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Antibiotic Susceptibility Profile and Slime Layer Formation of a Clinical Isolate of *Streptococcus sanguinis* from Infected Root Canals

Mohammed M. Ramadhan[1], Jassim F. Ali[2]
[1,2]University of Mosul, Iraq

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ABSTRACT

Objective: *This study examined susceptibility of the clinical isolate Streptococcus sanguinis, isolated from an infected root canal to various antibiotics, and tested its capacity to form a slime layer.* ***Method:*** *PCR and gel electrophoresis both confirmed it as bacterial identification. The 16S rRNA gene sequence of S. sanguinis was determined and compared to previously determined sequences in the NCBI (National Center for Biotechnology Information) database. Growth of the isolate was determined to consist of a strong ability to form a slime layer when grown on Congo red-modified Schaedler agar medium.* ***Results:*** *GenBank records for the resulting sequences are under the accession numbers PQ654829 and PQ654911. The antibiotic susceptibility profile was high to levofloxacin, moderate to gentamicin, tobramycin and vancomycin, and complete resistance to cefixime.* ***Novelty:*** *The results of this study provide important information about the bacterium's virulence and antibiotic resistance.*

INTRODUCTION

Microbial invasion and biofilm accumulation in the pulp cavity begin a root canal infection when the dental pulp is damaged, causing pain and may progress further into an abscess and systemic involvement[1]. The viridans group streptococci comprises of *Streptococcus sanguinis* that is facultative anaerobic Gram positive cocci which is usually present in the human oral cavity as a commensal[2]. *S. sanguinis* was first isolated in 1946 and until 1984, the species was usually classified under the synonym of Streptococcus mitis, because of its phonetic and genetic differences, that in 1984 were recognized as distinct species[3]. This microorganism has been reported to grow slowly on enriched media, and form alpha hemolytic colonies on blood agar under microaerophilic conditions at 37°C[4]. They are dental biofilms comprised of a complex microbial community embedded within a polysaccharide matrix that serve as a protected niche for pathogens like *S. sanguinis* that is resistant to antimicrobial agents and immune responses[5]. The extracellular matrix, rich in polysaccharides, is integral to biofilm formation, aiding bacterial persistence in challenging environments[6]. This biofilm structure is essential to the pathogenesis of 'endodontic' infection and constrains endodontic treatment. In addition, recent information indicate that *S. sanguinis* is capable of exploiting seemingly virulence traits, namely mechanisms that evade host immunity, survive antimicrobial exposure[7]. The increasing concern about antibiotic resistance in oral bacteria, including S. sanguinis, underscores the need for regional studies[8]. Local

antimicrobial susceptibility testing is crucial for guiding appropriate and effective therapy, especially in infections involving biofilm-forming pathogens[9].

The aim of this study is to investigate the antibiotic susceptibility profile and slime layer formation ability of a local clinical isolate of *Streptococcus sanguinis* obtained from infected root canals, using both phenotypic and molecular techniques.

RESEARCH METHOD

A dentist at the Dental Specialized Center/Gulan General Hospital collected 22 samples from patients diagnosed with root canal inflammation. To gather sufficient material, sterile paper points (size #20) were inserted into the root canal for 30-60 seconds. For transportation, these samples were promptly placed into broth tubes containing modified thioglycollate. Among the participants were 10 females and 12 males, ages ranging from 15 to 54ؘ. Prior to sample collection, all participants were confirmed to be free of systemic diseases, nonsmokers, and had not used antibiotics for at least three months[10].

Antibiotic Sensitivity

The Kirby-Bauer disk diffusion method (1966) used to assess the antibiotic sensitivity of *S. sanguinis*. In this method, antibiotic-coated disks are put on an agar plate that has the test organism on it. After incubation, the inhibition zone around each disk is measured. The diameter of this zone is measured to determine whether the bacteria are sensitive (or not) against each antibiotic. *S. sanguinis* was the bacterial species examined in this study. To prepare the inoculum, fresh colonies of *S. sanguinis* were sub cultured from Modified Schaedler agar to Todd-Hewitt broth. The broth cultures were maintained at 37°C for 24 hours to enable bacterial growth before testing antibiotic sensitivity. Antibiotics used in the present study were purchased from BioRex company (United Kingdom), including Gentamicin (10µg/disk), Tobramycin (10µg/disk), Cefixime (10µg/disk), Levofloxacin (10µg/disk), Vancomycin (10µg/disk)[11].

