

Original Research Article

Biofilm: Comparison between the *Staphylococcus aureus* and coagulase negative staphylococcus species isolated from a rural medical college hospital in North Kerala, India

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A B S T R A C T

Keywords

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Biofilm is a serious threat for the patient with infected with the biofilm producing microbes, as it may cause therapeutic failure with regular antibacterial therapy. The Gram positive bacteria are also known to produce biofilm. This study was conducted to know the biofilm producer among the *Staphylococcus* species isolated from the patients attending a medical college in North Kerala. The clinical isolates of *Staphylococcus* species were subjected to speciation and antibacterial susceptibility test by standard methods, then for biofilm producing by tissue culture plate method. Fifty isolates of *Staphylococcus aureus* and Coagulase Negative (CoN) *Staphylococcus* were included in the study. The antimicrobial susceptibility pattern showed that both *Staphylococcus aureus* and CoN *Staphylococcus* species are resistant to Ampicillin, Penicillin and Erythromycin. The rate of MRSA and MRCoN isolation was 6% and 4% respectively. CoN *Staphylococcus* (12%) were more biofilm producer than compared to *Staphylococcus aureus* (4%). Coagulase Negative *Staphylococcus* species have a higher capability of biofilm production and thus a therapeutic failure in infections. Hence CoN *Staphylococcus* cannot be neglected if isolated from the nosocomial infection sample.

Introduction

Biofilm are a group of microbes which are encased in an exo-polysaccharide matrix on both biotic and abiotic surfaces. Various changes occur during their transition from a planktonic to a surface attached community. This causes a number of persistent infections which respond poorly to conventional antibiotic therapy. After adherence to a surface,

organisms adapt to the environment of the biofilm by increasing the secretion of exopolysaccharide. This helps the microorganisms to escape their killing by antibiotics. Biofilm formation is commonly regulated by inter and intraspecies quorum sensing mechanisms. Availability of nutrients, chemotaxis towards the surface, motility of bacteria,

surface adhesion and the presence of surfactants influence biofilm formation in microorganisms (Seema *et al.*, 2011).

Microscopy of biofilm formation *in-vitro* suggests that two steps involved: (1) the attachment of the bacterial cells to the surface, which may occur rapidly; and (2) the growth dependent accumulation to form multilayered cell clusters surrounded by a slimy matrix (Friedrich *et al.*, 2002). Biofilm may be formed by one or several types of microorganism's. The cultural morphology of biofilm forming bacteria is usually different from those strains which do not form biofilms. It has been observed that biofilm forming bacteria attach themselves to solid surfaces by using their sticky appendages and employing a rolling motion, which results in their continuous attachment and detachment to the surface and the formation of micro aggregation. First they detach from the top of microorganisms where apparently the shearing force overcomes the attachment forces; then the micro aggregations roll slightly and attach some place downstream (Gerald *et al.*, 2009).

The two important species which producing biofilm are *Staphylococcus aureus* and Coagulase Negative *Staphylococci* e.g., *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*. *Staphylococcus aureus* is a ubiquitous pathogen. Its role in a variety of clinical conditions has been recognized to include nosocomial infections, septicemia, wound sepsis, septic abortion, osteomyelitis, septic arthritis, post-surgical infections and toxic shock syndrome. *Staphylococcus aureus* infection is one of the most common bacterial infections in AIDS patients (Shittu *et al.*, 2006).

Staphylococcus aureus has emerged as one of the pathogen, and has over the past numerous decades, been a leading

foundation of hospital acquired infections. *Staphylococcus aureus* is well characterized and known to have a diverse arsenal of virulence factors that cause a prominent inflammatory response. Biofilm formation does not only occur with foreign body infections. If one closely examine native tissues removed from patients with recurrent *Staphylococcus aureus* infection the cells are organized with a confluent colonies (Longauerova *et al.*, 2006).

Coagulase negative *Staphylococci* are among the bacteria routinely isolated at various microbiological departments. There were presently 41 taxon, designated Coagulase Negative *Staphylococci* (Longauerova, 2006). Coagulase negative *Staphylococci* can be divided into two groups depending on whether they resistant to or susceptible to novobiocin. Novobiocin susceptible species are *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus chleiferi*, as well as novobiocin resistant species *Staphylococcus saprophyticus* (Christof, 2002).

