AL-QADISIYAH MEDICAL JOURNAL , QAD.MED.J.19 (1): JANUARY-JUNE 2023
WWW.qmj.qu.edu.iq

REVIEW ARTICLE

Streptococcus angriness isolated from Patients with Acute Infection following Surgical Tooth Extraction

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Abstract

In this study, 250 individuals with acute infection following surgical tooth extraction were treated at a Hilla dental clinic and cultivated at 37°C for 18–24 hours. They cultured on several selective media at 37°C for 18–24 hours. Identification of Streptococcus anginosus was based on colonial morphology, microscopically, and biochemical tests, and all scientific specimens were successful cultures. Of 250 specimens, only 25(10%) were biochemically identified as Strep. anginosus, while 225(90%) were associated with other microorganisms. Hemolysin-producing Strep. anginosus isolates have been studied. 10(66.66%) isolates produced hemolysin in blood agar that formed a clearance sector around the colonies and a streak on the plate after 24 hours of incubation at 37°C. Strep. anginosus isolates were also tested for siderophores production, 5(33.33%) Strep. anginosus isolates produce siderophores. All bacterial isolates provide protease to hydrolyze protein. After 24 hours of incubation, all Strep. anginosus isolates were able to hydrolyze protein using protease enzyme (100%) when tested using M_9 (supported by 20% glucose and 1% csaien). Adding 3ml of 5% Trichloroacetic acid gave the colony a clean hallo. Quantitative biofilm development tests were performed in a microtiter using Trypticase Soy Broth with 1% glucose. To improve accuracy, this assay was done three times. All Strep. anginosus isolates were biofilm former (100%), 12/15(80%) were strong biofilm formers, and 3/15 (20%) were mild biofilm formers. However, the antibiotic disc diffusion test using six clinically important antibiotics showed that most Strep. anginosus isolates were extremely resistant to the antibiotics, especially the β-lactams, including Amoxicillin 13(86.6%), Cefixime 12(80%), Ceftriaxone 12(80%), Meropenem, 11(73.33%) Cefotaxime 10(66.66%), and Imipenem 9(60%).

Aim to study:

The study aims to test Streptococcus anginosus biofilm formation, hemolysin, siderophores, protease synthesis, and β-lactam antibiotic resistance.

Keywords:

Acute inflammation, Surgical tooth extraction, virulence factors, and biofilm and *Streptococcus anginosus*.

Introduction:

In order to remove a tooth surgically, additional surgical processes must be taken, which are unnecessary for non-surgical extractions. The extraction procedure necessitates an incision in the gum tissue, which the dentist will make (Seedat et al., 2018). Until the late 1900s, dental abscesses were little discussed in medical literature (Inchingolo et al., 2020). The mortality and morbidity associated with this clinical entity were commonly underappreciated (Dong et al., 2018). An abscess in the alveolar bone near the tip of a tooth's root is referred to as a dental abscess or dentoalveolar abscess. Dental cavities, trauma, extensive fillings, and unsuccessful root canal therapy are common underlying causes (Zarban et al., 2017). Root canals are colonized by a wide variety of bacteria once the pulp chamber has been compromised (Vieira et al., 2020). The ability of these microbes to create biofilms in root canals lends credence to the "biofilm concept" as a useful framework for understanding and treating root canal infections (Tejashree & Annaji, 2021). Dentoalveolar abscess is caused by a microbial community that includes facultative anaerobes such Streptococcus viridans and Streptococcus anginosus, as well as stringent anaerobes like Anaerobicus spp., Prevotella spp., and Fusobacterium spp (Nwaokorie et al., 2020). Septicemia, cavernous sinus thrombosis, brain abscess, shock, and occa-

