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RESEARCH ARTICLE OPEN ACCESS

PCR-Based investigation of virulence genes in Streptococcus sobrinus isolated from dental caries

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Abstract
Objective: Streptococcus sobrinus is a highly cariogenic, acidogenic, and strong biofilm-forming species. This may be the initial study to report the prevalence of eno1, atl, and S1 serine protease genes in S. sobrinus from Iraqi patients.
Methods: Carious tooth samples were obtained from patients between 6 and 60 years. Bacteria were isolated by conventional culture methods and were characterised by Gram staining, colony morphology, and biochemical properties. Twenty S. sobrinus isolates were additionally tested by PCR using primers specific for eno1 atl, and s1 family serine protease. The PCR products were examined by agarose gel electrophoresis.
Results: Bacterial growth was observed in 90 (81%) of the 110 samples. S. sobrinus was the most common (22.2%), followed by Lactobacillus spp., Enterococcus spp., Fusobacterium spp., and other species. The S. sobrinus isolates were Gram-positive, catalase-positive, non-motile, with sticky mucoid colonies. The eno1 gene was present in 7 of the 20 isolates (35%) as determined by PCR, indicating strain variation in distribution. The atl gene was present in 17 isolates (85%), and the S1 family serine protease gene, which was present in all the tested isolates (20; 100%), was conserved.
Conclusion: This research emphasises the prevalence of S. sobrinus in carious lesions and the variation of virulence gene distribution. The ubiquitous presence of S1 protease and the high incidence of atl suggest that they are fundamental to pathogenicity, although the presence of a percentage of eno1 implies genetic diversity. Identified virulence genes may guide early diagnostics and targeted preventive strategies for dental caries.

Keywords: Streptococcus sobrinus, Virulence genes, eno1, atl, S1 serine protease, Dental caries, PCR

Plain English Summary

Tooth decay is a complex disease where a group of bacterial communities play an important role in the infection's persistence and resistance to treatment. Streptococcus sobrinus highly causes tooth decay. This is among the first studies to culture and molecularly identify bacteria from dental caries lesions in this region. Bacterial growth was observed in 81% of the total sample. Streptococcus sobrinus was commonly found (22%). Protein-coding gene (the atl gene) was found in 85% of the isolates, and a group of enzymes (S1 family serine protease gene) was present in all the tested isolates. This study reiterated the presence of Streptococcus sobrinus in cavities and the variation of how infectious bacteria are in tooth decay.

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Introduction

Dental caries is a multifactorial, biofilm-mediated disease characterised by the localised destruction of dental hard tissues due to the acidogenic and aciduric activity of cariogenic microorganisms. Despite advances in preventive dentistry, dental caries remains one of the most prevalent chronic infectious diseases globally, affecting individuals of all ages and posing a significant public health burden (1). Among the microbial agents implicated, Streptococcus mutans has historically been recognised as the principal pathogen. However, accumulating molecular and clinical evidence underscores the equally critical role of Streptococcus sobrinus in the aetiology and progression of caries, particularly in populations with high caries risk, such as children, elderly individuals, and immunocompromised patients (2). S. sobrinus is a non-motile, facultatively anaerobic, Gram-positive coccus- a member of the mutans streptococci. It produces high acid (acidogenativity), acid resistance (aciduricity), and extracellular polysaccharide (EPS), which increases its potential to form biofilm and to adhere to the teeth. Unlike S. mutans, S. sobrinus is not naturally competent for horizontal gene transfer, resulting in less genetic diversity and evolutionary disconnectedness (3). This genetic distinctness may impact variation in virulence phenotypes, disease presentation, and the ability to survive under challenging environmental conditions.

Molecular epidemiological studies have demonstrated that S. sobrinus is commonly involved in more severe carious lesions and accelerating lesion progression, particularly in smooth-surface caries of primary teeth. In addition, co-infection with S. mutans and S. sobrinus exhibits a synergistically greater cariogenic potential than infection with S. mutans alone (4). These results have also generated growing attention from researchers in the molecular analysis of S. sobrinus, especially focusing on its virulence-related genes.

