Original Article

Molecular Detection of Some Virulence Genes Among *Streptococcus mutans*

Zahraa Dhafer Shamsulddin, Ilham A. Bunyan
Department of Microbiology, College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Background: Microbes greatly influence oral illnesses. Regulatory mechanisms and bidirectional communication maintain host-mouth bacterial equilibrium throughout life. **Objectives:** Current study is aimed at the molecular detection of some virulence genes among *Streptococcus mutans*. **Materials and Methods:** From November 2022 to March 2023, 100 College of Dentistry, University of Al-Kufa, medical educational clinic patients contributed soft, deep caries lesions. Samples from different media were incubated for 18–24 h at 37°C. *S. mutans* was found in colony morphology, microscopy, and biochemistry. **Results:** About 20% of the 100 positive clinical samples were *S. mutans*. A VITEK 2 automated system examined 64 biochemical GP-ID card *S. mutans* isolates. It promptly and accurately identified 20 bacterial isolates (94%–99.7%). The 497-bp *SM479* gene was amplified by standard polymerase chain reaction (PCR) using all-isolated DNA. All isolates were amplified by standard PCR. The virulence factor gene was found in *S. mutans* isolates. Unlike an allelic ladder, *S. mutans* possesses all its virulence genes since all 20 samples have the *cnm* gene. All twenty *S. mutans* isolates had PCR-detected 647-bp *Cbm* genes. All 20 *S. mutans* isolates had *gtfB*. The (517 bp) amplicon was found using a gel electrophoresis allelic ladder. **Conclusions:** The automated VITEK 2 system tested 64 biochemical GP-ID card *S. mutans* isolates. It quickly and reliably identified 20 bacterial isolates (94%–99.7%). All isolate DNA was used for standard PCR amplification of the *SM479* gene. Each previously isolated bacteria was amplified by conventional PCR. Virulence factor gene identification in *S. mutans* isolates. All 20 samples had *cnm*, *Cbm*, and *gtfB*.

Keywords: *Cbm* gene, *cnm* gene, *SM479* gene, *Streptococcus mutans*, virulence genes

Introduction

There is a strong link between oral disease and the presence of microorganisms in the mouth. It has been shown that once children acquire their first colonizing bacteria, the variety of their mouth microbiome grows. Different forms of bidirectional communication and regulatory mechanisms keep the host and the oral microbial population in homeostatic equilibrium throughout a person's lifetime. Caries, periodontal disease, and oral candidiasis are only some of the oral infectious disorders that can be brought on by a dysbiosis of the oral microbiota.[1]

Several systemic disorders, such as rheumatoid arthritis, Alzheimer's disease, and systemic lupus erythematosus, have been linked to changes in the oral microbiota, including malignancies of the head and neck, pancreas, and colon.[2] Studies of the oral microbiome have historically zeroed in on bacteria due to the constraints of conventional pure culture techniques. Oral fungi, the candidate phyla radiation group (a unique class of bacteria that co-parasitize the host), and viruses were previously not major topics in the ecology of the human oral microbiota; however, this has changed in recent years due to advances in sequencing technologies and molecular investigative techniques. However, it has been difficult to ascertain how oral microbes and oral disorders communicate with one another and how they are controlled.[3] According to Mosaddad *et al*. (2019)[4], dental caries is the breakdown

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_50_24

Address for correspondence: Dr. Zahraa Dhafer Shamsulddin, Department of Microbiology, College of Medicine, University of Babylon, Hilla, Iraq.
E-mail: zahraad.bashboosh@uokufa.edu.iq

Submission: 18-Mar-2024 **Accepted:** 16-Apr-2024 **Published:** 28-Jun-2025

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Shamsulddin ZD, Bunyan IA. Molecular detection of some virulence genes among *Streptococcus mutans*. Med J Babylon 2025;22:542-8.

of enamel and dentin caused by bacterial fermentation in the mouth. *Streptococcus mutans* is considered as a major pathogen of dental caries due to its ability to adhere and accumulate on the surface. Additionally, it is a cariogenic microorganism that breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth.[5] Cavitated and noncavitated carious lesions result from a progression of demineralization of hard tooth tissue that begins within the bacterial biofilm. Caries can lead to severe dental discomfort and even tooth loss if left untreated. Once a child has teeth, they are at risk for dental caries, a disease caused by bacteria that can last a lifetime. It is influenced by a number of variables, such as cariogenic bacteria, saliva flow, fluoride exposure, oral hygiene practices, and sugar intake.[6]