sionally death can occur if not treated promptly after the onset of these symptoms (Chigurupati & Shemkus, 2020). Abscesses and systemic infections caused by Streptococcus anginosus species were later recognized as normal human flora (S. milleri strains have been isolated from oral, throat, stool, and vagina cultures) (Sasaki et al., 2018). Apart from other pathogenic streptococci, S. anginosus is notable for its capacity to induce abscesses (Altay, 2019). Once isolated from a human host, especially a youngster, S. anginosus should be treated as a genuine pathogen. Gram-positive, catalase-negative Streptococcus anginosus is a common gut and oral commensal (Nayak et al., 2019). The highly virulent Streptococcus anginosus is known to produce invasive pyogenic infection, which may require immediate surgical intervention (Patel et al., 2020). Disease-causing and virulence-factor-producing Streptococcus species are common, although not all of them. Virulence mechanisms within Streptococcus anginosus have been found, however, which allow for invasion of host cells, evasion of host immunological activity, dissemination, and colonization of host tissues (Sitkiewicz, 2018). Beta-lactam antibiotics are used to manage and treat bacterial infections, and Streptococcus anginosus displays the -hemolytic phenotype on sheep's blood agar (Chang et al., 2021). In the case of bacterial infections, beta-lactam antibiotics are the first line of treatment. Members of an interdisciplinary healthcare team will benefit from this activity since it will focus on the mechanism of action, the adverse event profile, and other important elements (such as off-label usage, dosage, pharmacodynamics, pharmacokinetics, monitoring, relevant interactions) (Rizk et al., 2017).

Materials and methods:

A.Patients and collection of samples:

For this cross-sectional study, researchers looked at data for a full calendar year, from May (2021) to April (2022). Acute inflammation following surgical tooth extraction brought about 250 individuals to a Hilla city dental facility. Each case›s abscess was sampled using sterile cotton swabs in accordance with established protocols for subsequent microscopic investigation and bacterial isolation. In order to prevent contamination, specimens were carefully collected. At the bedside, an aliquot of the sample was promptly put into Blood agar media for aerobic culture. After inoculating Blood agar and Nutrient agar media, the remainder of the specimen was sent to the Department of Microbiology for additional analysis while being incubated aerobically at (37oC) for (24) hours. For Streptococcus anginosus identification, the gram stain, colony morphology, biochemical test, and Vitek 2 system were used to determine the bacteria›s identity.

Ethical Approval:

B.Each patient gave informed consent before they were enrolled in the trial.

C.Identification of bacterial isolates by gram stain, biochemical tests:

Individual isolates were identified using a battery of assays that considered cultural, morphological, and biochemical factors (Baron et al., 1994).

D.Identification of Streptococcus anginosus isolates with Compact VITEK-2 System:

The Compact VITEK-2 System identified all Streptococcus anginosus isolates (BioMerieux). Biochemical reactions identify isolates in this phenotypic identification. The Vitek-2 card has 64 wells for fluorescence biochemical experiments. 20 of 64 carbohydrate absorption tests were phosphatase, urea, nitrate, and actidione. The Vitek-2 machine automatically filled, sealed, and transferred cards to the 35°C incubator. Algorithms decode each output report. ID-GP databank identified the results. These systems› software suggests ID results. If the initial findings showed «poor discrimination» or «no ID,» the tests were repeated and used for data analysis. Inoculated culture media was incubated overnight at 37°C with all strains. Phenotypic VITEK-2 Systems was utilized to identify a single colony (BioMerieux).

E.Hemolysin production:

Inoculating bacterial isolates on blood agar medium at 37°C for 24–48 hours produced hemolysin. A clear zone surrounding the colonies indicated complete hemolysis (β-hemolysis), a greenish zone indicated partial hemolysis (α-hemolysis), and no zone indicated non-hemolysis (Johnson et al., 1980).

Iron uptake by Siderophore:

Siderophore-based iron uptake was tested in M9 medium. Bacteria were injected into media and incubated anaerobically at 37°C for 48 hours. Bacterial growth showed that siderophores might use FeSO4 as an iron source (Kvitko et al., 2012).

