

Association Between *Streptococcus Sobrinus* and *Helicobacter Pylori* within the 20-60 Year Age Range

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ABSTRACT

Objective: Dental caries and gum disease, along with their associated problems, harm human health. *Streptococcus sobrinus* bacteria are a major cause, forming the primary colonies in the oral cavity. The mouth is a key gateway to the digestive system, serving as a significant biological entry point for numerous microbes, including *Helicobacter pylori* (*H. pylori*) infection, which colonizes the gastric mucosa and causes serious upper gastrointestinal diseases. This study aimed to investigate the relationship between *S. sobrinus* and *H. pylori* infection. **Methods:** Nephrometric immunoassay was used to assess antibodies, and the study also focused on the VITEK2/AST-P592 system, and 16S rDNA (PCR). **Results:** The infection rate was 7 (63.6%) in females and 4 (36.4%) in males. Furthermore, the results of immunological evaluation for antibodies to *Helicobacter pylori* in 11 serum samples from patients diagnosed with *Streptococcus sobrinus* infection showed that males were more susceptible to double bacterial infection (75%), while the double infection rate in females was 71.4%. **Novelty:** *Streptococcus sobrinus* bacteria were first isolated and identified in Mosul, Iraq and identified as a new strain, HAA5, registered with the National Center for Biotechnology Information (NCBI).

INTRODUCTION

The changing conditions resulting from significant fluctuations in oral environmental parameters, along with human behavioral factors (such as dental hygiene, diet, and smoking), as well as genetic predisposition, age, and sex, contribute to the formation of a microbial system that plays a crucial role in human health. The mouth is an open ecosystem where nutrients and microbes are constantly exchanged. Humans have long suffered from oral diseases, such as tooth decay, which has existed since the dawn of civilization and remains one of the most common diseases worldwide. The oral cavity provides an environment for diverse and complex bacterial communities, most notably streptococci, including the dominant genus *Streptococcus sobrinus*, which is classified as a highly cariogenic bacterium. Its ability to produce acid and tolerate acidity is directly linked to its capacity to cause tooth decay. It is a type of opportunistic, spherical, Gram-positive bacterium belonging to the *Streptococcus mutans* group [1], [2], [3].

It possesses several mechanisms for adhering to teeth and forming biofilms, the most important of which are the SpaA protein and interaction with salivary proteins. It also possesses a large number of glucosyltransferase (GTF) enzyme genes responsible for the production of sticky glucans that bind sugars to teeth. In addition, this bacterium is a major cause of gingivitis and dental abscesses, especially in children and adults [4], [5].

Helicobacter pylori (*H. pylori*) is a bacterium that causes gastrointestinal diseases. It is a Gram-negative, spiral-shaped, flagellated bacterium belonging to the genus *Helicobacter* in the family *Helicobacteraceae*. This bacterium colonizes the gastric mucosa and causes a range of diseases, most notably chronic gastritis, gastric and duodenal ulcers, and may also be associated with gastric cancer. The mouth is the main route of transmission

Dental caries caused by *Streptococcus sobrinus* and *Helicobacter pylori* infection share similar risk factors, such as early childhood infection and low socioeconomic status. Therefore, it is possible that these two bacterial pathogens coexist in the same environment, supporting the idea that the mouth may be a reservoir for infection and increase the risk of recurrent infection. It has been hypothesized that infectious microbes of oral origin are involved in the development and progression of gastric cancer. However, the causal relationship between oral microbes and gastric cancer, and the role of *Helicobacter pylori* in this relationship, remains controversial [6], [7], [8], [9], [10].

This study focuses on the isolation and identification of *Streptococcus sobrinus*, the first bacterium isolated in Mosul, Iraq, and compares its relationship with *Helicobacter pylori* by detecting its specific antibodies in the serum of patients with oral diseases and dental caries. Our study was based on the molecular detection of two *Streptococcus sobrinus* genes, *gtf-T* and *gtf-L*, and the identification of a new strain in Mosul, Iraq.

RESEARCH METHOD

Ethics Committee Approval

We obtained approval from the Ninewa Health Directorate under Administrative Order No. 22744 dated June 10, 2024. were selected based on the following inclusion criteria: age between 20 and 60 years, having severe periodontitis and dental caries, and not having recently used antibiotics. Exclusion criteria included the absence of chronic diseases or immune disorders that could affect the study results

Bacterial Samples:

120 venous blood samples and oral swabs were collected from patients (with dental caries and periodontal sinusitis) who were seen at Al-Noor Specialized Dental Center and Al-Aysar Specialized Dental Center in Mosul, Iraq, between July 1, 2024, and February 1, 2025.