Caries in teeth occur when acidic byproducts of the oral bacterial fermentation of food carbohydrates dissolve minerals from hard tooth tissue. When oral biofilms are permitted to persist for extended periods of time on the tooth surface, caries develops. Because streptococci, which make up the majority of lactic acid bacteria, ferment dietary carbohydrates to create acid within the biofilm structure on the tooth surface,[7] the demineralization of dental enamel is caused by the acid's ability to lower pH below a key point (pH 5.5). Tooth components (hydroxyapatite) like calcium, phosphate, and fluoride diffuse due to demineralization. Allowing this demineralization to continue will lead to the development of a carious lesion.[8]

Action (GTFs) in glucan production is the primary adhesion mechanism dependent on sucrose. Sucrose activity is generated by GTFs, resulting in the cleavage of sucrose into glucose and fructose. The glucose fraction is subsequently incorporated into a progressively larger glucan polymer.[9] There are three GTFs encoded by genes in *S. mutans*: *gtfB*, *gtfC*, and *gtfD*. The GTFs work as a group to produce both water-soluble and WIGs. While dextran, the most common form of water-soluble glucan, is a linear polymer linked by alpha 1,6-glycosidic links, mutan, the most common form of water-insoluble glucan (WIG), is a polymer with a higher degree of branching linked by 1,3-linkages. Sucrose-dependent colonization and decay are thought to involve both polymers; however, WIG may be more crucial for smooth surface decay.[10]

Aim of study

Current study is aimed at the molecular detection of some virulence genes among *S. mutans.*

Materials and Methods

One hundred infected patients, ranging in age from 25 to 60 years old, were involved in this research. Patients seen at the medical education clinics of the College of Dentistry, University of Al-Kufa, between November 2022 and the end of March 2023 provided the samples taken from soft deep caries lesions. The standard procedure for collecting samples from soft, deep caries lesions is to rolate a sterile cotton swab twice, then put the swab in sterile transport media and immediately transport it to the laboratory. It was incubated on the swab aerobically at 37°C for 24 h after inoculating it in culture medium. In order to identify the bacteria and conduct laboratory analyses, the samples were sent to the Dentistry Laboratory College, Al-Kufa University, in a chilled box.

Ethical approval

Acquiring the required ethical clearance from the medical educational clinics' ethics committees, patients, and their supporters is essential. Furthermore, prior to sample collection, all individuals were verbally informed and given the necessary consent to conduct the tests and publish the results. A local ethics committee examined and approved the study protocol, subject information, and permission form at the College of Medicine, University of Babylon, Iraq, under reference No. 3–6 on July 72022.

A GP-ID assay using the VITEK 2 compact for *Streptococcus mutans* identification

The BioMerieux Compact VITEK 2 System recognized all *S. mutans* strains. This phenotypic identification distinguishes isolates biochemically. The 64-well VITEK 2 card is for fluorescence biochemical research. In 20 of 64 carbohydrate absorption experiments, phosphatase, urea, nitrate, and actidione were tested. The VITEK 2 machine filled, sealed, and transported cards to the incubator at 35°C. Output reports are decoded using algorithms. The ID-GP database identified the results. These systems' ID software suggests results. If the initial findings showed "poor discrimination" or "no ID," further tests were done and assessed. Each strain was incubated overnight at 37°C with injected culture media. One colony was found utilizing phenotypic VITEK 2 system (BioMerieux).

DNA extraction

Korean manufacturer Geneaid's genomic DNA purification kit was used for this procedure. All PCR experiments utilized chromosomal DNA templates. PCR was done in a thermal cycler. The following steps were used to profile bacterial DNA and load buffer without heat cycling before the PCR assay:

Detection of some of the *S. mutans* virulence genes

DNA (extract from bacterial cells) was used as a template in specific PCRs for the detection of some of the *S. mutans* virulence genes. DNA was purified from bacterial cells by using the Geneaid DNA extraction kit. The primers used for the amplification of a fragmented gene are listed in Table 1.