With the novel methods of molecular biology, in particular polymerase chain reaction (PCR) methods, it is now feasible to detect and quantify with high specificity and sensitivity specific bacterial species and the corresponding virulence factors. PCR-based methods to detect a variety of virulence genes of S. sobrinus, such as eno1 (encoding enolase), atl (encoding autolysin), and members of the serine protease gene family, have been tested (5, 6). These genes have a role in the key virulence functions (adhesion, metabolic adaptation, autolysis, biofilm production, and escape from the immune system) of the pathogen.

The eno1-encoded enolase is both a glycolytic enzyme of paramount importance and a surface adhesin that favours bacterial binding to host tissues and salivary factors. Its bimodal function as a metabolic and adhesive factor makes it an important molecular marker in virulence investigations (7). In contrast, the product of the atl gene, autolysin, is an enzyme involved in the degradation of the cell wall, which in turn also contributes to the structuring of biofilm and the integrity of the biomass, and its expression has been positively associated with increased biofilm density and biofilm structure (8). In addition, serine proteases, especially those belonging to the S1 family, are also known to be associated with the degradation of host tissue, immune modulation, and nutrient supply, which also highlights their importance to bacterial pathogenicity (9).

It is important to understand the genetic foundation of S. sobrinus virulence, not only to understand its pathogenesis, but also for the development of specific diagnostics and therapeutics. Despite its clinical significance, S. sobrinus is relatively less well-studied than S. mutans, at least in part because this organism is significantly less amenable to cultivation and genetic manipulation. Thus, further molecular analysis is needed to define its virulence arsenal and evaluate its epidemiological relevance in various populations (10).

Recent studies have shown that different strains of S. sobrinus have unique virulence gene patterns. Interestingly, they tend to carry more of certain glucosyltransferase genes, which might explain why they could be more likely to cause cavities compared to S. mutans (11). This emphasises how important it is to look more into S. sobrinus, especially since its different gene makeup might play a big role in how dental cavities develop, especially in communities where this bacterium is common. Despite its recognised importance in clinical settings, there remains a scarcity of data regarding the distribution of virulence genes of *S. sobrinus* among Middle Eastern populations. This gap limits our comprehensive understanding of regional differences in the pathogen's epidemiology.

The goal of the present study was to assess the prevalence and distribution of selected virulence genes (eno1, atl, and S1 family serine protease) in S. sobrinus strains isolated from patients with dental caries. This study aims to explain the molecular basis of the cariogenic potential of S. sobrinus, using routine PCR for further decay-preventive mechanisms. We hypothesised that atl

and S1 protease genes are conserved, while eno1 distribution varies among strains

Materials and Methods

Sample Collection

The individuals participating in this study were diagnosed with dental caries through clinical assessment. They had not received antibiotic treatment nor undergone any dental procedures within the preceding four weeks. Besides, they had no history of widespread conditions known to influence the oral microbiota.

A total of 110 decayed teeth were obtained from male and female patients with age ranges of 6–60 years who attended Al-Mishkhab General Hospital and dental clinic in Al-Najaf province from September 1 to November 15/2024. Teeth were aseptically extracted and then immediately transferred into sterile tubes with Brain Heart Infusion Broth (BHI) for transportation and maintenance at 4°C before the processing (12).

Culturing and Bacterial Isolation

Samples were inoculated into Nutrient Broth and incubated at 37°C for 24 hours to enrich bacterial growth. Subcultures were performed on Blood Agar and MacConkey Agar to isolate Gram-positive and Gram-negative bacteria, respectively. The media were prepared according to manufacturer guidelines (HiMedia, India) and sterilised using an autoclave at 121°C, 15 psi for 15 minutes (13, 14).

Identification of Isolates

Bacterial isolates were preliminarily identified by Gram staining using commercial kits (ATOM, Japan) and confirmed via morphological characteristics and biochemical tests, including:

Oxidase test: using freshly prepared tetramethyl-p-phenylenediamine dihydrochloride solution (15).

Catalase test: using 3% hydrogen peroxide solution (16, 17).

Urease test: performed on Urea Agar Slants incubated at 37°C for 24 hours (18).

Haemolysis test: evaluated on Blood Agar plates after 24h incubation (13).

Motility and fermentation tests: using semi-solid media as described by MacFaddin (13).

Biochemical profiles were compared with standard references and supported by the Vitek-2 compact system (Biomerieux, France) for confirmatory identification (16).