All oral swabs were collected using sterile swabs placed in sterile transport medium (Ames' medium). The samples were transported directly to the Department of Biology, College of Science, University of Mosul, where the bacteria were identified using conventional methods (Gram staining, hemolysis on blood agar and Mitis salivarius Agar (MSA) in the presence of carbon dioxide, oxidase assay, and the VITEK 2 diagnostic system with antibiotic susceptibility testing (from bioMérieux, France) to confirm the diagnosis of *Streptococcus sobrinus* in addition to molecular detection of 16srDNA [11], [12], [13].

DNA was extracted using the Presto™ Mini gDNA Bacteria Kit Quick Protocol (Geneaid, Taiwan).

Briefly, 20 µL of the master mix with 16S rRNA primers (27F, sequence 5'-GTTTGATCCTGGCTCAG-3', and reverse primer 1492R, sequence 5'-AA GGAGGTGATCCAGCC-3') and 20 µL of a 100-base-pair ladder were loaded separately into the wells of an electrophoresis device on a 1.5% agarose gel for 80 minutes at 80 V. Ultraviolet light was used to image the bands (1492 base pairs) using a thermally cyclized polymerase chain reaction (PCR) system (Eppendorf, Germany). The program includes heating at 94°C for 2 minutes, followed by 33 cycles (separation of the strands at 94°C for 30 seconds, then annealing at 60°C for the 16srDNA gene for 30 seconds, then elongation at 72°C for 30 seconds), and finally a final elongation for 3 minutes at 72°C. Subsequently, the 16srDNA gene sequencing products were transferred to a Hitachi 3130 gene analyzer in Korea, where the sequencing was performed at the Korea Research Institute of Life Science and Biotechnology (KRIBB), [14], [15], [16], [17].

Detection of gene glucosyltransferase (*gtf-I*) (*gtf-T*)

The primers used were designed in Table 1. The program included: chain separation at 95 °C for 3 minutes, followed by chain separation at 95 °C for half a minute, then annealing at 56 °C for half a minute, then elongation at 72 °C for half a minute, and finally final elongation at 72 °C for 5 minutes, Table (1) [18], [19].

Table 1. The primer used in the study.

Species	Primer	Sequence	Amplicon size (bp)
<i>S. sobrinus</i>	<i>gtf T-F</i>	5'-GAAACCAACCCAACCTTTAGCTTGGAT-3'	319
	<i>gtf T-R</i>	5'-ATGGAGTGATTTTCCATCGGTACTION-3'	(Sato <i>et al.</i> ,2003)
	<i>gtf I- F</i>	5'- GATAACTACCTGACAGCTGACT-3'	712 (Soni <i>et al.</i> ,
	<i>gtf I -R</i>	5'- AAGCTGCCTTAAGGTAATCACT-3'	2015)

National Center for Biotechnology Information (NCBI)

Registration with the National Center for Biotechnology Information (NCBI) is an important tool for improving diagnostics. Through this database, researchers can access accurate information about *Streptococcus sobrinus* bacteria and their antibiotic resistance, providing valuable insights for medical research.

Nephrometer Immunoassay Technique

Helicobacter pylori antibodies were measured using a Hipro Biotechnology Nephrometer immunoassay (NIA) to assess the associated immune response in patients with *Streptococcus sobrinus* infection. A fresh blood sample (5 mL) was drawn from these patients, collected in a gel tube for serological testing, and then centrifuged at 4000 rpm for 5 minutes. The serum was collected in a sterile Eppendorf tube and stored at -70°C until use [20], [21].

The *Helicobacter pylori* assay kit from Shijiazhuang Hipro BH250708 (China) was used according to the manufacturer's protocol and incubation instructions. Serum samples were loaded into the instrument's special transparent test tube at room temperature (R2). These tubes contain *Helicobacter pylori*-specific antigens, which bind

to antibodies (R1), if present, to form an immunological complex that produces light scattering (turbidity measurement). The instrument measures the intensity of the scattering using standard parameters and instrument-specific reagents. The results are read automatically and displayed on the screen. The resulting values are analyzed based on the standard calibration curve integrated into the software system [22].

