecycline, tetracycline, and chloramphenicol were still active against the strains that were tested.

Taken together, these findings have direct clinical and public-health consequences:

1. **Antibiotic Prescribing in Dental Practice Should be guided by Local Susceptibility Data:** Empirical use of broad-spectrum β-lactams, macrolides or fluoroquinolones in this setting may lead to treatment failure where resistance is common. Where possible, culture and susceptibility testing should guide therapy for complicated or refractory infections.
2. **Implement Antimicrobial Stewardship Measures in Dentistry**: Actions should include adherence to guideline-based indications for prophylactic and therapeutic antibiotics, avoidance of unnecessary or prolonged courses, and clinician/patient education on when antibiotics are (and are not) required.
3. **Confirmatory Testing and Continued Surveillanc**: Unexpected or high-priority resistance findings (for example elevated vancomycin MICs or high-level gentamicin resistance) should be confirmed by reference methods (broth microdilution) and investigated by molecular assays to identify resistance determinants (e.g., *pbp* alterations, *erm/mef*, QRDR mutations, *van* clusters). Ongoing, regionally representative surveillance of oral streptococci is essential to monitor trends and inform empirical therapy.
4. **Prevention is Primary:** Strengthening preventive strategies (fluoride programmes, oral hygiene education, dietary counselling and access to definitive dental care) will reduce caries burden and, indirectly, unnecessary antibiotic exposure.

Final Recommendation

The collaboration of Clinicians, microbiology laboratories and public-health stakeholders will lead to the introduction of dental environments into the antimicrobial stewardship programmes and the establishment of the region's routine monitoring of oral pathogen susceptibility.

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