Staphylococcus species including MRSA can cause painful skin infection resembling a pimple, insect bites, and furuncle and have pus or drainage. Pus is formed by white blood cells that rush in to kill bacteria and enlarge blood vessels – a way for the body to purify infections. The resistance of biofilms to antibiotics is increased compared with what is normally seen with planktonic cells. Infact, when cells exist in a biofilm, they can become 10-1000 times more resistant to the effects of antimicrobial agents (Heikens *et al.*, 2005).

Regarding too many infections due to *Staphylococcus aureus* and coagulase

negative *Staphylococcus* species and increasingly resistant to antibiotics agents, the present study was conducted to identify frequency of *Staphylococcus aureus* and coagulase negative *Staphylococci* isolated from pyogenic infections and determination of biofilm productions and susceptibility pattern of these species to antibacterial agents. Aims and Objectives: To know the antibiogram and biofilm production of the staphylococcus species isolated from the pyogenic infection.

Materials and Methods

This was prospective study was conducted at the MES Medical College, Perinthalmanna for a period of 3 month from April 2013 to June 2013. The pus sample was collected from patients attending the rural medical college Hospital. The sample was inoculated on 5% Sheep Blood agar (SBA) and MacConkey agar, incubated at 37°C for 24-48 hours. The β hemolytic colonies on SBA were subjected to Gram's staining. The gram positive cocci in clusters, catalase positive were identified as *Staphylococcus* species. The tube coagulase test was done to differentiate between *Staphylococcus aureus* and Coagulase Negative *Staphylococcus* species.

The isolates identified as Coagulase negative Staphylococci and Coagulase positive Staphylococci were subjected to test for biofilm production described by (Christensen *et al.*, 1985) by Tissue Culture Plate and antibiotic susceptibility according to CLSI guidelines (Wilker *et al.*, 2009).

Results and Discussion

A total of 100 isolates of *Staphylococcus*

species were isolated during the study period, of which 50 isolates of *Staphylococcus aureus* and 50 Coagulase negative Staphylococci were included in the study.

The antimicrobial susceptibility pattern showed that both *Staphylococcus aureus* and CoN *Staphylococcus* species are resistant to Ampicillin, Penicillin and Erythromycin. It was observed that the CoN *Staphylococcus* Species were resistant to Cotrimoxazole (24%) compared to *Staphylococcus aureus* (4%). These are the common drugs used in the community for most of the infectious disease. Of the 100 *Staphylococcus* species isolated, 6% strains were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 4% strains were Methicillin Resistant Coagulase Negative *Staphylococcus* (MRCoNS). No isolates were resistant to Vancomycin, Linezolid and Teicoplanin. The above figure shows that the CoN *Staphylococcus* species were biofilm producer than the *Staphylococcus aureus*. Higher percentage of CoN *Staphylococcus* (12%) were highly biofilm producer than *Staphylococcus aureus* (4%).

Staphylococcus species are known to produce biofilm in device related infections. These biofilms prevent the entry and the action of the antibacterial administered and rendering the pathogen drug resistant.

In modified TCP method, extended incubation for 24hour could lead to a better discrimination between moderate and non-biofilm producing Staphylococci and biofilm forming formation was observed in 62% isolates. This observation suggested a strong dependence between growth condition and biofilm formation in *Staphylococci*.

Fig 1. Age-wise Distribution Of Patient In Whom *S. aureus* And Coagulase Negative *Staphylococcus* Isolated