RESULTS AND DISCUSSION

Results

Several bacterial samples belonging to the genus *Streptococcus* spp were identified, some of which were classified as *Streptococcus sobrinus* using conventional methods and the Vitek2 system. Subsequently, a limited number of isolates were selected for molecular analysis, sequencing, and gene expression (see Table 2, Figures 1 and 2).

Table 2. Summary of diagnostic results and demographic distribution of *Streptococcus sobrinus*.

Bacterial Samples	No.	Percentage (%)
<i>Streptococcus</i> spp.	72	60
Traditional methods for <i>Streptococcus sobrinus</i>	33	27.5
VITEK 2 system	21	17.5
16S rDNA and <i>gtf</i> gene	11	9.2
Total sample 120		

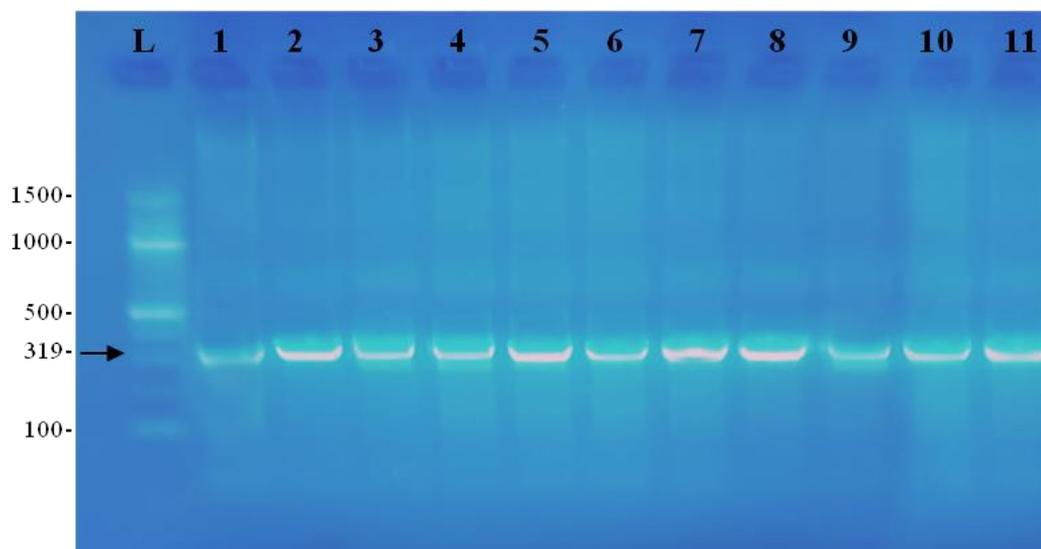


Figure 1. 16SRNA using primers, *gtf*-T from a selected group of *Streptococcus sobrinus* genomic DNA.

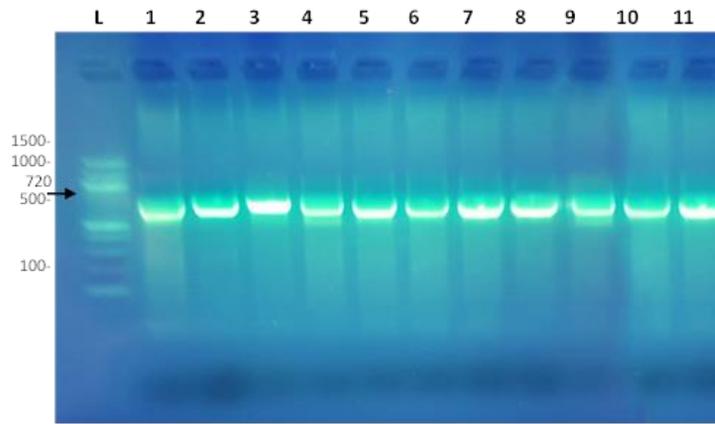


Figure 2. 16SRNA using primers, *gtf-I* from a selected group of *Streptococcus sobrinus* genomic DNA.

We recorded a new isolate in the NCBI database named (HAA5), marking the first such recording globally from Iraq. It obtained the serial number PQ814212.1(99%), as depicted in Figure 3.

