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PHENOTYPIC DETECTION OF SOME VIRULENCE FACTORS AMONG *STREPTOCOCCUS ANGINOSUS* ISOLATED FROM PATIENTS SUFFERING FROM ACUTE INFLAMMATION AFTER SURGICAL TOOTH EXTRACTION

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ABSTRACT:

In this study, one hundred fifty sufferers have been go to dental clinic in Hilla town affected by acute inflammation after surgical enamel extraction, the samples have been cultivated at (37ºC) for (18-24) hrs. Then, they cultured on numerous selective media at (37ºC) for (18-24) hrs. Identification of *Streptococcus anginosus* changed into trusted the colonial morphology, microscopically, and biochemical exams as preliminary identification, all of the scientific specimens have been effective culture; out of one hundred fifty specimens most effective 35(23%%) isolates have been belonged to *Streptococcus anginosus* via way of means of biochemical exams, whilst 115(77%) associated with different kinds of microorganisms, whilst *Streptococcus anginosus* scientific isolates have been recognized to be 30(20%) via way of means of particular effective playing cards of Vitek 2 system. *Strep. anginosus* isolates have been investigated for this to produce hemolysin. The outcomes confirmed that 16(53.33%) isolates have been capable of produce hemolysin in blood agar that shape of clearance sector surrounding the colonies indicated β-hemolytic hobby alongside the streak on blood agar plate inside 24 hours of incubation at (37ºC). In addition, *Strep. anginosus* isolates had been additionally investigated for this bacteria to supply Siderophores synthesis. The effects display that 14(46.66%) isolates of *Strep. anginosus* are capable of produce Siderophores. However, all isolates of bacteria to supply protease via way of means of hydrolyze the protein. Our effects monitor that *Strep. anginosus* isolates had been capable of hydrolyze the protein via way of means of protease enzyme (100%) while examined via way of means of the usage of M_9 (supported via way of means of 20% glucose and 1% Csaien), became investigated, and it became observed all isolates had the bacteria to supply extracellular protease after (24) hours of incubation. A clean hallo of obvious vicinity became observed across the colony after the addition of (3ml) of 5% Trichloroacetic acid. In addition, In a microtitter (biofilm test), the use of Trypticase Soy Broth complemented by (1 percent) glucose was completed for the quantitative biofilm formation experiment. This test was repeated three times to increase the precision of the assy. The findings showed that all the Strep. anginosus isolates had once been biofilm (100%), the solid biofilm was 23/30, (76,66%) and that some small biofilm forms were also 7/30 isolates (23.33 percent).However, the antibiotic disc diffusion take a look at become executed using (6) distinct clinically crucial antibiotics this take a look at confirmed that maximum of *Strep. anginosus* isolates had been extraordinarily resistant in opposition to the antibiotics used especially the β-lactams, along with Amoxicillin 29(96.66%), Cefixime 23(76.66%), Meropenem 20(66.66%), Cefotaxime 19(63.33%), Ceftriaxone 18(60%) and Imipenem 16(53.33%).

Aim to study: The aimed to phenotypic detection of some virulence factors such as heamolysin, siderophores, protease production and detection of biofilm formation in *Streptococcus anginosus* and resistance of these bacteria against β-lactams antibiotics.

Keywords: Surgical Tooth Extraction, Acute inflammation, *Streptococcus anginosus*, Virulence Factors, Biofilm formation.