Preservation of Isolates

For short-term storage, isolates were maintained on slanted Nutrient Agar at 4°C. For long-term preservation, pure cultures were stored in BHI broth with 15% glycerol at –20°C (12, 19).

Genomic DNA Extraction

To uphold representativeness without compromising practicality, twenty isolates of S. sobrinus were chosen at random from the ninety positive cultures for PCR analysis. This approach assisted in managing molecular processing and enabled an initial evaluation of the distribution of virulence genes within this population. Genomic DNA was extracted from the 20 selected *S. sobrinus* isolates using the Geneaid DNA extraction kit (Taiwan) following the manufacturer's protocol. DNA quality and purity were measured using a NanoDrop Spectrophotometer (ThermoFisher, USA) at 260/280 nm. Pure DNA had an absorbance ratio close to 1.8 (20, 21).

Primer Design and PCR Amplification

Primers targeting the virulence genes were synthesised by IDT (Canada) based on sequences obtained from NCBI GenBank. The primer sequences, expected product sizes, and annealing temperatures are presented in Table 1.

Table 1. Primer sequences, product sizes, and annealing temperatures

Gene	Primer Type	Primer Sequence (5′–3′)	Product Size (bp)	Annealing Temp (°C)
eno1	Forward	TTGGCGCACCAACATTCAAG	688	59°C
	Reverse	CGTGACAGTGAGCCTGTCTT		
Atl	Forward	CTTTGCCGTTTGGTCCAAGG	497	58°C
	Reverse	CAGTTTGTTGAACCGCCTGG		
S1 serine protease	Forward	ATTCTCAAGCCAAGGCTGCT	675	60°C
	Reverse	AGCTTGCGCTGCTTAAATCG		

PCR reactions were performed using 2× PCR Taq Master Mix (ABM, Korea). Each 25 µL reaction mixture included 12.5 µL of master mix, 1 µL each of forward and reverse primers (10 pmol/µL), 2 µL of template DNA, and 8.5 µL of nuclease-free water. Amplification was carried out in a PCR thermal cycler (Techne, UK) using gene-specific

cycling conditions based on individual annealing temperatures (Table 2) (22, 23, 24, 25) as follows:

Table 2. PCR Cycling Conditions for Virulence Gene Amplification

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	35
Annealing (eno1)	59°C	30 seconds	
Annealing (atl)	58°C	30 seconds	
Annealing (S1)	60°C	30 seconds	
Extension	72°C	45 seconds	
Final Extension	72°C	7 minutes	1

Positive and negative controls were included in each PCR run to validate the specificity and accuracy of amplification: a reference S. sobrinus strain served as the positive control, and nuclease-free water was used as the negative control.

Gel Electrophoresis

PCR products were resolved on 1.5% agarose gel prepared with TBE buffer (Bio Basic, Canada) and stained with ethidium bromide (1 µg/mL). A 3000 bp DNA ladder (Bioneer, Korea) was used to determine the product sizes. Gels were visualised using a UV transilluminator (UVP, USA) (21, 24).

Results

Sample Collection and Bacterial Growth

A total of 110 decayed teeth were collected from patients of both genders, aged 6 to 60 years. The samples were immediately transferred into tubes containing nutrient broth and stored under refrigerated conditions.

Following microbiological culturing procedures, 90 out of 110 samples (81%) exhibited positive bacterial growth, while the remaining 20 samples (18%) did not show any growth on culture media (Table 3). These results indicate a high prevalence of oral bacterial colonisation in carious lesions.

Table 3. Bacterial growth in dental caries samples

Parameter	Value
Total samples collected	110
Samples with bacterial growth	90 (81.8%)
Samples with no bacterial growth	20 (18.2%)
Total bacterial isolates	90
Gram-positive isolates	60 (66.7%)
Gram-negative isolates	30 (33.3%)

Isolation and Identification of Bacterial Species

All isolates of the 90 samples that exhibited bacterial growth were subjected to phenotypic identification using selective and differential culture media, as well as biochemical tests. Most isolates (67%) were Gram-positive, with Streptococcus sobrinus being the most frequently isolated species (22.2%, 20 isolates), followed by Lactobacillus spp. (16.6%), Enterococcus spp. (14.4%), and Streptococcus mutans (10%).