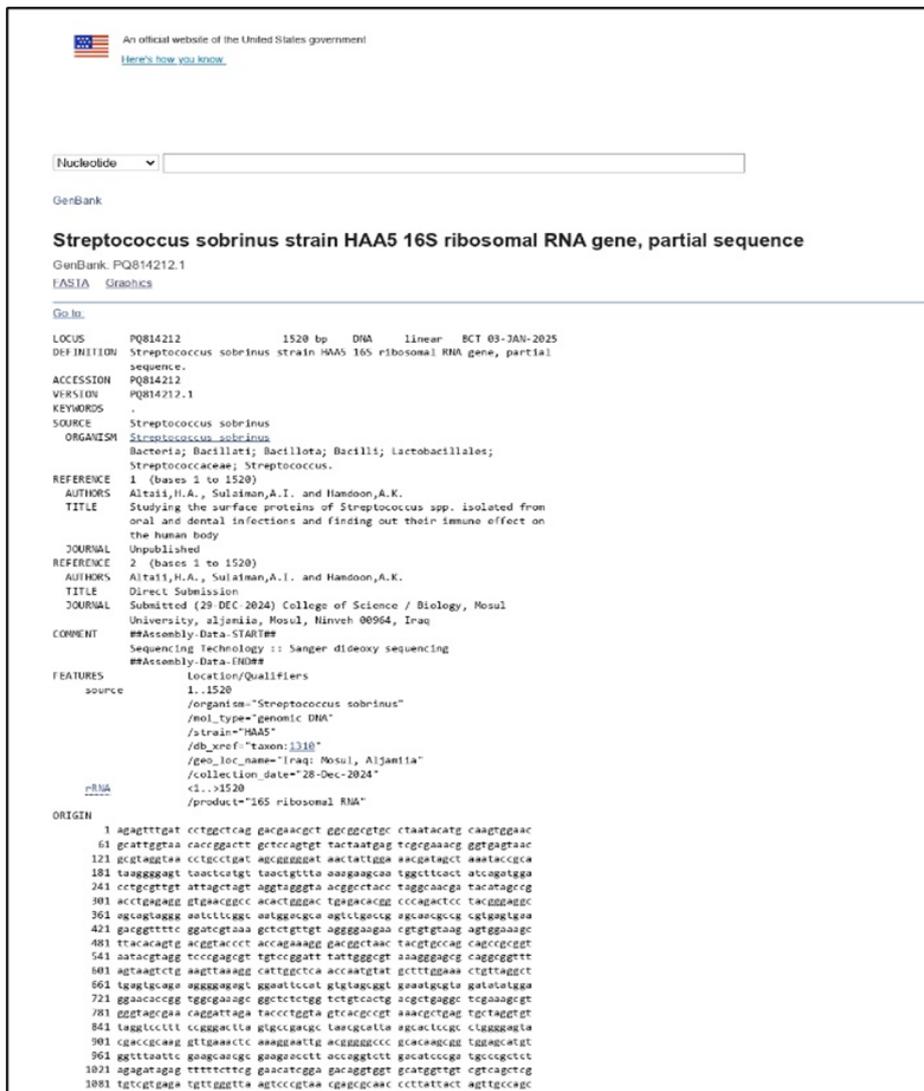


Figure 3. Gen Bank Registry Strain Ribosomal 16sRNA sequence HAA5 in *Streptococcus sobrinus*.

Antibiotic susceptibility testing using the VITEK® 2 AST-534 card showed that *Streptococcus sobrinus* isolates exhibited variability in their response to different antibiotics. High resistance rates were observed for some antibiotics, such as Ciprofloxacin and Sulfamethoxazole. In contrast, other antibiotics, such as Imipenem and Levofloxacin, showed high susceptibility rates, meaning they remain effective against the pathogens caused by these isolates. Still other antibiotics showed varying responses. The high resistance rates for some antibiotics suggest that the overuse or uncontrolled use of antibiotics has contributed to the emergence of resistant strains (Table 3).

Table 3. Antibiotics in AST-P592 Card for Gram-Positive *Streptococcus sobrinus* Bacteria.

