

BIOFILM FORMATION AND ISOLATION OF STAPHYLOCOCCUS AUREUS FROM BLOOD CULTURES OF DENTAL PATIENTS UNDERGOING ORAL SURGICAL PROCEDURES



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OBJECTIVE: The objective of this study was the detection and separation of *S. aureus* from blood cultures of patients undergoing oral surgical procedures. Antibiotic sensitivity pattern and biofilm formation of *S. aureus* were also performed.

METHODOLOGY: Total 250 patients undergoing oral surgical procedures were selected for bacteriological examination. 5ml of Blood sample was collected in blood culture bottles containing tryptone soya broth. Blood sample was incubated at 37°C for 7 days and after incubation subculturing was done on appropriate Media. The plates were then incubated at 37°C aerobically for 24 hours, after which isolated colonies were obtained. *S. aureus* was identified by Gram staining, colony morphology, pigment production, catalase, coagulase and often biochemical tests. Antibiotic susceptibility was performed by disc diffusion technique on isosensitivity agar. Strains of *S. aureus* were used for biofilm formation by simple tube method. With the help of spectrophotometer at 570 nm optical density was measured. *S. aureus* (ATTC 2523) was analyzed for biofilm production.

RESULTS: Bacterial isolates in descending order were *S. aureus* 56%, *E. coli* 25%, *Pseudomonas* spp. 13%, *S. typhi* 4% and *Shigella* spp 2%. *S. aureus* was resistant to different antibiotics. Biofilm production of *S. aureus* was detected in 16.17% of the *S. aureus* and mostly in association with antibiotic resistant bacteria.

CONCLUSION: *S. aureus* was the predominant group of bacteria isolated from blood cultures of dental patients. Increased antibiotic resistance of *S. aureus* may be due to biofilm production resulting in persistent dental infections.

KEYWORDS: *Staphylococcus aureus*, Biofilm, Antibiotic resistance.

INTRODUCTION

In hospitals and in our community, *Staphylococcus aureus* is a major human pathogen and a primary cause of bloodstream infections. In the form of biofilm, *S. aureus* colonizes the teeth of patients. Biofilms are the most common mode of bacterial growth in nature and are also important in clinical and dental infections which are common in our population.

Most common dental diseases are gingivitis,

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periodontitis, dental caries, pulpitis, pulp necrosis, peripheral abscess, cellulites and pericoronitis.¹ For the treatment of these different dental diseases, numerous dental procedures like tooth extraction, endodontic treatment, periodontal surgery, conservative dental procedures and scaling are performed, that causes significant bleeding and cause the spread of oral bacteria into the blood stream which result in bacteremia.²⁻³

S. aureus is a major human pathogen causing a wide range of infections. Over the last 25 years, the incidence of both community-acquired and hospital-acquired *S. aureus* infections has increased.⁴

Dental diseases are caused by microorganisms which are a part of normal flora that is present in the form of biofilm.⁵ Biofilm is defined as complex aggregation of microorganism surrounded by a protective and adhesive matrix of polymer substances that adheres to all surfaces either inert or living.^{6,7} Van Leeuwenhoek can be credited with the discovery of microbial biofilms who first observe

microorganisms on tooth surfaces. It can form on a wide variety of abiotic hydrophobic and hydrophilic surfaces. These environments are oligotrophic rendering the microbes in a starved state⁸. The biofilm regulate the developmental process that leads to a development of complex surface-attached bacterial community. This bacterial community has a number of distinct characteristics including the production of exopolysaccharides hydrodynamics of the bulk fluid. In this environment, biofilm formation can have profound negative and positive impact and as a consequence, it can increase the cost in term of both economics and human health⁹ Bacteria, in these environment, can grow as a biofilm.

Certain species (Spp) appear to have a predilection to form biofilms. Most of these Spp are members of the normal microflora of human beings and form biofilms at sites where they are found naturally. Streptococci form biofilms on the surfaces of teeth, are cariogenic and periodontal pathogenic bacteria¹⁰.

Bacteria in a biofilm are very resistant to biocides.¹¹ Gene transfer between bacteria is known to be an important means by which antibiotic resistance and virulence factors are spread between members of the same and different Spp.^{12,13} Transfer of gene occurs in biofilms and only a very limited number are involved with human diseases.¹⁴ In *S aureus* biofilm, *S.aureus* is an adaptable, pathogenic organism. It is an opportunistic pathogen and can infect humans resulting in a myriad of infections¹⁵. The close contact between bacteria within a biofilm and the matrix may inhibit the penetration of antibiotics through the exopolysaccharide matrix¹⁶.

The phenotypic heterogeneity of bacteria in a biofilm suggests the presence of persister cells or antibiotic-resistant non-dividing cells which are able to reestablish the biofilm after the threat has passed¹⁷. The oral cavity may also harbor antibiotic-resistant organisms, which are incriminated in many extra-oral systemic infectious diseases.¹⁸.

