Pharaonic Journal of Science
https://pjscience.org/
PJS
SPBH

Research article

Phylogenetic analysis of *Streptococcus cristatus* isolated from an infected root canal

Mohammad M. Salih

Department of Biology/ College of Science/ University of Mosul/ Mosul, Iraq

Email: mohsbio125@uomosul.edu.iq

DOI: https://doi.org/10.71428/PJS.2026.0114

ABSTRACT

Objectives: This research aimed to investigate the identification by molecular and morphological techniques of a new local strain of *Streptococcus cristatus*. This strain was detected in 30 cases of patients with infected root canals. **Methods:** In this study, a modification of Schaedler Agar Medium was done to encourage the isolation of this strain. Then tests of ability for slime layer production and Antibiotic sensitivity were done. **Results:** The results showed that only three out of the thirteen cases gave positive results for *S. cristatus*, estimating a 10% prevalence. PCR and gel electrophoresis were employed to further verify this outcome. *S. cristatus's* 16S rRNA gene sequence was ascertained and compared with the present information from the National Center for Biotechnology Information (NCBI) to determine the strain specifically. The accession number PV243282.1 was assigned to the sequence following its submission to GenBank. This strain was registered in GenBank under the name Mosul. Furthermore, the study confirmed that the isolate under study could create a slime layer and the capacity to produce biofilm. **Conclusion.** The results of this study enhance the knowledge about *S. cristatus* and its traits in relation to infected root canals, offering insightful information for future studies and Possible approaches to treatment.

Keywords: Modified Schaedler Agar, root canal infection, new local strain *Streptococcus cristatus,* PCR.

INTRODUCTION

Streptococcus cristatus belongs to the Mitis group of streptococci (mainly they inhabit the mouth). Although historically regarded as a commensal organism, recent case reports have served to bring to light its possible pathogenicity, especially in immunocompromised patients or poor oral hygienists. The bacteria *S. cristatus* have been associated with bacteremia and infective endocarditis, usually in individuals with underlying health problems (1). The *S. cristatus* is a significant bacterium that is present in infected root canals, which cause multiple dental pathologies. Its presence in the microbiome of the root canal indicates the complexity and polymorphism of these diseases. Research has found that infected root canals have a very great variety of bacteria, including *S. cristatus* as a streptococci. Other prevalent species include *Fusobacterium nucleatum* and *Porphyromonas gingivalis (2)*. *S. cristatus* has antagonist effects against *P. gingivalis,* which is a major cause of oral disease. An increase in ratios of *S. crystallatus* in oral microbiota can be associated with the possibility of a decreased virulence of pathogenic bacteria (3). A unique case of neonatal septic arthritis was also described and presented *S. cristatus* as a pathogen, which is uncommon among children (4). Molecular detection techniques, such as 16S rRNA gene sequencing and rRNA-based assays, have enhanced bacterial recognition; however, they

Received: February 28, 2025. Accepted: March 28, 2026. Published: April 12, 2026

cannot discriminate between viable and nonviable cells and thus make it difficult to determine the activity of the microbes (5). An integrated approach to the molecular technique has been implemented, such as the 16S rRNA or any other gene sequence, which has been ascertained to validate species, and this is more so when applied in clinical conditions (6). Moreover, only limited data exist on the antibiotic susceptibility of *S. cristatus* isolates of root canals, with almost all available data on other streptococcal species (7). *S. cristatus* has been found to be sensitive to a number of antibiotics, including vancomycin, as is evident in a case of septic arthritis in a neonate. The determination of the antibiotic resistance profile is necessary because the research shows that the composition of the oral microbiome can determine the virulence and resistance of *S. cristatus* (3). *S. cristatus* and other anaerobic bacteria make the treatment of root canals more difficult since the bacteria may remain even after the traditional methods (8).

MATERIALS AND METHODS:

By visiting the teaching hospital at the College of Dentistry\ University of Mosul, samples were collected from patients under the supervision of specialized dentists, accounting for 30 samples. The sample was taken using a paper point, inserted into the infected root canal, waiting for a full minute, then withdrawn and placed in the transport medium, which was brain heart infusion broth with Hemin 5 µg/mL. The patients (18 men and 12 women with ages of 19-65 years) demonstrated that they were healthy, were not smokers, and had not used antibiotics during the 3 months prior to sampling.