F.Extracellular protease production:

M9 medium detected protease enzyme. After sterilization in autoclave and cooling at 50°C, the medium was supplemented with 1% casein and 0.25 gm/L filtered glucose. Agar medium holes were inoculated with (20µl) bacterial broth and incubated at 37°C for 24 hours. Protein was precipitated with 3ml of 3% trichloroacetic acid. The colony›s transparent area showed success (Kobayashi et al., 2000).

G.Biofilm Production

Tissue culture plate method (TCP) assay, also known as semi quantitative micro titer plate test (biofilm assay), was the standard biofilm detection test (Pierce et al., 2008).

1.Isolates from fresh agar plates were inoculated in tryptcase soya broth (TSB) containing 1% glucose, incubated anaerobically for 72 hrs. at 37°C, and then diluted 1:100 with TSB.

2.Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates were filled with 150 µl of the diluted cultures. Only broth was used as a control to check media non-specific binding. Each isolate was triple-inoculated.

3.Tissue culture plates were incubated at 37°C for 24 hours. Tap water gently removed well contents after incubation. Four times with phosphate buffer saline (pH7.2) removed free-floating bacteria from the wells.

4.Plate biofilms were fixed in 37°C oven for 30 minutes.

5.Crystal violet (0.1% v/v) discolored all wells. Plates were dried after deionized water washed off excess discoloration.

6.150µl of acetone/ethanol (20:80, v/v) combination dissolved bounded crystal violet. The Table (1) interpreted the optical density (O.D.) at 570 nm.

Table 1: Differentiating biofilm types using the TCB approach

Mean of O.D. value at 630 nm	Biofilm formation
<0.120	Non
0.120-0.240	Moderate
>0.240	High

F. Susceptibility to Antibiotics Determined by a Disk Diffusion Assay (DDT):

A pure bacterial culture was used. The inoculum for this test was created by adding growth from (5) isolated colonies established on blood agar plates to (5 ml) of nutrient broth and

incubating for (2) hours to form a moderately turbid bacterial solution that compared to the ready-made (0.5) McFarland tube standard. The uniform culture inoculum was swabbed on Muller–Hinton agar plate with a sterilized swab.

1.Flamed forceps placed antibiotic discs on the medium at uniformly spaced intervals, then incubated at 37°C for 18 hours to find hetero-resistance cells.

2.Transparency rulers measured antibiotic inhibition zones. Comparing zone size to conventional zones determined antibiotic susceptibility (CLSI, 2016).

Results and discussion:

Samples were cultured at (37oC) for (18-24) hours from the mouths of 250 individuals who visited a dental facility in Hilla City after experiencing acute inflammation following surgical tooth extraction. They were then grown at (37oC) for (18-24 hours) on a variety of selective medium. Initially, Streptococcus anginosus was identified based on colonial morphology, microscopically, and biochemical tests; however, only 25(10%) of 250 isolates belonged to Streptococcus anginosus by biochemical tests, while 225(90%) were related to other types of microorganisms. Fifteen (60%) Streptococcus anginosus clinical isolates have been identified by specific positive cards of biochemical (Table 2).

Table 2: Strep. anginosus could be identified based on colony morphology, microscopy, biochemical testing, and the use of specific positive cards from the Vitek 2 system.

Total No. of samples	Initial identification of Strep. anginosus	Other microorganisms	Identification of Strep. anginosus by Vitek 2 system
250	25(10%)	225(90%)	15(60%)

These findings corroborated those of Hessan & Jassam, 2021), who also discovered that Strep. anginosus was the pathogenic bacterium isolated from dental abscesses following surgical tooth extraction. Issa et al., (2020) discovered that the microbiome of an acute dental abscess was polymicrobial, including both facultative anaerobes like Streptococcus anginosus and stringent anaerobes such anaerobic cocci like Prevotella. Dental infections and associated consequences have a complex etiology that is heavily influenced by a wide range of host variables. Certain subsets of the population have been identified as being particularly vulnerable (Wan et al., 2020). Dental abscesses are caused by a diverse group of bacteria, including both stringent and facultative anaerobes (Bhambri, 2020). About 20% of dental abscesses are caused by stringent anaerobes (Ibrahim et al., 2021). Even though there is a wide range depending on the patient's prognosis, it has been noted that pure cultures from an acute dental abscess are uncommon, and mixed aerobic infections are even rarer, accounting for just 6% of abscesses (Guet-Revillet et al., 2017).