METHODOLOGY

Total 250 bacterial isolates were collected from blood cultures of dental patients, who underwent different oral surgical procedures. Clinical history and consent was obtained from every patient. Ethical committee of Fatima Jinnah Dental College and Hospital Karachi has given the approval of the study. Blood samples were collected for bacteriological examination immediately after the essential steps of the oral surgical procedures had been

performed. 5ml Blood sample were collected in Tryptic Soya broth (diaphasic media) and incubated at 37°C for 7 days and then subcultured on blood agar and MacConkey's agar.

The plates were incubated at 37°C aerobically, after 24 hours growth was observed. Colonies were identified by gram's staining and biochemical test. Biochemical characteristics were observed by performing catalase, coagulase and other biochemical tests. With the help of disc diffusion technique on isosensitivity agar, antibiotic susceptibility was done.

Test was performed according to the clinical laboratory Standard Institute (CLSI) guidelines. Antibiotic sensitivity test was done by using the discs of ampicillin, clindamycin, vancomycin, tetracycline, cephalexin and cefoxitin. Biofilm assay was done by simple tube method and analyzed by spectrophotometer. *S aureus* strain (ATTC 2523) was used to compare the biofilm production. 68 strains of *S.aureus* were selected for biofilm analysis. Sterilized plastic tubes were taken and under aseptic condition, isolated cultures were inoculated in 2ml TSB (tryptone soya broth) tubes. TSB tubes were then incubated at 37°C for 24 hours. After incubation, to each tube 2ml of TSB with 2% glucose were added. Tubes were reincubated at 37°C for 24 hours. The growth medium was discarded. Each tube was washed 3 times with Phosphate Buffer Saline (PBS) under aseptic condition to eliminate the unbound bacteria. To evaluate the formation of biofilm, remaining attached bacteria were fixed with 2ml of 99% methanol for 15 minutes and biofilm were stained with 0.2 ml of 2% crystal violet. Excess stain was rinsed off by placing the tubes under running tap water. Tubes were air dried and the adherent cells were solubilized with 1.5ml of 33% glacial acetic acid.

The optical densities of each tube were determined at 570 nm by using spectrophotometer. The blank (negative control) was determined for each tube by measuring the optical density (OD) of a tube filled with PBS. For the positive control, pure cultures were used to measure the optical density. Results were recorded and OD of the isolated microorganisms was compared with the OD of pure cultures of *S.aureus*. If the OD of isolated and identified cultures was greater than the OD of pure cultures then it indicated that isolated culture had the ability to form more powerful biofilm than the pure culture.

RESULTS

Total 114 *S.aureus* were isolated from blood cultures of different dental patients. 68 strains of *S.aureus* were used for biofilm production. Figure 1 indicates the bacterial isolates from blood cultures associated with oral surgical procedures. Bacteril isolated in descending order were *S.aureus* 56%, *E.coli* 25%, *Pseudomonas Spp.*13%, *S.typhi* 4% and *Shigella Spp.* 2%.

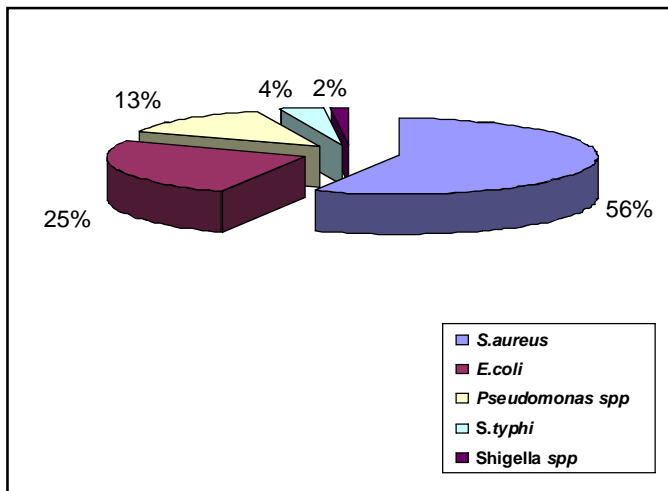


Figure 1: Percentages % of different Bacteria isolated from Blood Cultures of Dental Patients with Oral Surgical Procedures.

Figure 2 indicates the antibiotic sensitivity pattern of *S.aureus*. It was sensitive to ampicillin (98%), clindamycin (79%), vancomycin (77%), tetracycline (77%), cephalexin (71%), and cefoxime (47%).

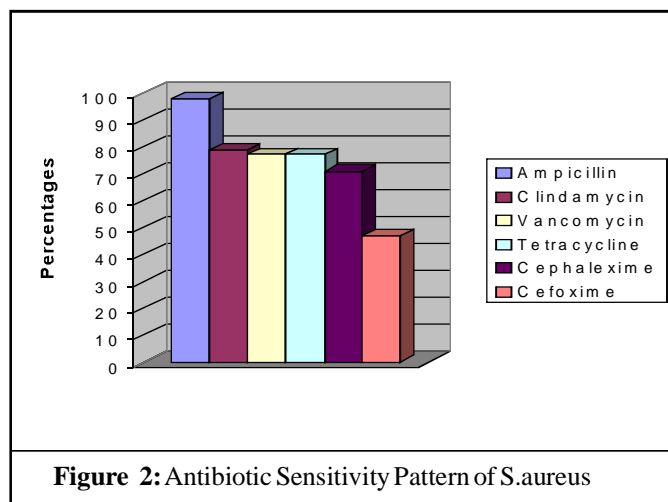


Figure 2: Antibiotic Sensitivity Pattern of *S.aureus*

Figure 3 indicates the Biofilm production of *S.aureus*. Biofilm was detected in 11(16.17%) of

S.aureus. ATCC culture of *S.aureus* (2523) was analyzed for biofilm production and it indicated weak biofilm production.