Results

The samples were collected from soft, deep caries lesions from patients who visited medical educational clinics of the College of Dentistry, University of Al-Kufa, during a period of five months from November 2022 to the end of March 2023, providing the study's 100 participants with soft, deep caries lesions specimens. The samples were grown in a number of different media. After that, they spent 18–24 h in a 37°C incubator. Colony morphology, microscopy, and biochemical testing were first used by scientists to identify *S. mutans*. Only 20% of the isolates were confirmed to be *S. mutans* from the 100 positive clinical samples. The automated VITEK 2 system deployed GP-ID cards, including 64 biochemical assays, to guarantee the isolates were indeed *S. mutans*. The approach promptly and accurately identified 20 distinct bacterial isolates with probabilities ranging from 94% to 99.7%.

All previously identified isolates' DNA was taken and used in conventional PCR to amplify the SM_{479} gene. Figure 1 displays the results of gel electrophoresis, which demonstrated that each of the 20 *S. mutans* isolates (100%) had, in fact, generated the same 497-bp DNA fragment when run alongside a ladder.

In this study, molecular detection of some virulence factor genes among *S. mutans* isolates was investigated.

Table 1: The primers, sequences, and PCR conditions for detection of some virulence gene

Gene name	Primer sequence (5´-3´)	Size of Bp	Conditions	References
cnm	F GACAAAGAAATGAAAGATGT R GCAAAGACTCTTGTCCCTGC	1600–1800	95°C, 5 min. 95°C, 45 s. 50°C, 45 s. 72°C, 1 min. 72°C, 10 min.	[11]
GtfD	F TTGACGGTGTTCGTGTTGAT R AAAGCGATAGGCGCAGTTTA	219	95°C, 5 min. 95°C, 45 s. 50°C, 45 s. 72°C, 1 min. 72°C, 10 min.	[12]
Cbm	F GACAAACTAATGAAATCTAA R TCATCAGGAACCAGCGCACA	647	95°C, 5 min. 95°C, 45 s. 50°C, 45 s. 72°C, 1 min. 72°C, 10 min.	[12]
SM479	F TCGCGAAAAAGATAAACAAACA R GCCCCTTCACAGTTGGTTAG	479	95°C, 5 min. 95°C, 45 s. 50°C, 45 s. 72°C, 1 min. 72°C, 10 min.	[12]

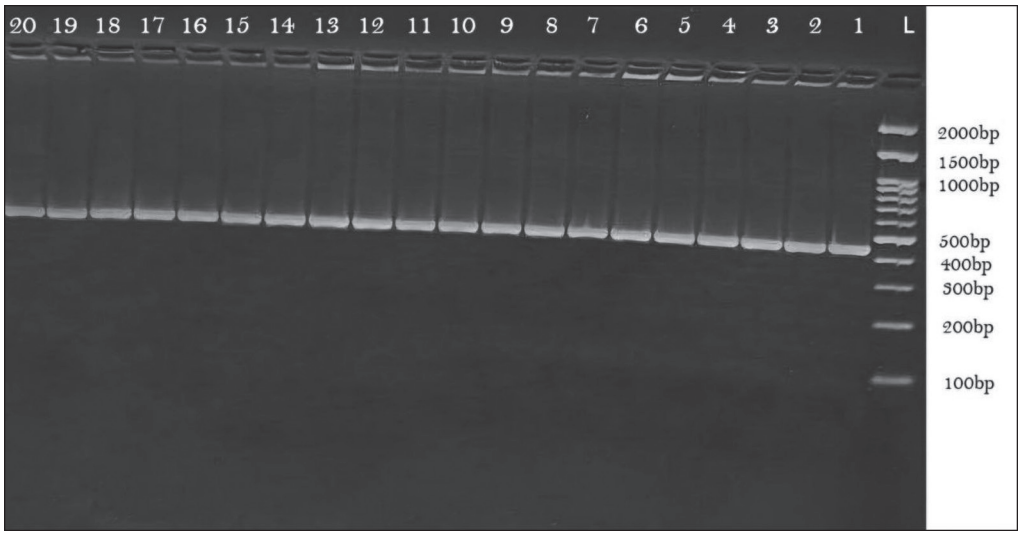

Figure 1: Agarose gel electrophoresis of PCR amplified of a specific SM_{479} gene at 479 bp of *S. mutans*. L: DNA ladder (100). Line 1–20 showed positive results of the SM_{479} gene