I. INTRODUCTION:

Surgical extraction of the tooth is a procedure that is used to remove a tooth which involves additional operational steps which are not necessary during routine extractions. The dentist makes an incision on a section of the gum tissue during the operational extraction (Enriquez, 2021). Dental abscess was a subject of medical science poorly discussed until the 1900s (Szreter, 2019). The morbidity and death of this clinical entity was often underrated (Dong et al., 2018). dentoalveolar abscess is a name used to describe a localized pus collection in the alveolar bone at the tooth's root apex. Dental cavities, traumas, deep fillings or failed treatment of the root canal are usually secondary (Zarban et al., 2017). Once the intact pulp chamber is broken, a diverse mix of bacteriological agents will colonize the root canals (Leelapornpisid, 2019). These microorganisms can form root canals in biofilm and thus make the "biofilm concept" in such infections plausible (Tejashree & Annaji, 2021). Dentoalveolar abscess pathogenesis is a polymicrobial in nature that consists of a range of faculty anaerobics such as streptococci, Streptococcus and stringent anaeraobics, particularly anaerobic cocci, prevotella and fusobacterium (Nwaokorie et al., 2020). Without early treatmentIt may grow quickly into adjacent anatomical structures that cause severe problems like septicemia, sinus cave thrombosis, abcessive brain, shock and sometimes death (Chigurupati & Shemkus, 2020).The pathogenesis of dentoalveolar abscesses consists of a variety of anaerobic faculties including straptococci and streptococcal and rigorous anaeraobic products (particularly anaerobic coccis, prevotella and fusobacterium) (Nwaokorie et al., 2020). It can grow fast and spread without early care to adjacent anatomical structures and can lead to serious complications including septicemia, cellular sinus thrombosis, abcessive brain and shock, as well as sometimes death (Chigurupati & Shemkus, 2020). While many species of Streptococcus have the ability to produce disease and virulence. However, within Streptococcus anginosus virulence mechanisms have been identified that permit host cell invasion, the evasion of host immune activities, propagation and colonization of host tissues (García López et al., 2020). Streptococcus anginosus also shows β-hemolytic sheep agar phenotype Beta-lactam antibiotics used to treat and manage bacterial infections (Chang et al., 2020). Antibodies to beta-lactam are used for bacterial infection management and treatment. This activity will highlight the mechanisms of intervention, adverse event profile and other key factors for members of the inter-professional healthcare team to treat patients (e, off-label use, dose, pharmacodynamics, pharmacokinetics, monitoring, relevant interactions) (Rizk et al., 2017).

II. MATERIALS AND METHODS:

Patients and collection of samples:

The cross-sectional trial took place from May (2020) until May for a period of 1 year (2021). The dental clinic in Hilla City was visited by 150 patients with acute inflammation following surgical dental extraction. The sample was collected by disposable cotton swabs at the site of inflame and by the standard microscopic testing and insulation procedure of Bacteria from abscess in each case. In order to avoid any contamination, specimens have been carefully collected. In Blood agar media an aliquot of collected specimens was instantly inoculated on aerobic culture side-by-side. The rest of the samples were transmitted to the Microbiology Department for further examination, inoculated in aerobically at (37oC) for (24) hours in blood agar and Nutrient agar medium. Gram stain, colon morphology, biochemistry testing and Streptococcus anginosus identification system Vitek 2 were diagnosed with isolated bacteria.

Ethical Approval:

A. Prior to their inclusion in the study, each patient received valid consent.

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

Identification tests were performed for each isolate according to the cultural, morphological and biochemical features (Baron et al., 1994, McFadden, 2000)

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

Through the compact VITEK-2 system, all Streptococcus anginosus isolates have been tested (BioMerieux). This type of identification is phenotypical, depending on biochemical reactions for the identification of isolates. The Vitek-2 card contains 64 wells containing various biochemical fluorescent tests. The phosphatase, urea, nitrate, and actimidione tests were 20 carbohydrate assimilations of the 64. The Vitek-2 machine automatically checks the card including filling, screening and transfer the cards to the connected incubator (35°C). Each output report is decoded by a certain algorithm. The results obtained were determined by ID-GP (Gram-positive bacteria

identity) database. The ID results of these systems are offered by the respective associated software automatically. The testing were repeated only if "low discrimination" or "no ID" were reported from the initial results, and repeated results were used for data analysis. All strains had been inoculated in crops and subsequently incubated at 37°C overnight. For identification using the VITEK-2 phenotypic method, a single isolated colony was used according to the directions of the producer (BioMerieux).