Gram-negative species were less prevalent, with Prevotella spp. (8.9%) and Fusobacterium spp. (12.2%) representing notable contributors. A limited number of Staphylococcus aureus isolates (3.3%) were also identified (Table 4).

Table 4. Bacterial species isolated from dental caries patients

Bacterial Species	No. of Isolates	Percentage (%)
Streptococcus sobrinus	20	22.2
Streptococcus mutans	9	10.0
Enterococcus spp.	13	14.4
Fusobacterium spp.	11	12.2
Lactobacillus spp.	15	16.6
Actinomyces spp.	11	12.2
Staphylococcus aureus	3	3.3
Prevotella spp.	8	8.9

Total	90	100.0

Phenotypic Characteristics of Streptococcus sobrinus

Colonies of *S. sobrinus* grown on blood agar were white to milky, convex, shiny, and smooth, with a mucoid, sticky appearance that increased in adherence over time. The isolates exhibited non-motile behaviour, with no signs of haemolysis. Biochemical profiling revealed that the isolates were Gram-positive, catalase-positive, oxidase-negative, urease-negative, and coagulase-negative, consistent with standard diagnostic criteria (Table 5).

Table 5. Biochemical tests for *S. sobrinus* isolates

Test	Result
Gram Stain	+
Oxidase Test	-
Catalase Test	+
Urease Test	-
Motility Fermentation Test	-
Haemolysis Test	-

Molecular Detection of Virulence Genes in Streptococcus sobrinus

PCR amplification was employed to detect three key virulence genes (*eno1*, *atl*, and *S1 family serine protease*) in 20 clinical isolates of *Streptococcus sobrinus*. The resulting amplicons were visualised on 1.5% agarose gels stained with ethidium bromide and examined under UV illumination.

Detection of the eno1 Gene

The eno1 gene of the protein under investigation was multidirectional (glycolysis enzyme and bacterial adhesion), and was present in 7/20 strains of S. sobrinus, 35% of which were detected. As shown in Figure 1, positive amplification products were obtained from lanes 1, 2, 4, 7, 11, 17, and 18, and each specific band for 688 bp was observed. The other 13 isolates were negative, and the result suggests that the gene was not present in these isolates or was expressed at a level below the detection limit of the PCR assay. These findings indicate that eno1 is not commonly present in all strains of S. sobrinus, which may be related to strain-dependent properties like enhanced adhesion levels in the oral environment.

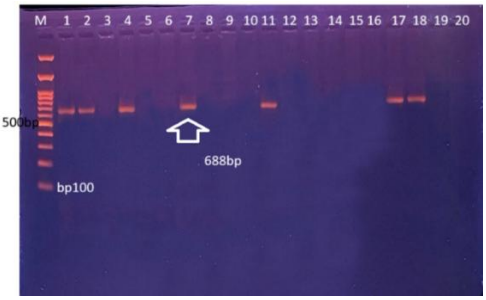

Figure 1: Agarose gel electrophoresis showing PCR amplification of the eno1 gene in Streptococcus sobrinus isolates. Bands are visible at 688 bp in lanes 1, 2, 4, 7, 11,17 and 18. Lane M: 3000 bp DNA ladder

Detection of the atl Gene

The gene encoding autolysin required for bacterial cell division, peptidoglycan turnover, and biofilm formation21 was found in 17/20 (85%) of S. sobrinus. As shown in Figure 2, lanes 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, and **20** had positive amplifications, and each showed a distinct band at 497 bp, the expected product size. No signal in lanes **4**, **18** and **19**. These data suggest that even though atl is highly conserved among S. sobrinus strains, it was not found in all strains, and variation may be a result of genetic polymorphism, primer mismatch, or loss of the gene in a minority of clinical isolates.

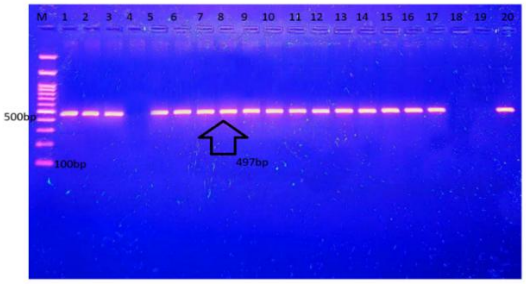

Figure 2: Agarose gel electrophoresis showing PCR amplification of the *atl* gene in *Streptococcus sobrinus* isolates. Clear bands at 497 bp are observed in lanes 1, 2, 3, 5–11, 13–17, and 20. No amplification bands are seen in lanes 4, 18, and 19. Lane M: 3000 bp DNA ladder.