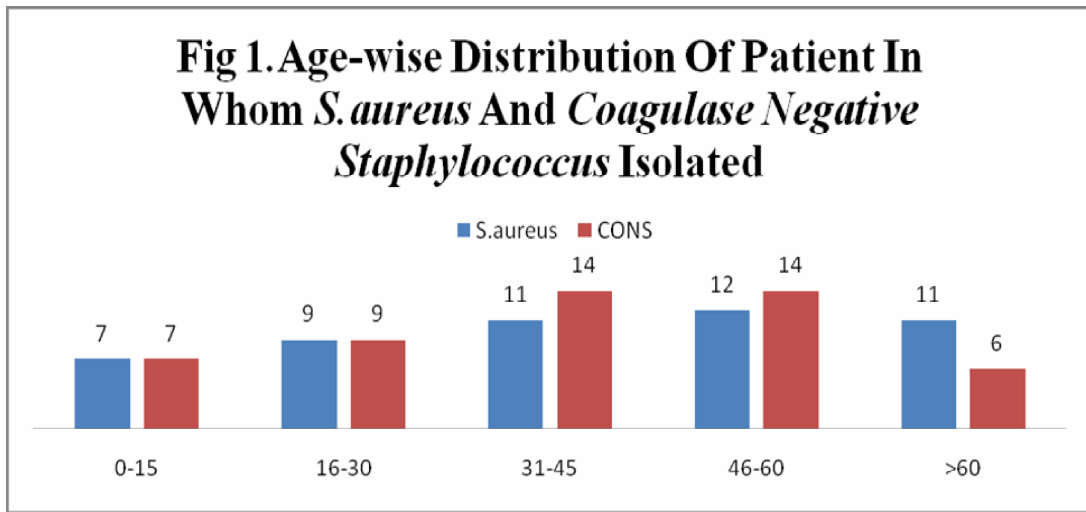


Fig 2. Antibiogram of Isolates Studied

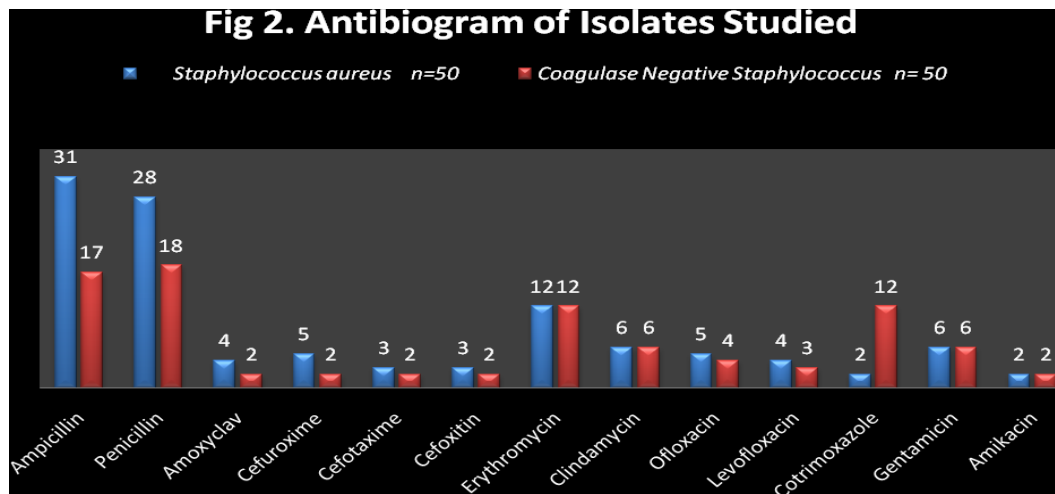
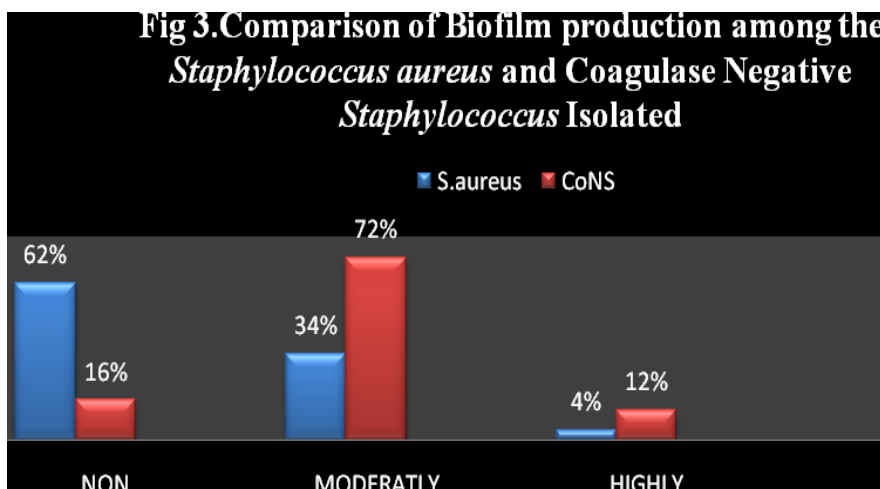


Fig 3. Comparison of Biofilm production among the *Staphylococcus aureus* and Coagulase Negative *Staphylococcus* Isolated



This study was similar to that of study done by (Christensen *et al.*, 1985) shows that 53.2% were producing biofilm.

