

BIOFILM DETECTION AND CLINICAL SIGNIFICANCE OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATESV. Sudha Rani¹, S. Ravindra², R. Kondal Rao³**HOW TO CITE THIS ARTICLE:**

V. Sudha Rani, S. Ravindra, R. Kondal Rao. "Biofilm Detection and Clinical Significance of Staphylococcus Epidermidis Isolates". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 28, April 06; Page: 4844-4849, DOI: 10.14260/jemds/2015/702

ABSTRACT: CONTEXT: Isolates of Staphylococcus epidermidis. **AIMS:** To detect biofilm among clinical isolates of Staphylococcus epidermidis. **MATERIAL AND METHODS:** 50 Staphylococcus epidermidis isolates were collected from clinical samples like blood, IV catheter tips, catheterized urine, wound swabs and exudates received from various clinical departments. Biofilm formation was studied in these isolates. The study was carried out over a period of one year i. e from March 2011-February 2012. The specimens received were processed by conventional methods. Tissue Culture plate method was used for detection of biofilm. **RESULTS:** IV catheter tip samples revealed 25%, implant device associated infections revealed 20%, the Catheterized urine samples showed 16%, blood culture 8%, ventilator associated infections 10%, post-operative wound infections 11% & exudates 5% of Staphylococcus epidermidis isolates. Isolates with O. D. values more than 0.2 were considered as high biofilm producers. 40% of S. epidermidis isolates were weak biofilm producers, 24% were moderate biofilm producers and 36% were high biofilm producers. Isolates from IV catheter tips showed high biofilm formation. **CONCLUSIONS:** In the modern health care set up, various devices such as IV catheters, urine catheters, shunts, implanted prosthetic devices etc are being increasingly used thereby causing the device associated infections particularly of Staphylococcus epidermidis. Device associated infections caused by Staphylococcus epidermidis are mainly due to biofilm formation which ultimately makes the treatment difficult.

KEYWORDS: Coagulase negative Staphylococci, Biofilm, nosocomial infections, Staphylococcus epidermidis.

INTRODUCTION: Coagulase-negative staphylococci are among the most commonly isolated organisms in the clinical microbiology laboratory. Coagulase-negative staphylococci (CONS) colonize skin and mucous membrane of humans and animals.^[1,2,3] Previously, these bacteria were mostly regarded as contaminants of clinical specimens from humans. During the last few decades, CONS have emerged as important nosocomial pathogens.^[4,2,3,5] Their role as significant pathogens following ophthalmologic, neurologic or cardiothoracic surgery, in immune compromised patients and in the patients with prosthetic devices has been established. Besides this we can see now that the infections of CONS are generally associated with the use of catheter and other medical devices. The capacity of adherence to polymer surfaces and consequent biofilm production are main virulence factors of CONS, and numerous species of CONS have been recognized as pathogens. S. epidermidis is the CONS species most frequently isolated from infections. It has been implicated as the etiological agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin. Biofilms can be defined as multicellular communities of bacteria, immobilized by an extracellular polymeric matrix produced by the bacteria, which can be attached to various biotic and abiotic surfaces.^[6,7] This three-dimensional biofilm structure is made up in 85% cases by the extracellular matrix which

ORIGINAL ARTICLE

comprises polysaccharides, proteins, enzymes, DNA, bacterial glycolipids, water, and in 15% cases by aggregates of microorganism cells.^[8] Biofilm development depends on many physical, chemical and biological factors ^[3]. In staphylococci, the main molecule responsible for intercellular adhesion is a polysaccharide intercellular adhesin (PIA), also known as a poly-N-acetylglucosamine (PNAG^[9]). It is a partially deacylated polymer of β -1, 6-N-acetylglucosamine, which, with the other polymers such as teichoic acids and proteins, can form a major part of the extracellular matrix. Recently, PIA homologues were identified in many pathogens with biofilm formation ability, which points out towards the assumption that three-dimensional matrix formation plays a crucial role in bacterial virulence in biofilm-associated infections.^[9,10,11]