Hemolysin production:

A. Inoculating bacterial isolate on blood agar media (37 daC) at (24-48) hours was used to produce hemolysine, Simple presence of the area known as a complete hemolysis (β hemolysis), a greenish area around settlements known as partial hemaolysis (α-hemolysis), unknown to the region known as nonhemolysis (α-hemolysis) (α-hemolysis) (Johnson et al., 1980).

B. Iron uptake by Siderophore:

C. The media used in this test was M9 to evaluate Siderophore's ability of iron. The medium was inoculated and incubated bacterially for 48 hours at 37°C. FeSO4 can be used by the bacteria as the iron source by using siderophores if bacterial growth occurs (Kvitko et al., 2012).

D. Extracellular protease production:

E. For the sensing of the protease enzyme, M9 medium was used. The medium was then added by casino(1%) after autoclave sterilization and cooling (50°C) and (0.25) gm/L glucose (filtration-sterilized). The pores were produced with (20 ml) of bacterial broth in agar medium and inoculation of (37° C) by each pores for (24) hours, and the protein was precipitated with (3 ml) (3 percent) of trichloroacetic acid. A positive result was the creation of transparent area around the colony (Kobayashi et al., 2000).

A. Biofilm Production

Assays for the tissue culture plate method (TCP) were considered standard assays for the detection of biofilm formation (also referred to as the semi-QT test (biofilm assay) described by Pierce et al., 2008):

The tryptcase-soy broth (TSB) was inoculated with 1 percent glucose and anaerobically incubated for 72 hours at 37°C and then diluted to 1:100 TSB.

1 Individual pools of sterile, polystyrene plates were filled with 150 µl diluted crops and only broth served for checking the non-specular binding of the medium, of 96 well-flat bottom tissual crop platform. Each isolate has three times been inoculated.

2 The plates were incubated at 37°C for 24 hours. The contents of each well were removed gently by tapping water following incubation. In order that bacterias were removed free-floating, the wells were washed four times with saline phosphate buffer (pH7.2)

3 Biofilms made up of sessile adherent organisms on the plate were fastened to 37°C for 30 minutes by placing them in the oven.

4 5- All violet crystal wells (0.1% v/v) have been stained. Deionized water washing was removed and drying plates held excess stain.

5 A 120 fifty (150) µl acetone and ethanol (20:80, v/v) blend supplemented the dissolution of the border violet crystal. The optical density (O.D.) is recorded at 570 nm and results in the table have been interpreted (1)

Table (1) Classification of bacteria biofilm formation by TCB method

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

F. Antibiotics Susceptibility Test by Disk Diffusion Test (DDT):

It was done using a pure cultivation of a bacterial organism previously identified. The inoculum used for this test has been prepared by adding growth from (5) isolated blood agar colonies to (5 ml) nutrient broth, incubating for (2) hours to a moderate turbidity bacterial suspension, as compared to the ready-made (0.5) standard McFarland tube turbidity. A standardized culture used a sterile swab to obtain an inoculum; this inoculum was then swabbed on the agar plate of Muller–Hinton.

1. The medium's antibiotic disks were evenly spared with forceps flamed and then burnt (37oC) for a full (18) hours before the results could be read to identify the hetero-resistant cells. 1. 1. 1.

2. The inhibition areas of antibiotics were measured with a ruler of transparency. Areas in order to determine organism susceptibility to each antibiotic were compared to standard zones (CLSI, 2016).

III. RESULTS AND DISCUSSION:

150 patients who had been visited in Hilla City Dental Clinic with acute inflammation from surgical tooth extraction were (37oC) grown for (18-24) hrs in this study. Then at (37oC) for (18-24) hours they cultured on several selective media. Streptococcal anginoidal identification was initially determined by colonial morphology, microscopic and biochemical tests. The positive culture was found in the clinical specimens; only 35%(23%) of the 150 specimens belonged to Streptococcosus anginosus through biochemical tests., While other types of microorganisms are 115(77%) as shown in figure (1), clinical isolates of streptococcus anginosus have been identified to be 30(20%) with specific positive Vitek 2 cards as shown in the Table Table (2).