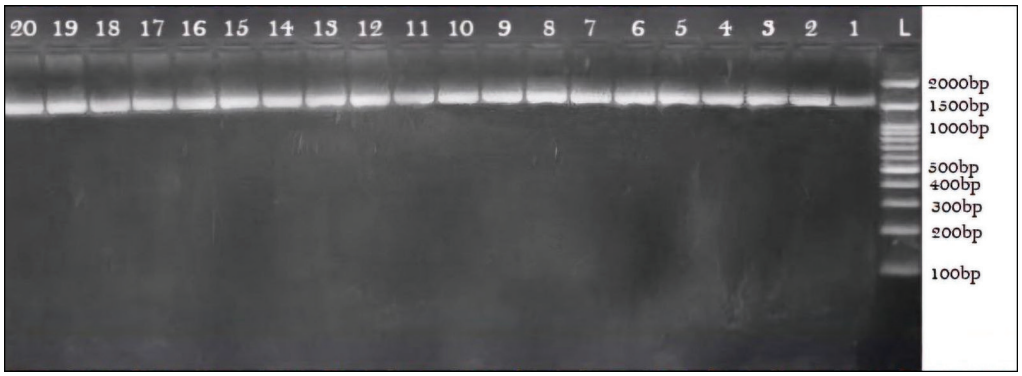

Figure 2: Agarose gel electrophoresis of PCR amplified of *cnm* gene at 1600–1800 bp of *S. mutans* L: DNA ladder (100). Lines 1–20 showed positive results of the *cnm* gene

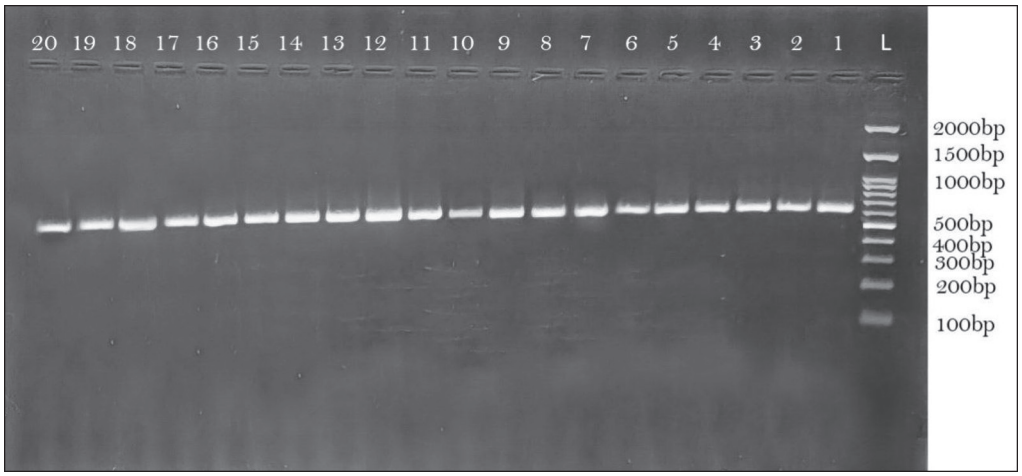

Figure 3: Agarose gel electrophoresis of PCR-amplified *Cbm* gene at 647-bp of *S. mutans*. L: DNA ladder (100). Lines 1–20 showed positive results of the *Cbm* gene

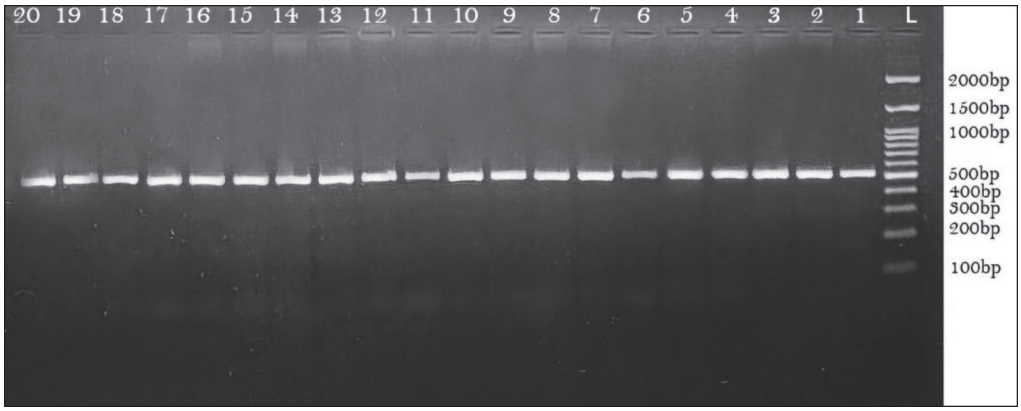

Figure 4: Agarose gel electrophoresis of PCR-amplified of the *gtfB* gene at 517 bp of *S. mutans*. L: DNA ladder (100). Lines 1–20 showed positive results of the *gtfB* gene