PIA biosynthesis is carried out by the proteins encoded by the *ica* gene operon: N-acetylglucosamine transferase (*icaA* and *icaD*), PIA deacylase (*icaB*), PIA exporter (*icaC*) and the regulatory gene (*icaR*) ^[12, 13]. *Ica* locus expression is regulated by a variety of environmental factors and internal regulatory proteins. Biosynthesis and deacetylation of PIA are recognized as crucial virulence factors in *Staphylococcus epidermidis*-associated infections.^[14,15,16]

Hence we have studied biofilm detection and clinical significance of *S. epidermidis* from different clinical samples received in the microbiology laboratory of a tertiary care hospital.

MATERIAL AND METHODS: 50 *Staphylococcal epidermidis* isolates were collected from clinical samples like blood, IV catheter tips, catheterized urine, wound swabs and exudates received from various clinical departments of MGM (Mahatma Gandhi Memorial) Hospital Warangal. Biofilm formation was studied in these isolates. The study was carried out over a period of one year i. e. from March 2011- February 2012. The specimens received were processed by conventional methods. Repeat isolations (twice) from blood culture with pure growth were given significance. 15 cfu from IV catheter tips were given significance.

Processing of the Specimens: Isolates were identified by standard microbiological procedures (Gram staining, colonial morphology, slide and tube coagulase test, biochemical tests).

Detection of biofilm production: Tissue Culture plate method was used for detection of biofilm.

Identification of *Staphylococcus epidermidis*: Colonies from blood agar plates were picked up and Gram stained. Catalase and coagulase tests were done. All the coagulase negative strains were tested for Novobiocin sensitivity, and, sensitive strains were identified as *Staphylococcus epidermidis*. *Staphylococcus epidermidis* isolates were collected after identification and preserved in 20% glycerol broth. Isolates of *Staphylococcus epidermidis* were screened for their ability to form biofilm by Tissue culture plate method.

PROCEDURE: Clinical isolates from fresh agar plates were inoculated in BHI (Brain heart infusion broth) with 2% sucrose and incubated for 18 hrs at 37°C in stationary condition. The cultured broth was diluted 1 in 100 with fresh medium. Sterile ELISA plate was taken and filled with 0.2 ml of the diluted cultures. The ELISA plate was incubated for 24hrs at 37°C. After incubation, contents of each well were gently removed. The wells were washed 4 times with 0.2 ml of phosphate buffer saline to remove the free floating bacteria. Biofilms formed by adherent organisms in plate were fixed with

ORIGINAL ARTICLE

2% sodium acetate and stained with 0.1% Crystal Violet. Excess stain was rinsed off by thorough washing with deionized water, plates were kept for drying. Adherent Staphylococcal cells usually formed biofilms on all side walls and were uniformly stained with Crystal Violet. Optical density of stained adherent bacteria were determined using micro ELISA autoreader at wavelength between 400-600 nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms.

RESULTS: A total of 50 Staphylococcus epidermidis isolates were collected from clinical samples like IV catheter tips, pus from implant device associated infections, catheterized urine, blood culture, pus from ventilator associated infections, exudates and post-operative wound swabs. IV catheter tip samples revealed 25%, implant device associated infections revealed 20%, the Catheterized urine samples showed 16%, blood culture 8%, ventilator associated infections 10%, post-operative wound infections 11% & exudates 5% of Staphylococcus epidermidis isolates (Table 1).

Nature of specimen	Number of samples	Number of S. epidermidis isolates	% of S. epidermidis
Post-operative wound infections	140	16	11
IV catheter tips	40	10	25
Pus from Ventilator associated infections	20	2	10
Pus from Implant device associated infections	10	2	20
Catheterized urine	60	10	16
Exudates	36	2	5
Blood cultures	100	8	8

Table 1: Staphylococcus epidermidis isolates from various clinical samples

40% of S. epidermidis isolates were weak biofilm producers, 24% were moderate biofilm producers and 36% were high biofilm producers (Table 2).