The ability of Strep. anginosus isolates to produce hemolysin was studied. After 24 hours incubation at (37oC), the results showed that 10(66.66%) of the isolates were able to produce hemolysin in blood agar, as evidenced by a clearance zone around the colonies that suggested -hemolytic activity along the streak on blood agar plate as shown in Table (3). Moreover, the capacity of Strep. anginosus isolates to synthesize Siderophores was also examined. Five (33.33%) of the Strep. anginosus isolates tested positive for Siderophore production. Nonetheless, all of the isolates demonstrated protease activity by hydrolyzing a protein substrate. After (24) hours of aerobic incubation, our data show that all Strep. anginosus isolates exhibited the potential to produce extracellular protease. This was determined by testing isolates on brain heart infusion agar with M9 (supported by 20% glucose and 1% casaien). After adding 3ml of 5% trichloroacetic acid, a transparent halo formed around the colony as shown in Table (3).

Table (3): some virulence factors of *Strep. anginosus*

Virulence factors	Clinical sample No.	%
Hemolysin	10	66.66%
Siderophore	5	33.33%
Protease	30	100%

It has been proven to be cytolytic for both erythrocytes and mammalian cells in culture, which is consistent with the findings of Tuipulotu et al., (2020). All of the isolates demonstrated erythrocyte lysis activity. Scientists have found evidence that certain clinical isolates were highly harmful and accountable for human diseases by virtue of the poisons they secreted (Olchowik-Grabarek et al., 2020). Strep. anginosus produces an enzyme and a toxin (together referred to as cytotoxic factors), and there is evidence to suggest that this enzyme has a tight association to β-heamolysin synthesis (Malovichko et al., 2019). In order to survive, pathogenic bacteria have developed a variety of strategies for scavenging iron from their natural habitats. The release of iron complex from heme and hemoglobin within cells is one such mechanism (Klebba et al., 2021). The haemolysins produced by bacteria are a crucial virulence factor. Haemolysins are a type of bacterial cytolysin that belongs to a broad family of pore-forming cytolysins that can cause cytoplasmic content leakage and cell death by rupturing the cellular membrane (Banerji et al., 2021). For pathogenic bacteria, the ability to absorb iron from the host is crucial. In order to obtain the iron, they need for their metabolic processes, bacteria produce and secrete small organic molecules called siderophores (Ganz, 2018). These findings corroborate those of Aldarhami et al., (2020) who showed that Strep. anginosus generated a protease enzyme. One of the virulence factors vital for Strep. anginosus bacteria is a protease enzyme that is secreted outside of the cell during the growth process and accumulates significantly in the phase stability of the bacterium (Yumoto et al., 2019).

Quantitative biofilm development investigations in a microtiter (biofilm assay) were also conducted with Trypticase Soy Broth containing 1% glucose. To ensure the highest level of precision in this test, it was performed three times. The results were broken down into categories of non-biofilm forming (mean OD value 0.120), moderate biofilm forming (mean OD value 0.120–0.240), and severe biofilm forming (mean OD value >0.240). All of the Strep. anginosus isolates tested positive for biofilm formation

(100%), with 12/15 (80%) classified as strong biofilm formers and 3/30 (20%) as moderate biofilm formers. Table 4 displays the outcomes.