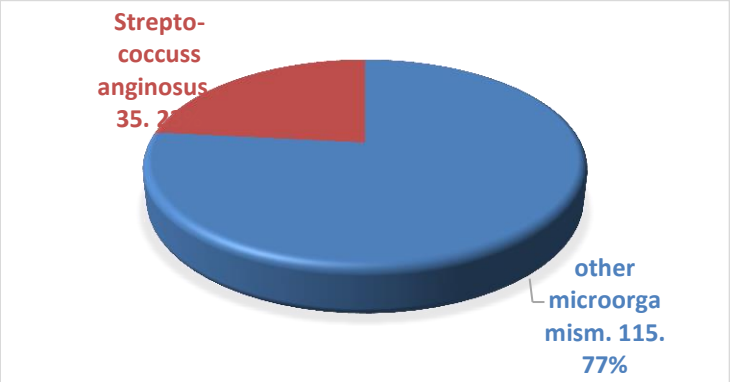

Figure (1): Strepe identification. anginosus was based as initial identification upon colonial morphology, microscopic, and biological tests

Table (2): Strep identification: anginosus was dependent on colonial morphology, microscopic, biochemical tests and Vitek 2 system specific positive cards

Total No. of samples	Initial identification of *Strep. anginosus*	Other microorganisms	Identification of *Strep. anginosus* by Vitek 2 system
150	35(23%)	115(77%)	30(20%)

These findings were identical to those from Chen et al. (2020), which found that after surgical dental extraction, Strep anginosus was pathogenic bacteria isolated from the abscess. Issa et al. (2020) found that anaerobic cocci (such as Prevotella, Fusobacterium and facultative anaerobic species) including strictly anaerobic abscess iwaspolymicrobe (such as Streptococcus anginosus). During the pathogenesis of dental infections and their complications, various host factors play an important role. Specific 'at-risk' populations have been identified (Wan et al., 2020). The complex mixture of strict anaerobic and optional anaerobic agents involved in the causation of dental abscesses (Bhambri, 2020). In approximately 20 percent of cases dental abscesses caused by severe anaerobics occur (Ibrahim et al., 2021). Although there are a large range of pure cultures from acute dental abscesses, depending on the conditions of the rehabilitation, mixed aerobic infections are uncommon and account for 6% of abscesses (Guet-Revillet et al (2017). *Strep. Angino isolates for their hemolysin production capabilities were investigated. The results showed, that, within 24 hours of incubation at (37o C), 16(53.33%) isolates were able to produce hemolysin in blood agar, which is a form of clearance zone around the colonies, indicating β-*

hemolytic activity along the streak on blood agar plate. The results have been shown in table (3). Furthermore, Strep anginosus isolates have also been studied in order to produce siderophores. Results indicate that Siderophore can produce 14 (46.66 per cent) Strep. anginosus isolates.. Tables showed the results (3). All isolates, however, were able to hydrolyze the protein to produce Protease. Our data show that Strep. anginosus isolates have hydrolyzed the protein through the protease enzyme (100 percent) Tested on M9 (supporting 20% glucose and 1% casaien) brain heart infusion agar, all isolates were found to be capable of producing extracellular protease after (24) hours of aerobic incubation. After adding 5 percent trichloroacetic acid (3ml), a clear hallo of transparent area has been found around the colony. Table showed the results (3).