Detection of the S1 Family Serine Protease Gene

In addition, the protease gene of S1 family serine protease was present in all isolates (20/20, 100%), and this gene is a proven core virulence factor in the streptococci species of S. sobrinus. Positive amplifications are shown in lanes 1-20, all showing a single strong and uniform band at an estimated size of ~675 bp (Figure 3). This gene encodes a serine protease enzyme for host protein degradation, immunoevasion, and nutrient acquisition, further demonstrating its significance in the survival and pathogenicity of S. sobrinus in the oral cavity.

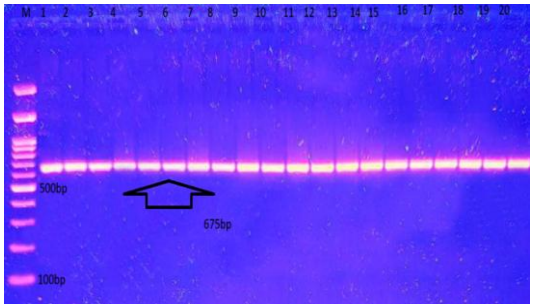

Figure 3: PCR amplification of the S1 family serine protease gene from Streptococcus sobrinus isolates. Strong bands at 675 bp are observed across all lanes. Lane M: 3000 bp DNA ladder

Overall, the molecular analysis showed that the S1 family serine protease gene was universally present in all isolates, while the atl was identified in almost all (85%), and the eno1 gene was found only in a subset of isolates (35%). This pattern might reflect genetic polymorphism and strain-level diversity of the virulence genes among S. sobrinus populations (Table 6)

Table 6. Summary of Gene Detection Results in *Streptococcus sobrinus* Isolates

Gene	Product Size (bp)	No. of Positive Samples	Detection Rate (%)
eno1	688	7 / 20	35%
Atl	497	17 / 20	85%
S1 family serine protease	675	20 / 20	100%

Discussion

Bacterial Growth from Clinical Samples

Out of 110 decayed teeth examined, 90 samples (81%) demonstrated bacterial growth, indicating widespread microbial colonisation of carious lesions. This high positivity rate is in line with findings from Tanner et al. (2011), who reported similar rates of cultivable bacteria in advanced caries, particularly in populations with poor oral hygiene (26). The absence of growth in 10% of cases may be linked to recent antibiotic exposure or the limitations of conventional culture techniques, which often fail to recover obligate anaerobes or fastidious organisms present in deeper dentin layers (27).

Distribution of Bacterial Isolates

Streptococcus sobrinus was the most frequently isolated species (22.2%), surpassing even *S. mutans* (10%), which is traditionally regarded as

the primary cariogenic agent. This supports studies suggesting that *S. sobrinus* plays a more aggressive role in smooth surface caries and is often associated with rapid lesion progression (28). Its presence, especially in paediatric samples, has been correlated with higher caries indices. The co-isolation of *Lactobacillus spp.* and *Enterococcus spp.* reflects the dynamic and polymicrobial ecology of caries, where both acidogenic and aciduric species work synergistically to sustain the cariogenic biofilm (29).

The increased prevalence of *S. sobrinus* relative to *S. mutans* within this cohort may be influenced by regional dietary habits that are high in fermentable carbohydrates, coupled with inadequate oral hygiene practices and environmental factors that promote the colonisation and persistence of *S. sobrinus* in the oral environment.

Phenotypic and Biochemical Characterisation of S. sobrinus

The observed colony morphology of *S. sobrinus,* mucoid, convex, and adhesive, is typical for strains with enhanced biofilm-forming capability. This correlates with findings by Song et al. (2021), who demonstrated that biofilm density in *S. sobrinus* was strongly associated with surface stickiness and EPS production (30). Biochemical profiling confirmed catalase positivity and oxidase negativity, consistent with previous descriptions of mutans streptococci. These traits support the organism's adaptation to acidic environments and its survival in mature plaque, where pH can fall below 5.5 for extended periods (31).