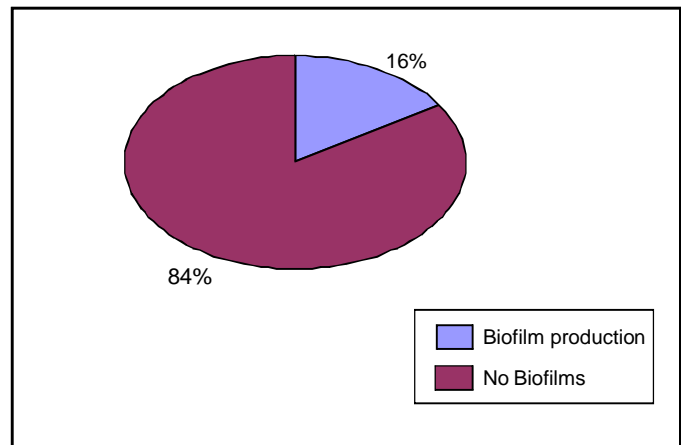


Figure 3 : Biofilm Production by *s.aureus*.

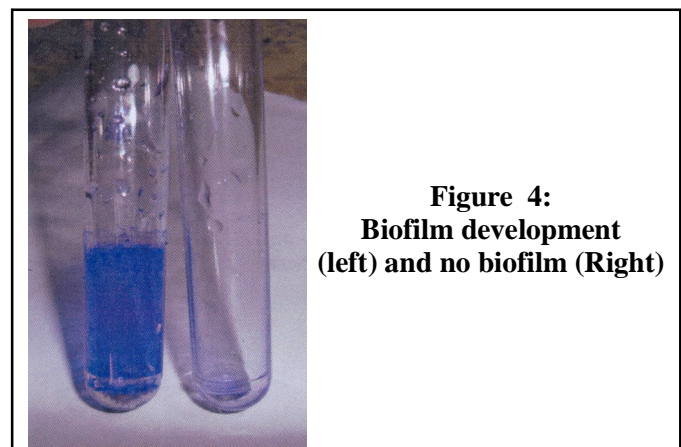


Figure 4: Biofilm development (left) and no biofilm (Right)

Figure 4 is showing a Biofilm production of *S.aureus*.



Figure 5: A Clinical presentation of dental plaque in the form of biofilm. Presence of plaque and calculus on tooth surfaces is major cause of gingivitis and other periodontal diseases.

DISCUSSION

Dental infections are mostly caused by different oral microorganisms due to antibiotic resistance and biofilm production. *S.aureus* is an opportunistic pathogen and approximately 30-50% of healthy children and adults are carriers of *S.aureus*²¹. It is a leading cause of *S .aureus* bacteremia (SAB) and other bloodstream infection.²² It gains entry into the host through breaches in the epidermal layer and cause severe illness.²³ In this study major bacterial isolates were *S.aureus* 56% and other bacterial isolates were *E.coli* 25%, *Pseudomonas Spp.* 13%, *S.typhi* 4% and *Shigella Spp.* 2%. *S.aureus* was sensitive to ampicillin (98%), clindamycin (79%), vancomycin (77%), tetracycline (77%), cephalexin (71%), and cefoxime (47%). 11(16.17%) *S. aureus* strains were positive for biofilm production. Biofilms are common cause of clinical infections and most common mode of bacterial growth in nature due to the high antibiotic resistance.²⁴⁻²⁶ *S. aureus* (ATTC 2523) was used to compare the biofilm production and it indicates weak biofilm formation. The properties of biofilms resulting in their increased resistance to antibiotics are not thoroughly understood. The close contact between bacteria within a biofilm and the matrix may inhibit the penetration of antibiotics through the exopolysaccharide matrix²⁸. Biofilm bacteria also exhibit a slow rate of metabolism and divide infrequently resulting in sensitivity to antibiotics targeted at cell wall synthesis such as penicillins.²⁹ There is an association between occurrence of biofilms and infections in certain human diseases. Oral Biofilm consists of bacterial cells, salivary polymers and bacterial extracellular products that adheres to teeth. These accumulations of microorganisms change the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease.³⁰ Dental plaque biofilms are responsible for different dental diseases³¹.

CONCLUSION

Dental infections are more common in our country due to poor oral hygiene that results in Biofilm production and high antibiotic resistance. It is therefore recommended to maintain the oral hygiene and control dental diseases with comprehensive mechanical and chemotherapeutic oral hygiene practices. Teaching patients to improve oral health by regular tooth brushing, interdental cleaning and use of antimicrobial mouthrinses and conduction of continuing dental education programs to prevent dental diseases and bacteremia during oral surgical procedures is recommended.

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