Iraqi journal of Bioscience and Biomedical
Published Online First: 30 / June /2026.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Isolation and phenotypic identification of Streptococcus mutans From oral cavity

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Received: 26/12/2025, Accepted: 2/2/2026, Published: 30/6/2026.

Abstract

Streptococcus mutans is a critical bacterium predominantly found in dental plaque biofilms and is considered one of the primary causative agents of dental caries in humans. *S. mutans* is known for producing acids that lower the pH and lead to the demineralization of teeth when it metabolizes dietary carbohydrates into organic acid. This study was designed for the isolate, identify, and characterize of multidrug-resistant *S. mutans* from dental sources. A total of 120 samples, including dental caries, saliva, and smooth tooth surface plaque were collected from different dental clinics and hospitals in Baghdad. The bacteria were isolated and identified using Mitis Salivarius Bacitracin Agar (MSBA), biochemical tests, confirmed by VITEK2 system and molecular detection. Antimicrobial susceptibility and resistance profile for the identified isolates were assessed using two methods. Test disk diffusion against 10 antibiotics was carried as an initial screening method, and the results were confirmed by VITEK2 system. The finding demonstrated that the isolated S. mutans strain exhibited resistance to multiple classes of antibiotics, highlighting the emerging concern of multidrug resistance among oral streptococci.

.**Keywords:** *S. mutans*, Dental Plaque, Mitis Salivarius Bacitracin Agar.

Introduction

S. mutans is a Gram-positive coccus, facultative anaerobic bacterium, that resides in dental plaque biofilms and is considered one of the most significant causative agents of dental caries in human[1]. *S. mutans* plays a central role in the initiation and progression of dental caries due to its remarkable ability to metabolize dietary carbohydrates into organic acids, which lower the pH of the surrounding environment and demineralizes tooth enamel [2].

These bacteria can effectively colonize the oral cavity due to its ability to create and tolerate acids, leading to the development of highly cariogenic plaque. As a result, *S. mutans* one of the most prevalent bacterium that is significantly more prevalent than other bacteria in dental plaque[3]. *S. mutans* the main

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Published Online First: 30 / June /2026.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

causes of this condition include fermentable carbohydrates, poor dental hygiene, and the pathogenic traits of cariogenic bacteria. Due to the participate of *S. mutans* in the glycolytic pathway, which breaks down dietary carbs, propanoic and butanoic acids are generated [4]. By upsetting the usual pH and encouraging the demineralization of the dental structure, this acidic environment leads to tooth loss[5]. This bacterium possesses several virulence factors that enhance its pathogenicity, including its strong ability to adhere to tooth surfaces, form biofilms, and produce extracellular polysaccharides that promote bacterial accumulation within dental plaque[6].

All these properties established *S. mutans* as a central agent in caries pathogenesis, making its accurate identification and characterization essential for effective disease management and prevention. In addition to its role in oral infections, S. mutans has been associated with systemic infections, including bacteremia and infective endocarditis, particularly in immunocompromised individuals, highlighting its broader clinical significance[7,8].

Antimicrobial resistance in *S. mutans* has gained increasing attention in dental application due to the broad and often uncontrolled use of antibiotics in oral infections. Previous studies have reported increase resistance to common antibiotics such as Tetracyclines, Macrolides, Clindamycin and rarely Vancomycin. This developed resistance decrease the effectiveness of treatments for dental infection and periodontal disease[9]. At the molecular level, resistance in *S. mutans* is related with specific genetic factors, including efflux pump gene and ribosomal protect gene or modification gene. In addition, biofilm formation specially participates to antimicrobial resistance by preventing antibiotic penetration and increasing bacterial survival within the oral cavity. Recent genomic and molecular research have underlined the significance of phenotypic antimicrobial susceptibility testing with molecular and whole genome sequencing methodologies to correctly uncover resistance mechanisms and understand their clinical implications.[10,11].

Therefore, the aim of this study to investigate multidrug-resistant *S. mutans* from different dental sites such as (dental caries, saliva, smooth tooth surface plaque) and evaluate their antibiotics susceptibility patterns.

Materials and Methods

Collection of clinical specimens:

From November 2024 to May 2025, a total of 120 clinical samples were collected from different intraoral sites known to harbor *S. mutans*, such as dental plaque, tooth caries, saliva and dental instruments[12]. The samples were carefully collected using dentist equipments from patients attending Al-Mahmoudia Hospital in Baghdad and . These samples were directly put in tubes contain sterile transport media and immediately transferred to a laboratory within a maximum period of 1-5 hours[13].