Distilled water was used to prepare all media utilized in this study, specifically Todd-Hewitt broth and modified Schaedler agar. The test was performed as follows: Fresh bacterial colonies were collected from each isolate under study and inoculated into Todd-Hewitt broth to activate bacterial growth, then incubated for 24 hours at 37°C. The following day, a bacterial solution was formulated to achieve an absorbance equivalent to the McFarland 0.5 standard, corresponding to a bacterial concentration of $1.5x10^8$ cells/cm^3. A portion of 0.1 mL from this suspension was aseptically transferred to a plate with modified Schaedler agar. The bacterial inoculum was uniformly distributed over the agar surface using an L-shaped spreader sterilized with an alcohol flame. Antibiotic disks were meticulously positioned on the medium's surface using sterile, alcohol-disinfected forceps. The inoculated plates were incubated for 24 hours at 37°C in a sealed jar under microaerophilic conditions. Post-incubation, the outcomes were documented by quantifying the diameter of the inhibitory zones surrounding each antibiotic disk[12].

Detection of the Slime Layer

The bacterial ability for the slime layer production was examined by modifying the Congo red (CR) agar medium with Congo red dye (0.8 g/L in distilled water) and sucrose (36 g) in the Schaedler agar medium. This method introduces a novel approach to our investigation. To ensure sterility, the modified Schaedler agar medium and CR dye were autoclaved individually, whereas Sucrose was sterilized through filtering as a concentrated water-based solution. After autoclaving, the sucrose solution and CR dye were incorporated into the modified Schaedler agar cooled to 45–50 °C prior to inoculating bacterial strains onto the CR-modified Schaedler agar plates and incubating for 48 hours at 37 °C, followed by an additional 48 hours at room temperature to facilitate slime layer development with modified[13].

RESULTS AND DISCUSSION

Results

Streptococcus sanguinis was isolated from patients with infected root canal in Akre city / Iraq. This bacterium was diagnosed by molecular methods; the sequence of 16S rRNA gene was determined for E. *Streptococcus sanguinis*, then compared with that of (NCBI) National Center Biotechnology Information. Deposition the sequence of 16S rRNA of *Streptococcus sanguinis* at GenBank, under the accession number PQ654829 and PQ654911, then this strain has given JAMOMOSUL and JAMOMOSUL1 as name, see Table 1.

Table 1. Investigated bacterial strains.

No	Bacteria	Strain	Accession Number	Concentration	Purity (A260/A280 Ratio)
1	*Streptococcus sanguinis*	JAMOMOSUL	PQ654829	91.2 ng/µl	1.71
2	*Streptococcus sanguinis*	JAMOMOSUL1	PQ654911	91.5 ng/µl	1.73

The susceptibility of *S. sanguinis* to various antibiotics measured by the Kirby-Bauer diffusion method, see Figure 1. Antibiotic disks are labeled with their names and concentrations; clear areas (zones of inhibition) surrounding the disks indicate successful bacterial growth inhibition by antibiotics. Below is an outline of the key findings: Gentamicin (10 µg): 22 mm zone of inhibition against S. *sanguinis*: moderate susceptibility. Tobramycin (10 µg): Showed a similar result, with a 21 mm zone of inhibition, indicating moderate effectiveness. Cefixime (5 µg): Had no visible zone of inhibition, meaning *S. sanguinis* was resistant to it. Levofloxacin (5µg) produced a large zone of inhibition of 31 mm, confirming its high efficacy against this organism. Vancomycin (30µg) showed an 18 mm inhibitory zone (moderate activity) but was less effective compared with that of levofloxacin. *S. sanguinis* is highly sensitive to

levofloxacin, moderately sensitive to gentamicin, tobramycin, and vancomycin, but resistant to cefixime, see Table 2.