Table (4) Production of biofilm in *Strep. anginosus*

Bacterial isolate No.	Biofilm			
	Strong	Moderate	Weak	% of biofilm Formation
Strep. anginosus (15)	12(80%)	3(20%)	0(0%)	100

Biofilms are communities of organisms attached to a surface and contained within an extracellular matrix mostly made of polysaccharides, proteins, nucleic acids, lipids, and other macromolecules and chemicals (Alves-Barroco et al., 2020). To be more specific, extracellular polysaccharides are an essential part of the matrix and serve multiple purposes, such as facilitating cell adhesion, constructing and maintaining biofilm architecture, and shielding cells from environmental stresses and predators like antimicrobials and host defenses (Karygianni et al., 2020). At least three polysaccharides (alginate, Pel, and Psl) produced by Strep. anginosus are determinant for the stability of the biofilm structure (Kanwar et al., 2019). Biofilms have been shown to be more resilient against antimicrobial agents (such antibiotics, surfactants, and disinfectants) than their planktonic counterparts (Zlotnicki et al., 2021).

Nonetheless, six distinct therapeutically relevant antibiotics were used in the disc diffusion test. The results of this investigation demonstrated that most Strep. anginosus isolates were extremely resistant to the antibiotics utilized, especially the -lactams, including Amoxicillin (13/86.6%), Cefixime (12/80%), Meropenem (10/66.66%), Cefotaxime (12/80%), Ceftriaxone (11/73.33%), and Imipenem (9/60%). Figure 2 displays the obtained results.

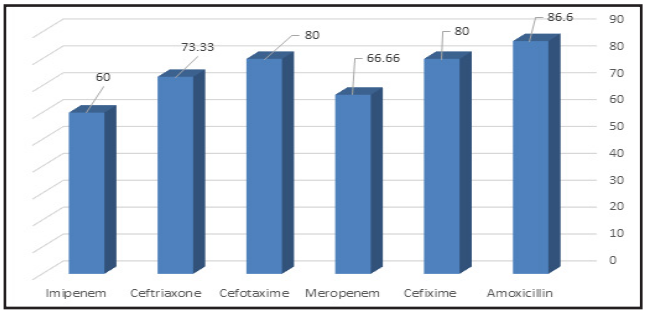

Figure (1): Antibiotic resistance among Strep. anginosus strains

This conclusion was consistent with the findings of a local investigation by Jubeh et al., (2020), who also discovered that Strep. anginosus had developed a resistance to -lactam antibiotics. When dealing with gram-positive infections, ceftriaxone, a cephalosporin of the third generation, is the drug of choice. A total of 63.33 percent of the Strep. anginosus isolates tested were resistant to this antibiotic, correlating with the results of Tan et al., (2018), who identified a 60 percent rate of resistance to ceftriaxone among the same strain of bacteria, as well as those of Tedijanto et al., (2018), who discovered that Ceftriaxone resistance was 55.9%. As a kind of Strep, anginosus, it is naturally resistant to a wide range of antibiotics. Resistance to many antibiotics, both initially and through chromosomal changes, poses a significant treatment challenge (Oechslin, 2018). In most cases, the presence of multidrug resistance is attributable to a constellation of several resistance mechanisms. Common to multidrug-resistant Staphylococcus anginosus isolates are efflux pumps, which limit the buildup of antibacterial medicines within the bacterium by expelling them from the cell before they can reach a sufficient concentration at the site of action (Aminov, 2019). Kim & Lee (2020) observed that 93.9% of Strep. anginosus isolates are resistant to Amoxicillin, which is consistent with the results of the present investigation, in which a similar percentage of isolates showed resistance to the antibiotic. Mutations in outer membrane proteins like OprD that reduce the drug's permeability, the carbapenem hydrolyzing enzymes carbapenemases, and efflux mechanisms are all potential causes of carbapenem resistance (Uppalapati et al., 2020). Other research showed that Strep. anginosus strains have a sensitivity profile to antimicrobial drugs typical for the genus, with the exception of resistance to Cefotaxime, a third-generation Cephalosporin, and Imipenem (Kärpänoja, 2017).

Conclusion:

Most Strep. anginosus clinical isolates produce many virulence factors like hemolysin, sidrophores, and protease; biofilm formation is considered an important ability to produce disease; and Strep. anginosus is highly resistant to -lactams, especially Amoxicillin and cefixime.

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