Detection of the eno1 Gene

The eno1 gene, encoding enolase, was detected in only 7 out of 20 *Streptococcus sobrinus* isolates, corresponding to a 35% detection rate. Enolase is a well-characterised moonlighting protein that performs both glycolytic and surface adhesion functions. In its cytoplasmic role, it catalyses the reversible conversion of 2-phosphoglycerate to phosphoenolpyruvate. On the cell surface, enolase binds to host extracellular matrix components such as plasminogen, promoting bacterial adhesion, colonisation, and tissue invasion (32).

The partial presence of this gene among *S. sobrinus* isolates suggests that eno1 is not a core virulence factor, but rather an accessory gene potentially associated with enhanced adhesion capabilities in specific strains. These observations are in line with previous reports highlighting the presence of a substantial strain-level genetic diversity found in S. sobrinus, especially genes involved in surface structure and host-interaction (33). Gera et al. (2020), enolase expression was associated with upregulation of biofilm mass and aciduricity in mutans streptococci, which could potentially be responsible for the higher cariogenic virulence of eno1-positive strains (34).

The differences observed in eno1 detection may also be indicative of variations in gene regulation depending on the environment, which is known to alter adhesin expression following changes in pH, nutrient conditions, and even immune pressure from the host (35). In addition, enolase has been identified as a surface-located antigen and could therefore serve as a diagnostic or immunological marker, although its non-universal distribution may preclude its use as a universal molecular target.

Collectively, these results suggest that although eno1 is important for virulence in some strains, it is not critical for S. sobrinus colonisation or persistence. But its presence may correspond to higher adhesion strength or competitive advantage in the early stages of caries than expected.

Although this investigation revealed a notably high prevalence of the eno1 gene among S. sobrinus isolates, it is important to recognise that prior research conducted in diverse populations, such as those in East Asia and Europe, has documented relatively lower detection rates (11). This pattern suggests potential variations influenced by geographic factors or host-related differences in the distribution of virulence genes.

Detection of the atl Gene

The presence of the autolysin gene atl was identified in all S. sobrinus isolates, indicating its conserved status among the cariogenic strains. Autolysins are involved in cell wall remodelling, daughter cell separation, and biofilm formation, which are required for bacterial colonisation and survival in the oral cavity (36).

The universal presence of atl supports its classification as a core virulence gene, analogous to the atlA gene described in *S. mutans*.

In addition to facilitating structural development, autolysins contribute to biofilm maturation through the release of surface-bound proteins and extracellular DNA (eDNA). These components enhance biofilm cohesion and promote resistance to antimicrobial agents and environmental stressors. Studies have shown that disruption of autolysin genes in *S. mutans* leads to reduced biomass, structural disorganisation, and attenuated cariogenicity in animal models (37).

Given the close genetic relationship between *S. sobrinus* and *S. mutans*, it is likely that atl in *S. sobrinus* functions similarly. Its consistent presence in all clinical isolates further suggests that atl is

essential for plaque establishment, especially under the acidic and competitive conditions of dental biofilms. Furthermore, autolysins may also serve as antigenic targets for host immune recognition, although *S. sobrinus* may deploy protective mechanisms to shield them from detection.

Detection of the S1 Family Serine Protease Gene

The S1 family serine protease gene was also detected in 100% of *S. sobrinus* isolates, signifying its conserved and likely indispensable role in virulence. S1 serine proteases facilitate host protein degradation, immune system modulation, and tissue invasion, functions critical for bacterial survival and pathogenicity (38). These enzymes contribute to immune evasion by cleaving immunoglobulins and complement proteins, effectively disrupting host defence pathways.

Moreover, S1 proteases assist in nutrient acquisition, especially in nutrient-depleted environments such as mature dental plaque, by breaking down host-derived peptides and salivary glycoproteins (39). In *S. pyogenes* and *S. pneumoniae*, homologous S1 enzymes have been shown to promote colonisation and increase disease severity in animal infection models. While functional studies in *S. sobrinus* are limited, the detection of this gene in all isolates supports its role as a key effector in oral pathogenicity.