Isolation and identification of bacteria were done when the samples had been processed in the laboratory within one to two hours of their collection. A paper point pre-impregnated into the transport medium was then plated onto the Schaedler agar by Hi Media (Mumbai, India), which was supplemented with Hemin 5 µg/mL and Vitamin K 10 µg/mL. The plates were then incubated facultatively anaerobically by utilizing an anaerobic container (jar) and the CampyGenTM 2.5L Sachet at 37 ° C for 48 hrs. Isolates of the *S. cristatus* were characterized morphologically by using the Gram staining and conventional biochemical tests, such as catalase and oxidase tests.

Molecular detection

After the bacteria gave clear growth on the modified Schaedler agar medium, the genomic DNA was extracted using the extraction kit supplied by Omega Company, and the extraction process was performed according to the manufacturer's instructions. This was followed by a process of measuring the concentration and purity of the isolated genomic DNA. The genetic material was then preserved for future use at - 20 C.

PCR kit to detect the 16s rDNA gene: Use universal primers based on (Al- Safar and Al-Sammak, 2014) to detect the 16s rDNA gene, prepared by Promega, at a final concentration of 10 pmol, as shown in (Table 1).

PCR reactions to detect the 16s rDNA gene: Prepare the master mixture in a volume of 25 microliters for all samples. The tubes were inserted into the PCR thermal amplifier to complete the replication using the following program (Table 2). The samples were electrophoresed with a voltage of 50 for 40 minutes, and then the gel was examined using ultraviolet radiation to detect the presence of the 16s rDNA gene bands at position 1350 bp.

Phylogenetic relationship of diagnosed strains:

The Phylogenetic relationship between lineages belonging to a species within Clustal W was obtained using Maximum likelihood using the Mega Program 11.

Antibiotic susceptibility test:

The sensitivity towards the antibiotics of bacterial isolates was tested on modified Schaedler agar by using the disc diffusion method, using five types of antibiotics (Bioanalyse, Turkey), as mentioned in Table 3 (9).

Table (1): Sequence of primers used to investigate a 16s rDNA gene

Gene name	Sequence of primers
16s rDNA gene	27F AGAGTTTGATCMTGGCTCAG
	1522R AAGGAGGTGATCCARCCGCA

Table (2): PCR cycling conditions

Steps	Temp.	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	94 °C	30 sec	30
Annealing	57 °C	30 sec	
Extension	72 °C	1 min	
Final extension	72 °C	3 min	3

Table 3: Antibiotic, Name Abbreviation, and Concentration (µg).

No.	**Antibiotic Name**	**Abbreviation**	**Concentration (µg)**
1.	Cefixime	CFM	5
2.	Vancomycin	VA	30
3.	Gentamicin	CN	10
4.	Levofloxacin	LEV	5
5.	Tobramycin	TOB	10

Slime layer study:

Schedler's medium supplemented with Hemin 5 µg/mL and Vitamin K 10 µg/mL was used; then the required components were added to study the ability of *S. cristatus* to form a slime layer. Congo red dye and sucrose were added in proportions of (0.8 g/L) and (36 g/L), respectively. In order to maintain sterility, the dye and sugar were sterilized separately. while sucrose was sterilized by filtration. Then, after completing the autoclave, waiting for the medium to cool to a temperature of 45 ° C after that, the components were added to the medium. The *S. cristatus* to be studied was cultured on the CR-modified Schaedler agar plates and then incubated with the appropriate facultative anaerobic conditions at 37 C for 48 hours. After completing the incubation, the sample was taken out of the incubator and placed at room temperature for two days to observe the increase in the degree of color (10).

RESULT

Twenty-seven samples of dental root canals in the primary isolation showed growth on the modified Schaedler agar medium, and the percentage of primary isolation was 90% (Figure 1). 16 and 11 samples showed positive growth for male and female, respectively. The appearance rate of the bacteria targeted in our study was 10%, as three isolates were diagnosed phenotypically as they grew on the modified Schaedler agar medium, and one of them was diagnosed molecularly based on the 16s rRNA gene, and the other two isolates were unable to grow in the subculture process.

It was described as Gram-positive, cocci that were roughly one µm in diameter and aggregated in chains, and was named *Streptococcus cristatus* (Figure 2).