The results showed that all 22 samples tested positive for the *cnm* gene (100%) in an analysis of the *S. mutans* virulence genes, as shown by bands between 1600 and 1800 bp when compared to an allelic ladder as shown in Figure 2. In addition, molecular analyses of the *Cbm* gene were performed on all *S. mutans* isolates using specific PCR markers. As may be observed in Figure 3, a lengthy *Cbm* gene (647-bp) was detected in all 20 (100%) *S. mutans* isolates.

The presence of the *gtfB* gene was checked in all 20 *S. mutans* isolates, and the results found that all these isolates were found to be positive. The (517 bp) amplicon was found by comparing it to an allelic ladder on gel electrophoresis, as illustrated in Figure 4.

Discussion

In this study, only 20% of the isolates were confirmed to be *S. mutans* from the 100 positive clinical samples. The automated VITEK 2 system deployed GP-ID cards, including 64 biochemical assays, to guarantee the isolates were indeed *S. mutans*. The approach promptly and accurately identified 20 distinct bacterial isolates with probabilities ranging from 94% to 99.7%. Research by Chen *et al*. (2020)[13] found that *S. mutans* was known for its ability to metabolize dietary sugars and produce acids as byproducts. These acids can demineralize the tooth enamel, leading to the formation of cavities. In deep caries lesions, the acidic environment can create conditions favorable for the survival and proliferation of *S. mutans*.[14] Also, *S. mutans* has adaptations that allow it to thrive in acidic conditions. It can survive and grow in an environment with a low pH, which is often present in caries lesions due to the acid produced during the fermentation of sugars, according to a study of caries. Sadeghinejad *et al*. (2017)[15] found *S. mutans* was considered one of the primary cariogenic (cavity-causing) bacteria. Its presence and activities contribute significantly to the initiation and progression of dental caries. The bacteria can adhere to tooth surfaces, produce acids, and contribute to the breakdown of teeth. In addition, Guan *et al.* (2020)[16] found *S. mutans* has specific mechanisms for utilizing sucrose, a common dietary sugar.

All previously identified isolates' DNA was taken and used in conventional PCR to amplify the SM_{479} gene. The results in this study were in agreement with the results of Al-mohammadawy *et al*. (2018)[17] and Al-Nafaee *et al*. (2023)[18], who found that specific SM_{479} genes were detected in *S. mutans* isolates. Also, a study by Al-Amin *et al*. (2022)[19] found genetic detection was done by using a specific SM_{479} gene for *S. mutans* for identification of this bacteria. Specific SM_{479} gene was expanded, widely used, and showed an elevated rank of effectiveness for the identification of microorganisms and the evaluation *S. mutans* foundation in mouth and dental caries specimens[20]. This study's findings demonstrate that conventional PCR with a specified target may detect *S. mutans* with more speed, simplicity, and accuracy than previous methods. The results of the PCR experiments confirmed that the comparative genomics method had successfully located the target sequence. PCR using species-specific primers is a useful tool that may replace complex molecular clustering techniques and routine microbiological tests for identifying species that are difficult to differentiate using phenotypic approaches[21].

In rare cases, a specific species of *S. mutans* may defy phenotypic identification because it lacks diagnostic characteristics. Better and more consistent methods would be ideal for use in microbiology laboratories. When compared to the culture approach, polymerase chain reaction (PCR) provides the highest accuracy, sensitivity, specificity, distinctiveness, and cost-effectiveness for detecting *S. mutans* infections.[22] Results from a molecular technique may be achieved in 24 h, whereas those from a convective method, such as normal culture followed by a biochemical test, require between 24 and 48 h.[23] PCR has enabled the rapid, culture-independent identification of streptococci in a wide range of clinical specimens. Results may be obtained in as little as a few hours. However, all existing PCR-based tests are exclusively species-specific for streptococci, which are of clinical importance.[24]

In this study, molecular detection of some virulence factor genes among *S. mutans* isolates was investigated, and the results showed that all 22 samples tested positive for the *cnm* gene. In addition, molecular analyses of the *Cbm* gene were performed on all *S. mutans*. The results in this study were in agreement with results obtained by Araújo Alves *et al*. (2018)[25], who found that the *cnm* gene, which codes for a collagen-binding protein, has been associated with certain strains of *S. mutans* and their ability to interact with host tissues, including collagen-rich surfaces. This interaction was considered important in the context of dental caries, as *S. mutans* was a primary causative agent in the formation of dental plaque and the subsequent development of caries.[26]