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

Virulence factors	Clinical sample No.	%
Hemolysin	16	53.33%
Siderophore	14	46.66%
Protease	30	100%

These results similar to Tuipulotu *et al*., (2020) as demonstrated to be cytolytic to crop mammalian cells and erythrocytes. The erythrocyte lysis of all isolates was possible. Researchers show that clinical isolates are highly pathogenic and responsible for human diseases, as various toxins are secreted (Olchowik-Grabarek et al., 2020).Certain reports showed that β-heamolysin, produced from anginosa, is very close to producing toxins in the cell enzyme and toxin, referred to as cytotoxic factors (Malovichko et al., 2019). A series of mechanisms for iron acquisition from their surroundings develop pathogenic bacteria. One is hemolysin, which releases iron complex into intracellular hema and hemoglobin (Klebba et al., 2021). Hemolysins are one of the major virulence factors in bacteria. The haemolysins belong to a large group of poreforming bacterial cytolysins which, breaking the membrane and finally the cell death, can cause cytoplasmic content leakage (Banerji et al., 2021). The ability of pathogenic microorganisms to produce host iron is crucial for their survival, as microorganisms need iron for a number of metabolic processes, thus synthesizing and secreting small organic molecules that are called siderophores (Ganz, 2018). Siderophors are low-molecular metal chelating agents that deliver an efficient Fe-acquisition system for microorganisms due to their great affinity with Fe (III) complexation (Chaudhary et al., 2017). Iron (Fe) is a critical element for the growth of nearly all living microorganisms as it acts as catalyst for enzymes, oxygen metabolism, transfers of electrons and synthesis of DNA and RNA (Sah et al., 2017). These findings have been agreed with Aldarhami et al. (2020) that Strep. Anginosus produced the protease enzyme. Protease enzyme secreted outside the cell by a growth process as it builds up in a significantly stable phase in bacteria, and it is one of the most important virulence factors of Strep (Yumoto et al., 2019). Extracellular protease plays a significant role in the survival and the communication of cells (Wan et al., 2018). In furthermore, quantitative biofilm training experiments with (1%) glucose were carried out in a microtitter (biofilm test) using trypticase SoyBroth. This test was repeated three times to enhance the precision of assis. The result was interpreted as none, modest and strong former BioFilm with the average of OD value in (63) nm when the average OD value was (<0.120, 0.120-0.240, and >0.240). The results show that all Strep. anginosus isolates were former biofilms (100 percent), the former strong biofilms (23/30(76.66%), while the modest biofilm isolates represented 7/30 of them (23.33 percent). The results have been shown in the table (3-3).

Table (3-3) Production of biofilm in *Strep. anginosus*

Bacterial isolate No.	Biofilm			
	Strong	Moderate	Weak	% of biofilm Formation
Strep. anginosus (30)	23(76.66%)	7(23.33%)	0(0%)	100

Biofilms consist primarily of polysaccharides, proteins, nuclear acids, lipids, other macromolecules and chemicals, and are surface-related communities within an extracellular matrix (Alves-Barroco et al.,2020). Extracellular polysaccharides are especially critical in the matrix and perform a variety, including the promotion of surface attachment and other cellular structure, the building and maintenance of biofilms, and the protection of cells against environmental attacks and predatory effects, including antimicrobials and host protection (Karygianni et al., 2020). Strep. anginosus provides nearly 3 polysaccharides (alginate, pel, and psl) which are decisive for biofilm stability (Kanwar et al., 2019). Biofilms have been documented to have higher resistance

than planktonic agents to antimicrobials, i.es. antibiotics, surfactants, disinfectants (Zlotnicki et al., 2021). Furthermore, their physiology differs substantially from planktonic cells. The biofilm's characteristics are based on a variety of factors such as matrix complexity, substrate available properties, EPS composition and micro-organism metabolism (Tallawi et al., 2017). Antibiotic disc diffusion testing was performed using (6) various clinically important antibiotics, however, most Strep. anginosus isolates were highly resistant to antibiotics used, in particular, βlactams like Amoxicillin 29(96,66 percent), Cefixime 23(76,66 percent), Meropenem 20(66,66 percent) (53.33 percent). The results were illustrated in the table (2).