Colony of *S. cristatus* on modified Schaedler agar was small, circular, and smooth, and normally appeared in greyish or white colour (Figure 3). They can also have a slightly raised morphology and can be seen to be surrounded by a clear zone, which is a feature of hemolytic activity typical of alpha-hemolytic streptococci.

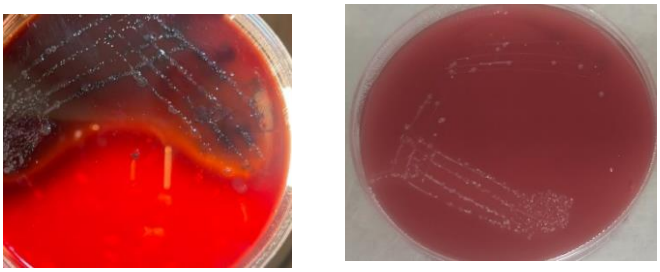

Figure 1: Primary isolation of *S. cristatus* on modified Schaedler agar

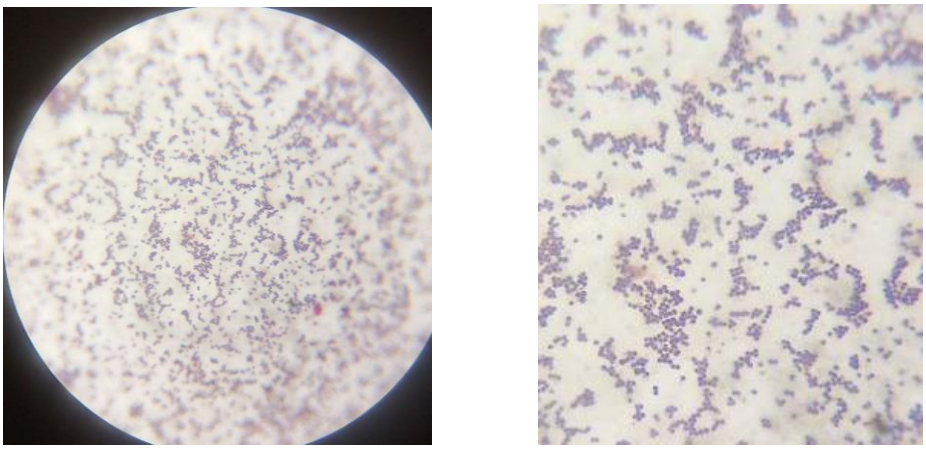

Figure 2: Gram-negative cells (100x) of *S. cristatus*

Figure 3: Growth of *S. cristatus* on modified Schaedler agar as a pure culture.

Incubation: The best growth is at 37 °C under anaerobic conditions, which is fundamental in the development of colonies of the correct type of *S. cristatus* on modified Schaedler agar.

Molecular detection:

The results of the 16s rRNA gene diagnostic were positive, where the result of the concentration and purity was 98.44 ng/µl and 1.823, respectively. *S. cristatus* was identified, and it was registered in the gene bank and given a specific accession number (PV243282). Then, our strain has been given the name Mosul. Thus, by browsing the NCBI, it becomes clear to us that our isolate was the first to be isolated and diagnosed at the local level.

Antibiotic susceptibility test:

The susceptibility of *S. cristatus* to several antibiotics was assessed using the Kirby-Bauer diffusion method. Antibiotic disks were recognized by their concentrations and names; the existence of distinct areas (zones of inhibition) surrounding the disks indicates that the antibiotics have been effective in preventing the growth of bacteria. According to an antibiotic susceptibility test, our isolate was responsive to the remaining medicines but resistant to cefixime (Table 4).

Slime Layer Production:

After inoculating of CR- Modified Schaedler Agar Medium plates with *S. cristatus* isolate (Figure 4), there was a change in color to black, which is an indication that this bacterium has the capacity to create a slime layer.

Phylogenetic genetic tree:

The results of the genetic analysis based on the genetic tree were that the isolate of the current study was present within one genetic cluster with the reference and international strains of *S. cristatus*

(Figure 5), with a high and precise connection to the strain *S. cristatus* 29972 PX419576.1. This result, based on the analysis and genetic tree, confirms the importance, validity, and accuracy of the molecular diagnosis for the study strain, as it was noted that the Bootstrap was 100% at the joint node. On the other hand, the success of the genetic analysis was clear when it showed the genetic differentiation of the current study strain with related species (*S. pneumoniae* and *S. timonensis*), which proves the affiliation of our isolate, which was diagnosed from tooth root canals, and its integration into the correct genetic path for this bacterial species globally in GenBank.