Bacterial isolation and identification:

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Samples were cultured on selective media for isolation of *S. mutans* Mitis Salivarius Bacitracin Agar (MSBA), the plates were incubated under anaerobic condition (candle jar) at 37°c for 24-48 hours. For further identification of *S. mutans*, VITEK2 system[14] and molecular test (PCR) were used.

Antimicrobial susceptibility test:

The disk diffusion method, as specified in Clinical and Laboratory Standards Institute (CLSI) guideline[15], was used to test the susceptibility profile of the isolates on Muller-Hinton agar media. Antibiotic susceptibility testing was performed on all 32 isolates. Bacterial suspensions was prepared and adjusted to match the 0.5 MacFarland turbidity standard, then evenly inoculated onto Muller-Hinton agar plates. The plates were incubated at 37°c for 24 hours under appropriated condtion.

Molecular study:

Molecular identification of *S. mutans* was done by polymerase chain reaction (PCR) using species-specific primers[16]. Genomic bacterial DNA was extracted from the isolates by using the ABIOpure™ Total DNA Extraction Kit (ABIOpure, USA) according to the steps detailed in the manufacturer's catalogue. The concentration and quality of the extracted DNA were measured by using Quantus Fluorometer (Promega, USA). DNA samples with appropriate concentration and purity were selected for downstream molecular analyses. Subsequently, PCR amplification was performed with GoTaq® Green Master Mix (Promega, USA) in a thermal cycler under normal circumstances, with an annealing temperature of 60°C. The amplified PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and examined with a gel imaging system. The size of amplicon was 415 bp.

Results and Discussion

Out of 120 clinical samples, 32 bacterial isolates were identified as *S. mutans*. As as they appear small 2-3 mm in size raised, rough, and dark blue to black on Mitis-salivarius Bacitracin agar (MSBA), as shown in **Figure1**. After 24–48 hours of incubation at 37°C under anaerobic conditions, a result that consistent with the characteristics of *S. mutans*. The dark coloration is associated with sucrose fermentation and glucan production, which contribute to biofilm formation and cariogenic potential[17].

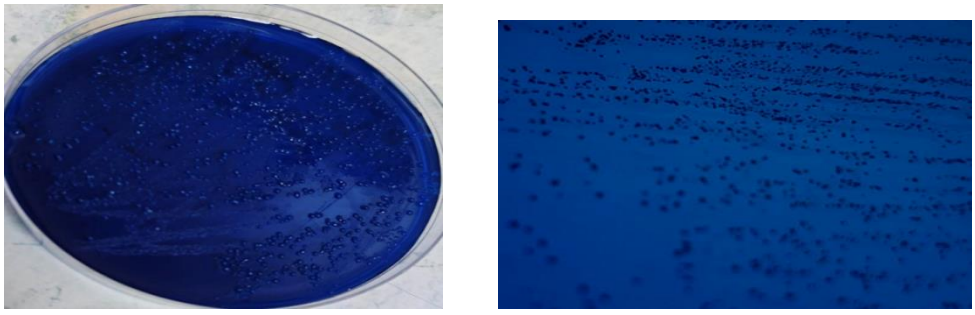

Figure (1) : *S. mutans* colonies on Mitis-salivarius Bacitracin agar (MSBA)After incubation, showing raised, rough, and adherent colonies characteristic of *S. mutans*.

Gram staining confirmed the presence of Gram-positive cocci, arranged in small chains, which is characteristic of *S. mutans* [18]. These morphological features are important for preliminary identification and

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correlate with their ability to adhere to tooth surfaces and produce extracellular polysaccharides, key factors in the initiation of dental caries[19]. *S. mutans* is catalase-negative, meaning it lacks the ability to produce catalase enzyme, so no bubbling occurs when exposed to hydrogen peroxide. This feature is a key characteristic for distinguishing *S. mutans* from catalase-positive bacteria such as *Staphylococcus*, and it serves as a reliable preliminary identification step in oral microbiology studies [7,17].

Growth on Blood Agar confirms that *S. mutans* can survive and multiply on nutrient-rich, non-selective media, (**Figure 2)**, which is important for phenotypic characterization and downstream biochemical tests. [20,21].