A study by Nomura *et al*. (2020)[27] found the collagen-binding protein gene, such as the *cnm* gene in *S. mutans*, was considered a virulence factor because it contributes to the ability of the bacterium to adhere to and interact with host tissues, particularly those rich in collagen. Virulence factors are microbial attributes or products that enhance the ability of a microorganism to cause disease in a host. *S. mutans*, which was a major contributor to dental caries, had the ability to adhere to tooth surfaces and form biofilms, which was critical for its pathogenicity. According to a study by OmerOglou *et al*. (2022)[28], collagen was a protein that was abundant in various host tissues, including those in the oral cavity. The collagen-binding protein produced by the *cnm* gene facilitates the adhesion of *S. mutans* to collagen-rich surfaces, including dentin in teeth. The ability of *S. mutans* to adhere to the surfaces of teeth and other oral tissues was a crucial step in the formation of dental plaque, which is a biofilm that provides a favorable environment for the growth of bacteria.[29] Adherence to collagen-rich tissues may also contribute to the ability of *S. mutans* to invade host tissues. This invasion can lead to persistent infections and contribute to the progression of dental caries, as a study by Garcia *et al*. (2021)[30] found. Collagen binding enhances the bacterium's ability to form biofilms on tooth surfaces. Biofilms provide a protected environment for bacteria, making them more resistant to antimicrobial agents and the host immune response.[31] The *cnm* gene played a crucial role in the adhesion of *S. mutans* to collagen-rich surfaces present on teeth, particularly dentin.[32] However, the ability of *S. mutans* to bind to collagen may also contribute to its ability to

invade dentin, the hard tissue beneath the enamel of a tooth. This invasion was associated with the progression of dental caries, as the bacteria can reach deeper layers of the tooth structure.[33]

The presence of the *gtfB* gene was checked in all 20 *S. mutans* isolates, and the results found that all these isolates were found to be positive. These results were identical with the results of Lozano *et al*. (2019)[34] and Wasfi *et al*. (2018)[35], who found that all *S. mutans* isolates were positive for specific *S. mutans* genes (*gtfB* and *gtfD*). A study by Xu *et al*. (2018)[36] found the *GtfB* gene in *S. mutans* encodes for the glucosyltransferase D enzyme, which is part of a group of enzymes called glucosyltransferases. These enzymes play a crucial role in the virulence of *S. mutans*, making the *GtfD* and *gtfB* gene a virulence factor gene because glucosyltransferases produced by the *GtfD* and *gtfB* gene were responsible for synthesizing glucans from sucrose. Glucans are important components of the extracellular matrix that forms dental plaque. This matrix, along with bacterial cells, makes up the biofilm on tooth surfaces[29]. Biofilm formation was a key step in the pathogenesis of dental caries, as it allowed the bacteria to adhere to and accumulate on the teeth, providing a protective environment for the bacteria[13].A study by Zhang *et al*. (2021)[37] found the *gtfB* gene in *S. mutans* encodes for the glucosyltransferase B enzyme. Glucosyltransferase B enzyme is an enzyme that plays a key role in the formation of dental plaque, which is associated with the development of dental caries. In addition, the glucans produced by *GtfD* contribute to the adherence of *S. mutans* to tooth surfaces. The bacteria use these glucans to anchor themselves to the tooth enamel and other surfaces in the oral cavity, facilitating the formation of dental plaque, according to the results of Rezaei *et al*. (2023)[38]. A study by Jurakova *et al.* (2023)[39] found that glucosyltransferases also contribute to the production of acids by *S. mutans*. When sucrose is metabolized by the bacteria, it leads to the production of acids as byproducts. These acids can contribute to the demineralization of tooth enamel, which is a key step in the development of dental caries. The ability of *S. mutans* to form biofilms, adhere to tooth surfaces, and produce acids contributes to its virulence in causing dental caries. Virulence factors are traits or products that enhance the ability of a microorganism to cause disease[33].