In our study among the *Staphylococcus aureus* isolates studied 62% were non biofilm producers, 34% were moderate biofilm producers and 4% strong biofilm producers, and in CoN *Staphylococci* 16% were non biofilm producers, 72% were moderate biofilm producers and 12% highly biofilm producers. It was found that CoN *Staphylococci* isolates were strong biofilm producer. In a study done in Western India, it was observed similar percentage of the Staphylococcal isolates were biofilm producer, 15.64 % were highly biofilm producers, 38.55% were moderate biofilm producers and 45.81% were non biofilm producers. (Bose S *et al.*, 2009).

In this study antibiotic susceptibility pattern of various biofilm producers and non-producer *Staphylococci* spp. isolated from clinical materials were obtained. The significant and clinically relevant observation was that the high resistance shown by biofilm producers to conventional antibiotics than non-biofilm producers. These observations supported the study by (Gürkan Mert *et al.*, 2011). All the strains were sensitive to linezolid and vancomycin.

The Susceptibility pattern of *Staphylococcus aureus* against antibacterial agents in this study showed that the majority of the resistance of *Staphylococcus* was belonging to Ampicillin (31%), Penicillin (28%), Cephalexin (24%) and Erythromycin (12%) and there is no resistance towards Vancomycin, Teicoplanin, and Linezolid. This study was supported by Asma Bashir *et al* (2007) that most effective antibiotic

for *Staphylococcus aureus* vancomycin showing 80.5% efficacy, then methicillin with 68.0% efficacy, erythromycin with 55.6% efficacy (Asma Bashira *et al.*, 2007).

Staphylococcus aureus is highly versatile and adaptable pathogen capable of causing a diverse array of infections in hospitals and community settings. *Staphylococcus aureus* has been reported to be the cause of most wound infections.

In this study prevalence of MRSA was 6%. The MRSA also showed resistance to cotrimoxazole, erythromycin, and gentamicin. This study was supported by (Murugan *et al.*, 2008), as expected all the strains were resistant to ampicillin and most of them to penicillin. But significant and clinically relevant observation of their study is moderate resistance shown by MRSA to other conventional antibiotics.

Coagulase negative *Staphylococci* have emerged in recent years as pathogens in a growing number of nosocomial infections. Production of an exopolysaccharide, allowing adherence and subsequent formation of a multilayered biofilm, appears to be essential for the pathogenesis of Coagulase negative *Staphylococcus* species. Because many isolates are multi-drug resistant, their infections are difficult to treat and can even be fatal. A detailed characterization of isolates of CoNS through speciation, genetics and antibiotic susceptibility may be necessary to distinguish infecting from contaminating isolates and to plan suitable therapy. In this study, the CoNS isolates were resistant to the common antibacterials used for wound infections; hence an accurate sensitivity pattern has to be determined before starting of the antibacterial therapy to prevent therapeutic

failure. Hence, CoNS cannot be neglected as nonpathogenic as infections with these can be life-threatening and the biofilm producing isolates may be multi-drug resistant too. It is better to isolate the patients if they are infected with MRCoN for the better infection control measures.

The Methicillin Resistant Staphylococcal isolates, ie both MRSA and MR CoN were strong biofilm producers. The similar findings were found by (Rachna *et al* ., 2010); there is a significantly decreased penetration of antibacterial drugs. This results indicate that either a high density growth phase or its metabolic state which is related to a biofilm growth mode rather than biofilm producing ability contribute significantly to the resistance of CoNS biofilm to antibiotics.

Thus, we conclude that the biofilm producing CoN *Staphylococcus* species are difficult to be eradicated from the site of infection using antibacterial drugs. These biofilm producing CoN *Staphylococcus* even though considered contaminant in the clinical specimens sometimes can cause serious infections in patient with reduced immunity. They can be multidrug resistant as biofilm restrict the entry of the drug to the point of action. Hence, when CoN *Staphylococcus* is isolated the biofilm producing capacity has to evaluate before treating the patients with the antibacterial.

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