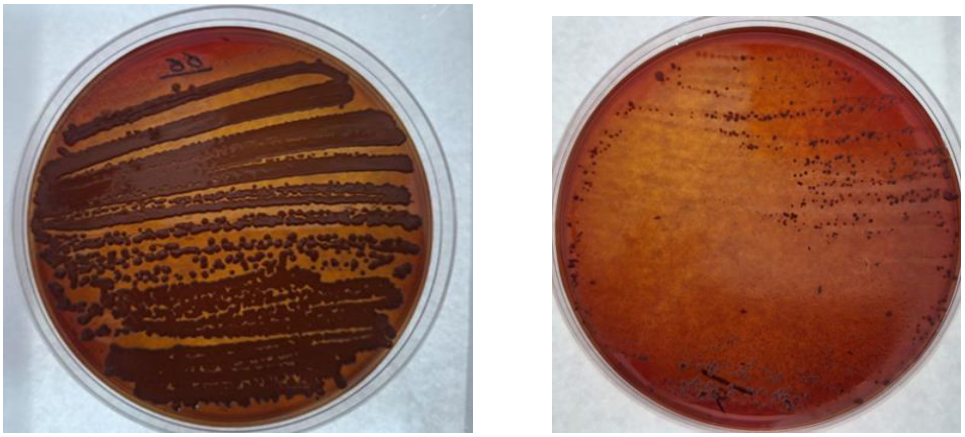

Figure (2) : Growth of *S. mutans* isolates on blood agar showing α-hemolysis, characterized by partial hemolysis and greenish discoloration surrounding the colonies after incubation.

The bacterial sensitivity test for antibiotics was performed using Muller-Hinton Agar medium and ten types of antibiotics. The current study showed that bacterial isolates exhibited a multidrug-resistance (MDR) phenotype, showing resistance to several antibiotic classes commonly used in dental practice. *S. mutans*, showed that have high resistance against Tetracycline(Doxycycline and Tetracycline), macrolides (Erythromycin), Lincosamide(Clindamycin), and glycopeptides (Vancomycin)[22]. According to CLSI criteria , resistance to more than three antimicrobial classes qualifies these isolates as MDR, which is of considerable clinical concern[23]. **(Table 1)**,

These findings are consistent with several local studies in Iraq that reported elevated resistance patterns among oral *S. mutans* isolates. For example, previous work from Baghdad demonstrated high resistance rates to erythromycin and tetracycline, suggesting persistent selective pressure from widespread antibiotic use in dental practice[24]. Similarly, a study from Diyala University reported high erythromycin resistance among dental caries patients [25]. These local reports reflect a similar MDR trend, highlighting the public health implications of antibiotic misuse and lack of susceptibility-guided therapy in Iraqi dental settings[26].

Globally, reports indicate that resistance to traditional first-line antibiotics for oral infections is no longer uncommon, particularly in regions where antibiotics are accessible without strict regulation [23].These

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international patterns reinforce the notion that AMR in oral pathogens is not a localized phenomenon but part of a broader, global antimicrobial resistance crisis.

In contrast to the high resistance observed, *S. mutans* isolates were susceptible to ofloxacin and amikacin, while rifampicin, imipenem, and cefepime showed intermediate susceptibility. The preserved activity of fluoroquinolones and aminoglycosides is consistent with regional and global studies, although their clinical use in dentistry is limited[27] . Intermediate responses may indicate emerging resistance and highlight the importance of ongoing surveillance and susceptibility-guided therapy[23].

Table (1): Antimicrobial Susceptibility pattern of *S. mutans* isolates against selected antibiotics.

Antimicrobial	Symbol	Resistant %	Sensitive %	Intermediate %
Doxycycline	DO	**16 (50%)**	**14 (43.8%)**	**2 (6.2%)**
Ofloxacin	OFX	**5 (15.6%)**	**24 (75%)**	**3 (9.4%)**
Tetracycline	TE	**15 (46.9%)**	**14 (43.8%)**	**3 (9.4%)**
Erythromycin	ERY	**18 (56.2 %)**	**12 (37.5%)**	**2 (6.3%)**
Vancomycin	VA	**17 (53.1 %)**	**13 (40.6%)**	**2 (6.3%)**
Clindamycin	CL	**18 (56.2 %)**	**12 (37.5%)**	**2 (6.3%)**
Rifampicin	RIF	**4 (12.5 %)**	**24 (75%)**	**4 (12.5%)**
Imipenem	IPM	**3 (9.4 %)**	**25 (78.1%)**	**4 (12.5%)**
Amikacin	AK	**2 (6.3 %)**	**28 (87.5%)**	**2 (6.3%)**
Cefepime	FEP	**4 (12.5 %)**	**25 (78.1%)**	**3 (9.4%)**

Interpretation of results was performed according to CLSI guidelines. S: Sensitive, I:Intermediate, R:Resistant.

Phenotypic identification of isolates using the VITEK2 system showed limitations in accurately differentiation. Out of total of 32 isolates, only 7 isolates were identified by VITEK2. *S. mutans*, consistent with reports that viridians group *streptococci* (VGS) share high biochemical similarity, often resulting in low-discrimination or ambiguous species-level identification [28]. To confirm species identity, PCR targeting housekeeping genes specific to *S. mutans*, was performed. This molecular approach provided definitive

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p-ISSN: 3007-5491 / e-ISSN:3007-5505

identification, overcoming the limitations of phenotypic methods and ensuring reliable species-level resolution[29].