Conclusions

The automated VITEK 2 system tested 64 GP-ID card biochemical *S. mutans* isolates. It correctly identified 20 bacterial isolates (94%–99.7%). The *SM479* gene was amplified by standard PCR utilizing all previously identified isolates' DNA. Standard PCR amplified all previously described isolates. Virulence factor gene identification in *S. mutans* isolates was molecularly examined. All 20 samples had *cnm*, *Cbm*, and *gtfB*.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Swaney MH, Kalan LR. Living in your skin: Microbes, molecules, and mechanisms. Infect Immun 2021;89:10-128.
2. Bacali C, Vulturar R, Buduru S, Cozma A, Fodor A, Chiș A, *et al*. Oral microbiome: Getting to know and befriend neighbors, a biological approach. Biomedicines 2022;10:671.
3. Kumar PS, Dabdoub SM, Ganesan SM. Probing periodontal microbial dark matter using metataxonomics and metagenomics. Periodontology 2000 2021;85:12-27.
4. Mosaddad SA, Tahmasebi E, Yazdanian A, Rezvani MB, Seifalian A, Yazdanian M, *et al*. Oral microbial biofilms: An update. Eur J Clin Microbiol Infect Dis 2019;38:2005-19.
5. Ali AB. Antibacterial pattern of lactic acid bacteria on Streptococcus mutans isolates from dental caries. Med J Babylon 2015;1:2015.
6. Mathur VP, Dhillon JK. Dental caries: A disease which needs attention. Indian J Pediatr 2018;85:202-6.
7. Nathaniel NJ. Dental caries and salivary secretion: The correlation? (Doctoral dissertation, Department of Physiology, Federal University of Technology, Akure).
8. Bandekar S, Patil S, Dudulwar D, Moogi PP, Ghosh S, Kshirsagar S. Remineralization potential of fluoride, amorphous calcium phosphate-casein phosphopeptide, and combination of hydroxylapatite and fluoride on enamel lesions: An: in vitro: comparative evaluation. J Conservat Dent 2019;22:305-9.
9. Alejandra BM, Daniel OM. Virulence factors of Streptococcus mutans related to dental caries. Staphylococcus Streptococcus 2020;11:9.
10. Wang Z, Zhou Y, Han Q, Ye X, Chen Y, Sun Y, *et al*. Synonymous point mutation of gtfB gene caused by therapeutic X-rays exposure reduced the biofilm formation and cariogenic abilities of Streptococcus mutans. Cell Biosci 2021;11:91.
11. Nakano K, Nomura R, Shimizu N, Nakagawa I, Hamada S, Ooshima T. Development of a PCR method for rapid identification of new Streptococcus mutans serotype k strains. J Clin Microbiol 2004;42:4925-30.
12. Maasi G, Štšepetova J, Jõesaar M, Olak J, Mändar R. Different patterns of virulence genes in Streptococcus mutans and Streptococcus sobrinus originating from Estonian Toddlers—Mothers Cohort. Microbiol Res 2022;13:928-36.
13. Chen X, Daliri EB, Kim N, Kim JR, Yoo D, Oh DH. Microbial etiology and prevention of dental caries: Exploiting natural products to inhibit cariogenic biofilms. Pathogens 2020;9:569.
14. Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. Int Endod J 2019;52:949-73.
15. Sadeghinejad L, Cvitkovitch DG, Siqueira WL, Merritt J, Santerre JP, Finer Y. Mechanistic, genomic and proteomic study on the effects of BisGMA-derived biodegradation product on cariogenic bacteria. Dent Mater 2017;33:175-90.
16. Guan C, Che F, Zhou H, Li Y, Li Y, Chu J. Effect of rubusoside, a natural sucrose substitute, on Streptococcus mutans biofilm cariogenic potential and virulence gene expression in vitro. Appl Environ Microbiol 2020;86:e01012-20.
17. Al-mohammadawy ZH, Aljarah AK, Saad AM. Isolation and identification of Streptococcus mutans from dental caries by using Sm479 gene. Indian J Pub Health Res Dev 2018;9:639.
18. Al-nafaee AK, Al-warid RJ, Abeas KA. Study the effect of a fixed orthodontic appliance on the oral microbial cavity. Med J Babylon 2023;20:168-74.
19. Al-Amin NT, Al-Jubouri YH, Shaker AP. Isolation and identification of dental caries bacteria and study of their sensitivity to some antibiotics and nanoparticles. World Bull Public Health 2022;7:43-54.