Table (4): Antibiotic, Concentration (µg), and Result

Antibiotic	Concentration (µg)	Result
Gentamicin	10	S
Tobramycin	10	S
Cefixime	5	R
Levofloxacin	5	S
Vancomycin	30	S

Figure 4: Production of Slime layer on CR- Modified Schaedler Agar Medium by *S. cristatus*.

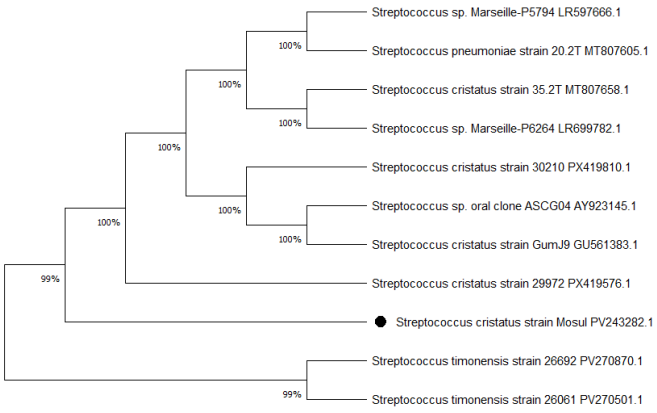

Figure 5: phylogenetic tree of *S. cristatus*

DISCUSSION

One of the Iraqi studies conducted in the year 2024 showed that the percentage of males was the highest, and they constituted the group most exposed to the risks of tooth loss due to dental root canal diseases. Also in Babil Governorate, the highest percentage of tooth decay was among women, but the percentage of tooth loss was highest for males, and the same percentage was also in Iraqi Kurdistan (11), and this matches the results of the current study. This may be due to the fact that males may ignore the initial symptoms of infection, which leads to the complication of the disease (12), or that they have a more complex canal than women; this may also be linked to poor oral health awareness (13). Although being naturally occurring, *S. cristatus* has been linked to a number of human illnesses, such as endocarditis, osteomyelitis, endophthalmitis, tooth abscesses, and acute septic arthritis (1). When comparing our study with international studies, one of them was able to isolate and diagnose *S. cristatus*, but using blood agar medium (1). Guzman's study was able to isolate *S. cristatus* using blood and chocolate agar medium, but they did not succeed in growing it on MacConkey agar medium (14). The results of biochemical tests showed that *S. cristatus* was catalase-negative and oxidase-positive. In the study of Guzman and his colleagues was clarified that the biochemical profile was negative for mannitol, urea hydrolysis, and VP, but variable for arginine hydrolysis, esculin, and sorbitol (14). Comparing our current study with a local study conducted at our same university, it was found that the researchers used the same modified medium as our study, but they isolated a different bacterial species, *Streptococcus sanguinis*. Therefore, we conclude that this modified medium is not selective for either isolated species. The researchers also used this medium to study the sensitivity of *S. sanguinis* to antibiotics and their ability to form a slime layer. Our study is consistent with this local study, as both isolated bacterial species were from infected root canals, and they share a common resistance to Cefixime (7).

Biofilm formation may vary according to the environmental conditions. A study was conducted in 2006, which revealed that there was a reduction in biofilm formation by the luxS mutant; however, that was not related to the media used or to whether the cells were grown in 5% CO_2 atmosphere or under anaerobic conditions (15). When compared with international studies, the study by Glazunova and his colleagues showed that *S. cristatus* has a genetic affinity with both *S. sinensis* and *S. sanguinis*, through a study of their analysis and genetic tree (16). Phylogenomic studies show that *S. cristatus* has a high degree of genetic congruence with other species belonging to the Mitis group, thus suggesting a common evolutionary ancestor (17).

CONCLUSION

The research was a genetic analysis of *S. cristatus* bacteria isolated from the root canals in Mosul. The phylogenetic tree showed that the local isolate (Mosul PV243282.1) is closely related to the global one (PX419576.1) with 100% bootstrap support. This molecular diagnosis helps in the documentation of the local oral pathogens and provides a genetic linkage to the global isolates, ensuring the accuracy of the clinical analysis and consequently the development of treatment strategies.