PCR Amplification

The PCR amplification results indicated successful amplification of the *S. mutans* gene from the *S. mutans* samples, as evidenced by the bands observed on the agarose gel.

- **Gel Electrophoresis:** The use of a 1.5% agarose gel allowed for effective separation of the PCR products. The presence of a clear band corresponding to 415 bp signifies successful amplification of the target gene, **(Figure 3).**
- **PCR products of the *S. mutans*:** The consistent appearance of bands in these lanes suggests that the PCR reaction was reproducible across different samples. This is an important indicator of the reliability of the PCR assay used.
- **100 bp Ladder Marker**: The ladder provides a reference for estimating the size of the amplified products, confirming that the observed bands align with the expected size of the *S. mutans* gene.

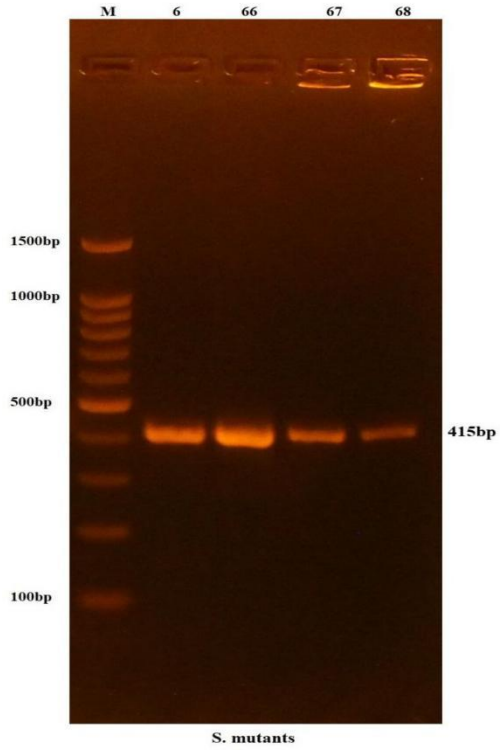

Figure (3) : Amplification of *S. mutans* gene of *Streptococcus mutans* samples species were fractionatedon 1.5% agarose gel electrophoresis stained with Eth.Br. Lane M: Molecular DNA ladder (100 bp), Lanes 6 - 68: resemble 415bp PCR products.

The results indicate that the extraction and amplification processes for the *S. mutans* gene were generally effective. The range of DNA concentration detected between 2 to 44 ng/µl is consistent with other studies and there is general variability in DNA extraction efficiency among sources and method applications. For instance, study by (23), determined that DNA yield from oral samples may vary in magnitude considerably and is dependent on the sampling, extraction procedure, and inhibitor-interference factors. Reported successful *S. mutans* gene amplification with a similar PCR strategy, confirming the reliability of our method. Gel Electrophoresis: Lane 6—Lane 68, the purity and uniform bands of different samples was an indicator which reflected in PCR assays that have high fidelity and specificity for *S. mutans* detection. 100 bp Ladder Marker The electrophoresis band pattern was estimated by using a standard ladder

marker which is compatible with the band products separation of DNA observed, as described significant 24. Confirming the size of bands with expected product could designate a reliable PCR method. The relationship between our result and previous reports demonstrates the quality of DNA extraction and PCR amplification methods. Successful gene amplification and visualization of the DNA levels clearly indicate that our approach is comparable to standard methods used in this area. Further investigations might pursue these results to provide quotative information of PCR products and their specificity, in order to more validate the possibility of our methods use in human diagnostics and research.

Conclusions

The present study highlights the significant role of Streptococcus mutans in dental caries through its ability to form cariogenic biofilms and express key virulence traits. The successful isolation and molecular identification of S. mutans using PCR confirmed the reliability and accuracy of molecular techniques in overcoming the limitations of conventional phenotypic methods. Furthermore, the effectiveness of DNA extraction and amplification supports the use of standardized molecular protocols for reproducible detection of S. mutans. Collectively, these findings emphasize the importance of rapid and reliable diagnostic approaches and provide a scientific basis for developing targeted preventive and control strategies against S. mutans–associated oral infections.

Acknowledgments

The author would like to express our deepest gratitude to all those who contributed to the completion of this study. Our sincere thanks go to the healthcare providers and staff at (Mahmoudiya Hospital and Milano Dental clinic). for their invaluable assistance in sample collection. extend our heartfelt appreciation to the faculty and staff of the College of Biotechnology, Al-Nahrain University, for their support and guidance throughout this research.

Author's Declaration

Rahma F. Ameer: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

Dhafar N. Al-Ugaili: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

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