20. Shehab EY, Abdullah BA, AlTaie AA. Phenotypic identification of Streptococcus mutans and attendant bacteria from dentin caries and Necrotic pulp, and its correlation with caries activities tests. Med J Babylon 2024;21(Suppl 2):S189-S194.
21. Franco-Duarte R, Černáková L, Kadam S, Kaushik K S, Salehi B, Bevilacqua A, *et al*. Advances in chemical and biological methods to identify microorganisms—From past to present. Microorganisms 2019;7:130.
22. Hmood AM, Al-Shukri MSM, Al-Charrakh AH. Molecular detection and antimicrobial resistance of clostridium perfringens isolated from diabetic patients and bullet wounds. J Appl Biol Biotechnol 2019;7:54-59.
23. Pinheiro BG, Hahn RC, Camargo ZP, Rodrigues AM. Molecular tools for detection and identification of Paracoccidioides species: Current status and future perspectives. J Fungi (Basel, Switzerland) 2020;6:293.
24. Sabat AJ, van Zanten E, Akkerboom V, Wisselink G, van Slochteren K, de Boer RF, *et al*. Targeted next-generation sequencing of the 16S-23S rRNA region for culture-independent bacterial identification-increased discrimination of closely related species. Sci Rep 2017;7:3434.
25. Araújo Alves L, Ganguly T, Mattos-Graner RO, Kajfasz J, Harth-Chu EN, Lemos JA, *et al*. CovR and VicRKX regulate transcription of the collagen binding protein Cnm of Streptococcus mutans. J Bacteriol 2018;200:10-128.
26. Mallya PS, Mallya S. Microbiology and clinical implications of dental caries—A review. J Evol Med Dent Sci 2020;9:3670-5.
27. Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K. Contribution of severe dental caries induced by Streptococcus mutans to the pathogenicity of infective endocarditis. Infect Immun 2020;88:10-128.
28. OmerOglou E, Karaca B, Kibar H, Haliscelik O, Kiran F. The role of microbiota-derived postbiotic mediators on biofilm formation and quorum sensing-mediated virulence of Streptococcus mutans: A perspective on preventing dental caries. Microb Pathog 2022;164:105390.
29. Jakubovics NS, Goodman SD, Mashburn-Warren L, Stafford GP, Cieplik F. The dental plaque biofilm matrix. Periodontology 2000. 2021;86:32-56.
30. Garcia BA, Acosta NC, Tomar SL, Roesch LFW, Lemos JA, Mugayar LRF, *et al*. Association of Candida albicans and Cbp+ Streptococcus mutans with early childhood caries recurrence. Sci Rep 2021;11:10802.
31. Matsumoto-Nakano M. Role of Streptococcus mutans surface proteins for biofilm formation. Japan Dent Sci Rev 2018;54:22-9.
32. Zamperini CA, Aydin B, Sroussi HY, Bedran-Russo AK. In vitro study of the role of human neutrophil enzymes on root caries progression. Caries Res 2021;55:99-107.
33. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, *et al*. The biology of Streptococcus mutans. Microbiol Spect 2019;7:10-128.
34. Lozano Moraga C, Díaz Garrido N, Kreth J, Giacaman RA. Streptococcus mutans and Streptococcus sanguinis expression of competition-related genes, under sucrose.
35. Wasfi R, Abd El-Rahman OA, Zafer MM, Ashour HM. Probiotic Lactobacillus sp. inhibit growth, biofilm formation and gene expression of caries-inducing Streptococcus mutans. J Cell Mol Med 2018;22:1972-83.
36. Xu RR, Wang B. An update on the evolution of glucosyltransferase (Gtf) genes in Streptococcus. Front Microbiol 2018;9:417544.
37. Zhang Q, Ma Q, Wang Y, Wu H, Zou J. Molecular mechanisms of inhibiting glucosyltransferases for biofilm formation in Streptococcus mutans. Int J Oral Sci 2021;13:30.
38. Rezaei T, Mehramouz B, Gholizadeh P, Yousefi L, Ganbarov K, Ghotaslou R, *et al*. Factors associated with Streptococcus mutans pathogenicity in the oral cavity. Biointerface Res Appl Chem 2023;13:368.
39. Jurakova V, Farková V, Kucera J, Dadakova K, Zapletalova M, Paskova K, *et al*. Gene expression and metabolic activity of *Streptococcus mutans* during exposure to dietary carbohydrates glucose, sucrose, lactose, and xylitol. Mol Oral Microbiol. 2023;38:424-41.