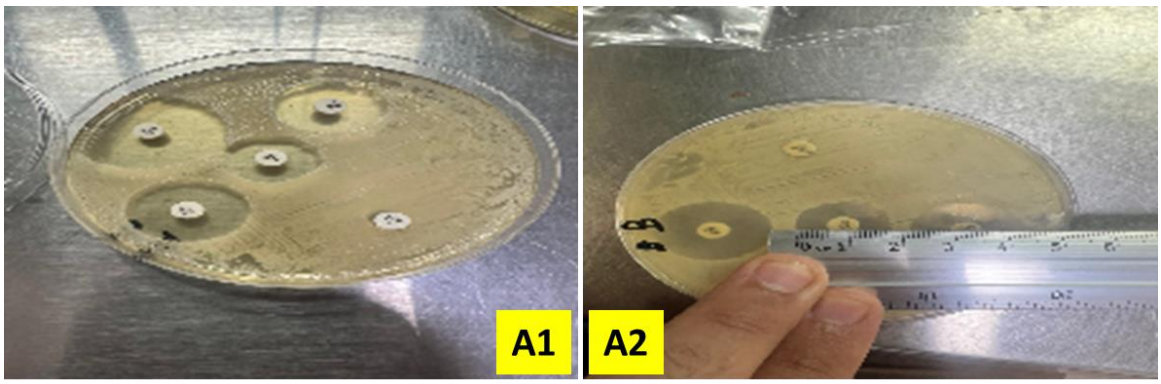

Figure 1. Depictive representation of (A1) antibiotic sensitivity of *Streptococcus sanguinis* on modify Schaedler agar (A2) measurement of inhibition zone.

Table 2. Susceptibility of *Streptococcus sanguinis* to Antibiotics Compared with CLSI Guidelines.

Antibiotic	Concentration (µg)	Zone of Inhibition (mm)	CLSI Interpretation	Comment
Gentamicin	10	22	Susceptible (≥15 mm)	Effective against *S. sanguinis*; activity comparable to tobramycin.
Tobramycin	10	21	Susceptible (≥15 mm)	Similar activity to gentamicin.
Cefixime	**5**	No zone	Resistant	No inhibition zone observed
Levofloxacin	5	31	Susceptible (≥17 mm)	Highly effective against *S. sanguinis.*
Vancomycin	30	18	Susceptible (≥15 mm)	Effective, but less active compared to levofloxacin.

After inoculating CR-modified Schaedler agar medium with *S. sanguinis* isolates, see Figure 2, a change in color to black was observed, indicating the bacterium's ability to produce a slime layer. After three days, the plates were re-evaluated, revealing a more notable black colouration, so proving the bacterium's capacity to generate a slime layer.

Figure 2. Generation of slime layer on CR- modified Schaedler agar medium by *Streptococcus sanguinis*.

Discussion

The antibiotic susceptibility profile of *S. sanguinis* isolated from an infected root canal demonstrates variability in response to various antibiotic classes, underscoring the necessity for tailored antimicrobial therapy. Levofloxacin exhibited the greatest efficacy among the tested antibiotics, indicating its potential as an effective treatment option. The widespread application of fluoroquinolones is associated with the development of resistance, requiring careful and prudent use in clinical environments[14].

Aminoglycosides, including gentamicin and tobramycin, exhibited significant activity. The use of these antibiotics in endodontic infections is restricted; nonetheless, they may be beneficial in localized treatments, including intracanal medicaments, especially for biofilm-associated infections[15]. Consistent with the inherent resistance mechanisms of *Viridans*-group *streptococci* to particular cephalosporin, cefixime did not show any effect. Nepal et al. (2022) highlight the limited effectiveness of cephalosporin in treating endodontic infections and emphasize the importance of using antibiotics with proven efficacy, such as penicillin or amoxicillin, commonly used for oral infections[16]. Vancomycin exhibits consistent efficacy and continues to be an important choice for the treatment of severe or resistant infections attributed to *S. sanguinis*. Systemic use is typically limited to severe cases, as localized infections, such as those in the root canal, are often more effectively treated with less toxic alternatives[17].