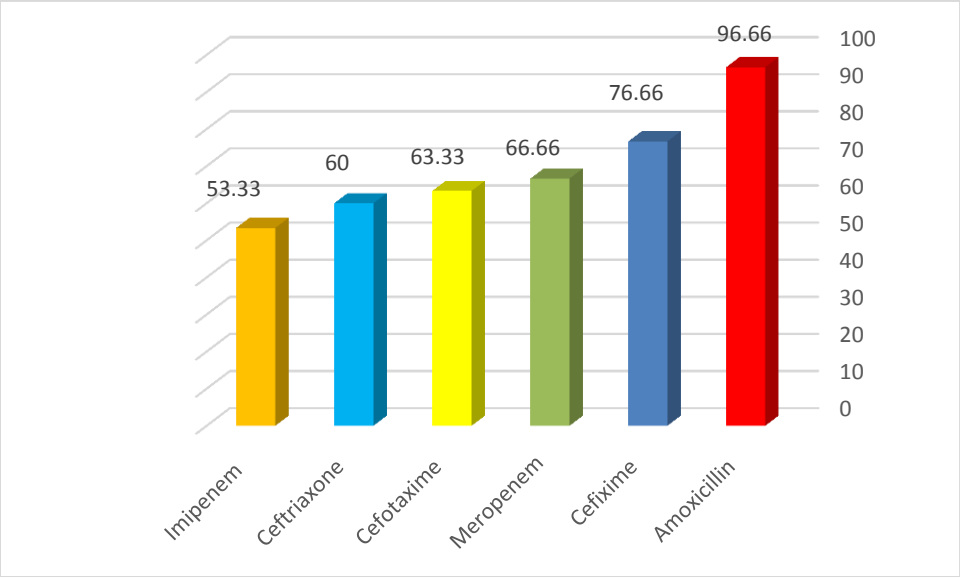

Figure (2): resistant of *Strep. anginosus* isolates against the β-lactams antibiotics

This was based on results from a local Jubeh et al. study (2020), which showed that strep anginosis is an increased resistance to β-lactam antibiotic. Ceftriaxone is the third generation widely used in treating gram-positive inflammation of cephalosporin. The antibiotic resistance rate (63.33 percent) and Tan et al (2018) findings showed that Strep. anginosis was resistant to ceftriaxone (60 percent).Nonetheless, the findings disagreed with those obtained by Rodríguez et al (2020) who said the ceftriaxon-resistance percentage was (34.6 percent) and with the Tedijanto et al. results (55.9 percent). Stretch anginosis that has inherent tolerance to a variety of antifungal agents. A major therapeutical issue arises from the resistance to many antifungal products and its ability to establish multidrug resistance and mutual antibiotic resistance through chrome mutations (Oechslin, 2018). It is also a mixture of mechanisms of resistance that reflects multilateral resistance. Efflux pumps are typical components of multi-drug resistance. Stretches anginosus isolates and prevents an accumulation of antibacterial drugs in the bacterium, which extrudes the cell drugs before they reach a suitable concentration at the place of action (Aminov, 2019). The findings of this study were in line with the results of Kim & Lee (2020) which demonstrated that both Amoxicellin and Amoxicellin have 93,9 per cent of Strep' s isolates (96.66 percent).Carbapenem resistance results from different mechanisms, including mutations in external membrane proteins like OprD, which lead to reduced drug permeability, carbapenem hydrolyzing enzymes carbapenemases and efflux mechanisms (Uppalapati et al., 2020). In addition, the susceptibility profile of Strep. anginosus strains was typical of the genus but was resistant to cefotaxime, the 3rd generation of cephalosporins and imipenem (Kärpänoja, 2017).

IV. CONCLUSION:

Strep anginosus is a cause of the agent of acute inflammation after surgical extraction of the tooth. It is the most frequent of the clinical isolates of the Strep. Anginosus causes numerous virulence factors like hemolysin, sidrophores and protease, can produce biofilm by the method of quantitative processing and the forming of biofilms is considered a major capacity for disease.

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