Conflict of interest: NIL

Funding: NIL

References

1. Silva AS, Baptista M, Soares I, Macedo D, Neto RR. A Case of Native Valve Infective Endocarditis and Bacteremia Due to Streptococcus cristatus. Cureus. 2024;16(8):e67122.
2. Nóbrega LM, Montagner F, Ribeiro AC, Mayer MA, Gomes BP. Molecular Identification of Cultivable Bacteria From Infected Root Canals

Associated With Acute Apical Abscess. Braz Dent J. 2016;27(3):318-24.

3. Wang Q, Wang BY, Pratap S, Xie H. Oral microbiome associated with differential ratios of Porphyromonas gingivalis and Streptococcus cristatus. Res Sq. 2023.
4. Gupta G, Chaudhary M, Khunt A, Shah V, Shah MM. An unreported case of Streptococcus cristatus septic arthritis of wrist in a neonate. J Clin Orthop Trauma. 2020;11(2):328-31.
5. Prado LC, Gavini G, Silveira ADC, Nakamura VC, Mayer MPA, Pinheiro ET. Comparison of rRNA-based reverse transcription PCR and rDNA-based PCR for the detection of streptococci in root canal infections. J Appl Oral Sci. 2019;27:e20180256.
6. Kosecka-Strojek M, Wolska M, Żabicka D, Sadowy E, Międzobrodzki J. Identification of Clinically Relevant Streptococcus and Enterococcus Species Based on Biochemical Methods and 16S rRNA, sodA, tuf, rpoB, and recA Gene Sequencing. Pathogens. 2020;9(11).
7. Ramadhan MM, Ramadhan MM, Ali JF, Ali JF. Antibiotic Susceptibility Profile and Slime Layer Formation of a Clinical Isolate of Streptococcus sanguinis from Infected Root Canals. Antibiotic Susceptibility Profile and Slime Layer Formation of a Clinical Isolate of Streptococcus sanguinis from Infected Root Canals. 2025;2(05):193-200.
8. Ito Y, Sato T, Yamaki K, Mayanagi G, Hashimoto K, Shimauchi H, Takahashi N. Microflora profiling of infected root canal before and after treatment using culture-independent methods. J Microbiol. 2012;50(1):58-62.
9. Salih MM, Thanoon Altaee MF, Mohammed MI. Comparative study between two types of water on the ability of growth of some pathogenic and ecological bacterial species. Biochemical & Cellular Archives. 2021;21.
10. M. Ramadhan M, F. Ali J. Morphological and molecular identification of a local strain of Streptococcus sanguinis isolated from an infected root canal. Health Biotechnology and Biopharma (HBB). 2025;9(2):21-41.
11. HAMONARI NH, AL DABBAGH SA, MUDHIR A. Prevalence of Dental Caries and Periodontal Diseases among Secondary School Students in Duhok, Kurdistan Region, Iraq. Journal of Clinical & Diagnostic Research. 2021;15(3).
12. Suvarna A, Thennarasu P, Sojan S, Gables O, Browne DJ, Amaral RR. Epidemiology of Endodontic Treatment in Far North Queensland, Australia: A Retrospective Observational Study of 11 Years. Clin Exp Dent Res. 2025;11(5):e70232.
13. Elsayed MA, Elmesellawy MY, Schäfer E. Prevalence of multiple roots and complex canal morphology in mandibular premolars among a selected Southern Egyptian sub-population: a CBCT-analysis. Odontology. 2024;112(4):1257-64.
14. Guzman C, Zaclli A, Molinari J. Streptococcus cristatus bacteremia in a patient with poor oral hygiene: a case report. J Med Case Rep. 2023;17(1):218.
15. Petersen FC, Ahmed NA, Naemi A, Scheie AA. LuxS-mediated signalling in Streptococcus anginosus and its role in biofilm formation. Antonie Van Leeuwenhoek. 2006;90(2):109-21.
16. Glazunova OO, Raoult D, Roux V. Partial recN gene sequencing: a new tool for identification and phylogeny within the genus Streptococcus. Int J Syst Evol Microbiol. 2010;60(Pt 9):2140-8.
17. Richards VP, Palmer SR, Pavinski Bitar PD, Qin X, Weinstock GM, Highlander SK, et al. Phylogenomics and the dynamic genome evolution of the genus Streptococcus. Genome Biol Evol. 2014;6(4):741-53.