Earlier study reported that resistance strain could potentially resists vancomycin [18]. These results highlight the necessity for routine antibiotic susceptibility testing that drives effective treatment strategies. Levofloxacin and aminoglycosides seem to be good alternatives, but interventions should be considered judiciously to prevent resistance and avoid systemic toxicity. In addition, the cefixime resistance underscores the dangers of empiric antibiotic use in the absence of susceptibility data. An ultimate success in the management of root canal infection can only be achieved through proper antibiotic stewardship along with the use of locally effective regimes.

A wide range of methods can be employed to detect the presence of slime layers and biofilm capacities, including the Congo Red Agar (CRA) method. Similar recent modifications of the CRA method now render it far more sensitive to the biofilm

formation of clinical isolates of resistant bacteria, thus making it an even more applicable tool in the screening for biofilm-forming isolates from the field[19].

Bacterial isolates exhibiting morphological differences can be identified using the CRA (Congo Red Agar) methodology. The differences in the organizational structures mentioned above are imperative when it comes to identifying the pathogenic kinds of bacteria that create slime layers. The method directly differentiates between biofilm-producing bacteria and their nonproducing bacteria, which is critical for assessing the severity of an infection and informing empirical treatment. Natural variation in the expression of polysaccharide intercellular adhesion (PIA) represents changes in genetic control contributing to the variability of biofilm formation[20]. Bacteria embedded in biofilms exhibit unique morphologies compared to planktonic bacteria. The observed differences encompass modified motility, elevated extracellular polysaccharide synthesis, and augmented antibiotic resistance. The matrixome, or extracellular matrix, is critical during these transformation processes by providing structural firmness, a locus for bacterial binding, and a shield from antimicrobial agents, creating a virtuous cycle for biofilm-mediated virulence[21].

CONCLUSION

Fundamental Finding : This study revealed that *Streptococcus sanguinis* isolated from infected root canals demonstrated variable susceptibility to commonly used antibiotics. Levofloxacin showed the highest efficacy with a significant zone of inhibition, while cefixime exhibited no inhibitory effect, indicating complete resistance. Additionally, phenotypic detection using modified Congo Red agar confirmed the strain's ability to form a slime layer, emphasizing its biofilm-forming capacity—a key virulence factor contributing to persistence in endodontic infections. **Implication :** The findings underscore the critical need for region-specific antibiotic susceptibility profiling in the treatment of endodontic infections, particularly those involving biofilm-producing pathogens like *S. sanguinis*. The high efficacy of levofloxacin suggests it could be considered in local antimicrobial therapy, but with caution due to the risk of resistance development. The confirmation of slime layer production reinforces the necessity to integrate biofilm-targeted strategies into endodontic infection management protocols. **Limitation :** A primary limitation of this study is its relatively small sample size, which may not fully represent the genetic or phenotypic diversity of *S. sanguinis* in broader populations. Furthermore, the study was limited to phenotypic antibiotic resistance profiling and basic biofilm detection; comprehensive genomic or proteomic analyses were not conducted, which could have provided deeper insights into resistance mechanisms and virulence factors. **Future Research :** Further studies are recommended to explore the genetic determinants of antibiotic resistance and biofilm formation in S. sanguinis using whole-genome sequencing and transcriptomic analysis. Expanding the sample size and including isolates from various geographical locations will enhance the generalizability of findings. Additionally, evaluating the effectiveness of biofilm-

disrupting agents in combination with antibiotics may offer promising strategies for improving therapeutic outcomes in persistent endodontic infections.

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* **Mohammed M. Ramadhan (Corresponding Author)**
University of Mosul, Iraq
Email: biology.ak@gmail.com

Jassim F. Ali
University of Mosul, Iraq
Email: jassim.fathi@uomosul.edu.iq