No.	Antimicrobial agents	Code	S 100%	R 100%	Mic	Interpretation
1.	Cefoxithin	CFX	5(45.5%)	6(54.5%)	>=1.0	I
2.	Benzylpenicillin	P	7(63.3%)	4(36.3%)	>=0.5µg	I
3.	Gentamicin	GM	5(45.5%)	6(54.5%)	>=16µg	R
4.	Imipenem	IMP	10(91%)	1(9%)	>=1 µg	S
5.	Oxacillin	OXI	8(72.3%)	3(27.2%)	> = 4µg	S
6.	Ciprofloxacin	CIP	2(18.1%)	9(81.8%)	> = 8µg	R
7.	Levofloxacin	LEV	10(91%)	1(9.0%)	> =0.12µg	S
8.	Moxifloxacin	MOX	9(81.8%)	2(18.2%)	>=0.5µg	S
9.	Fusidic Acid	FA	5(45.5%)	6(54.5%)	> = 16µg	R
10.	Tetracycline	TE	6(54.5%)	5(45.5%)	> = 1 µg	I
11.	Rifampicin	RA	8(72.3%)	3(27.2%)	> = 0.5µg	S
12.	Sulfamethoxazole	SXT	2(18.1%)	8(72.3%)	> = 10µg	R
13.	Linezolid	LNZ	6(45.5)	5(45.5%)	> = 8 µg	I
14.	Tigecycline	TIC	7(63.3%)	4(36.3%)	> = 0.2µg	S
15.	Erythromycin	E	7(63.3%)	4(36.3%)	> =0.25 µg	S
16.	Clindamycin	CM	4(36.3%)	7(63.3%)	> = 16µg	R

Susceptible : S
Intermediate : I
Resistant : R

The results of the immunological evaluation of antibodies to *Helicobacter pylori* in the serum of patients infected with *Streptococcus sobrinus* (four males and seven females) showed that males were more susceptible to double bacterial infection (75%), while the double infection rate among females was 71.4%. It should be noted that *Streptococcus sobrinus* infection was higher in females than in males, according to the isolation results in our study (Table 4).

Table 4. Immunological testing using the Hipro device to detect co-infection of *S. sobrinus* and *H. pylori*.

	Control+	>160 AU/ml	Result
	Control -	< 5 AU/ml	
Male	1	< 5	-ve
	2	9.82	+ve
	3	29.3	+ve
	4	139	+ve
Female	1	< 5	-ve
	2	<5	-ve
	3	9.3	+ve
	4	11.8	+ve
	5	16.6	+ve
	6	58.4	+ve
	7	92.2	+ve

Discussion

Our results show strong agreement with those of Sánchez-Acedo, who found that 18.9% of *S. sobrinus* bacteria were isolated from individuals with dental caries, while in a similar study by Mohammed, the isolation rate was 5.1% (6/118). The difference in isolation values may be attributed to several factors, including age, sex, immune factors, and dietary type. However, all studies agree that this type of bacteria causes dental caries. [23], [24].

The *gtf-T* and *gtf-I* primers showed high efficacy in diagnosing this bacterium, a result consistent with the findings of researchers [(Sato *et al.*, 2003), (Soni *et al.*, 2015)] who were able to analyze all samples belonging to *S. sobrinus* [25].

Co-infection between dental caries bacteria and *H. pylori* is common and plays a role in increasing the severity of oral and infectious diseases. However, further research is still needed to fully understand the relationship between *H. pylori* in the mouth and the development of gastritis [26], [27].

S.sobrinu bacteria carry the *gtfl* and *gtfT* genes responsible for adhesion and biofilm formation on dental plaque, giving them resistance mechanisms that may lead to increased virulence in the future, and attention to good oral hygiene and treatment of decay may contribute to reducing the risk of *H.pylori* infection or its recurrence [28].

CONCLUSION

Fundamental Finding : This study confirms the presence of *Streptococcus sobrinus* among patients with dental caries and periodontal disease in Mosul for the first time, including the identification of a novel strain (HAA5). The bacterium demonstrated opportunistic behavior and carried key biofilm-associated genes (*gtfl* and *gtfT*), which may enhance its virulence and antibiotic resistance. A high prevalence of co-infection with *Helicobacter pylori* was observed, suggesting a potential epidemiological

association between oral and gastrointestinal infections. **Implication :** These findings highlight the oral cavity as a possible reservoir for *H. pylori* and emphasize the importance of oral hygiene and caries management in reducing the risk of co-infection. **Limitation :** However, no definitive causal relationship could be established. **Future Research :** Further molecular and clinical studies are needed to clarify the role of oral bacteria in *H. pylori* transmission and disease progression.

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