Proteomic analyses have confirmed that S1 proteases are often surface-associated or secreted, making them accessible to the host immune system. This localisation may render them attractive vaccine candidates or targets for structure-based drug development (40). Their highly conserved catalytic domains also provide potential for selective inhibition, minimising off-target effects on human proteases. Therefore, the ubiquitous detection of S1 protease presented here is not only indicative of the biophysiological importance dominated by S1 but also proves the potential to use this enzyme as a molecular tool for diagnostic and therapeutic purposes.

The distribution of genes reported in this study indicates a genomic hierarchy for virulence genes in S. sobrinus. Although the atl and S1 protease genes were present in all isolates, representing core virulence determinants, the eno1 gene was detected in 30% of the isolates, indicating that it may be an accessory virulence factor and provide a selective advantage in certain conditions as adhesion or colonisation.

This differentiation between conserved and variable genes carries some important implications. Diagnostically, the presence of atl and S1 in molecular detection panels may increase sensitivity and specificity, whereas detection of eno1 might provide additional value in diagnostic of hyper-virulent strains related to severe or recurrent caries.

From a scientific point of view, gene regulation is an important concept. Further studies to analyse transcriptional profiles of such virulence genes under various other environments, such as acidic pH, sugar, and oxidative stress, would help to clarify the extent of environmental signals that are integrated into the expression of virulence. Real-time PCR, RNA sequencing, and proteomic analyses can be used to determine whether the presence of genes involves functional activity in vivo.

Last, these results corroborate the requirement to incorporate multi-gene PCR methods into diagnostic investigations and to address the intra-species variability of virulence genes when developing vaccines or targeted interventions for high-risk groups. The ubiquitous presence of the S1 serine protease gene among S. sobrinus isolates highlights its potential as a diagnostic biomarker and a therapeutic target, as inhibitors of bacterial serine proteases may disrupt tissue degradation and biofilm formation central to caries progression

Study limitations

While this study provides valuable insights, it is important to recognise certain limitations. Firstly, only 20 out of the 90 *S. sobrinus* isolates were subjected to PCR analysis, which may restrict the broader applicability of the findings regarding virulence gene prevalence. Secondly, the study did not include sequencing of the PCR amplicons to verify the specific identity of the amplified gene fragments, potentially allowing for non-specific amplification and uncertainty in results. Besides, primer bias cannot be entirely discounted, as differences in primer efficiency could affect amplification success across different strains. Lastly, the investigation was confined to three virulence genes, notably omitting other important factors such as the gtf and gbp gene families, both of which are recognised for their roles in cariogenicity. Future research with larger sample sizes and more comprehensive gene panels is advisable to enhance understanding and accuracy.

Conclusion

Our study contributes to evidence at the molecular level of the presence of streptococcal virulence genes, eno1, atl, and S1 family serine protease, in clinical Streptococcus sobrinus isolates from dental

caries patients. The presence of atl and S1 protease genes in all isolates indicates their genomic conservation and indispensability for biofilm formation, tissue penetration, and immune evasion. In contrast, with the eno1 gene detected only partially in about 30% of isolates, it appears to be an auxiliary factor that would enable adhesion only in certain strains. These results support the hierarchical model of a virulence gene locus in S. sobrinus as both core- and strain-specific virulence determinants are identified. The use of gene-specific PCR protocols was efficient in describing the genetic virulence profile and could be used as an important tool for initial diagnosis, risk assessment, and epidemiological surveillance of cariogenic species.

List of Abbreviations

Atl: Encoding Autolysin
eDNA: Extracellular DNA
eno1: Encoding Enolase
EPS: Extracellular Polysaccharide
PCR: Polymerase Chain Reaction

Declarations

Ethics considerations and consent to participate

This study was approved by the Institutional Review Board of [University of Al-Qadisiyah] (Approval No: 63/2024), and written informed consent was obtained from all participants or their legal guardians before sample collection."

Consent for publication

All the authors consented to publishing the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Nil.

Authors' contributions

KHSA is the idea inventor and study design, did the laboratory work, and data collection. KHSA participated in the interpretation and analysis of the data and drafted the manuscript. AKM reviewed, supervised the research and guidance of the methodology and PCR techniques, and critically reviewed the manuscript for intellectual content. AKM also contributed to the final approval of the published version.

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