Mean O. D. values	No. of S. epidermidis isolates	% of S. epidermidis isolates
<0.1	20	40
0.1-0.2	12	24
>0.2	18	36

Table 2: O. D values of biofilm producing S. epidermidis isolates

Isolates with O. D. values more than 0.2 were considered as high biofilm producers. Isolates from IV catheter tips showed high biofilm formation. (Table-3).

ORIGINAL ARTICLE

Nature of specimen	OD Values		
	>0.2	0.1-0.2	<0.1
IV catheter tips	8	1	1
Implant device associated	2	0	0
Catheterized urine	4	4	2
Blood cultures	3	2	3
Ventilator associated infection	1	1	0
Exudates	0	1	1
Post-operative wound swabs	2	6	8

Table 3: O. D values of biofilms formed by *S. epidermidis* strains from different clinical samples

DISCUSSION: Earlier, Coagulase negative staphylococci (CONS) were considered as harmless skin commensals and dismissed as culture contaminants. But in recent years they are increasingly being recognized as important human pathogens. Among all CONS, *S. epidermidis* strains represent the most frequent cause of nosocomial sepsis and the most common agents of infections with implanted devices.

In a study done by Shubhra Singh, Gopa Banerjee et al showed 72 among 150 strains of CONS (60%) were isolated from blood samples, 36 from pus samples, 15 from urinary catheter tip and 12 from the urine samples.^[16] In our study, IV catheter tip samples revealed 25%, implant device associated revealed 20%, the catheterized urine samples showed 16%, blood culture 8%, ventilator associated infections 10%, postoperative wound infections 11% & exudates 5% of *S. epidermidis* associated infections. In another study done by Azuka Azih and Idahosa Enabulele et al CONS were most commonly isolated from infected wounds (17.7%), followed by urine from cases of urinary tract infections.(16.5%) and least isolated from ear infections (1.26%). Infected wounds were mainly from surgical wounds, diabetic foot ulcer and prosthetic devices.^[17]

Previously, many workers have demonstrated biofilm formation by *S. epidermidis* from clinical isolates. Biofilm was detected mainly from the strains isolated from device associated infection followed by IV catheter associated septicemias. *S. epidermidis* is an opportunistic pathogen of foreign bodies' particularly prosthetic cardiac valves, CSF shunts, orthopedic appliances and other devices. In a study done by Rohde H, Burdelskic et al showed that because of its biofilm forming capacity *S. epidermidis* has evolved as a leading cause of device related infections.^[18] In the present study we found that *S. epidermidis* forms biofilms.

The findings correlate well with the others mentioned above. In another study T. Mathur et al evaluated three methods for detection of biofilm formation in *S. epidermidis* by tissue culture plate (TCP) method, Tube method and Congo red agar (CRA) method. Of these they found the TCP method was the most sensitive and accurate method for detection of bio film formation.^[19] In another study done by Afreenish Hassan, Javaid Usman et al the TCP method was considered to be superior to TM and CRA. From the total of 110 clinical isolates, TCP method detected 22.7% as high, 41% moderate and 36.3% as weak or non-biofilm producers.^[20]

We studied biofilm formation by tissue culture plate method because it was considered as standard test for detection of biofilm formation. In the present study, we screened all isolates of *S.*

ORIGINAL ARTICLE

epidermidis from clinical samples from blood cultures, IV catheter tips, ventilator associated infections, implant device associated, catheterized urine, exudates and postoperative wound infections.

SUMMARY AND CONCLUSION: In the modern health care setup, various devices such as IV catheters, urine catheters, shunts, implanted prosthetic devices etc are used increasingly thereby causing device associated infections particularly of *Staphylococcus epidermidis*. In the present study we have been able to demonstrate biofilm production by the clinical isolates of *Staphylococcus epidermidis*, mainly from device associated infections. The optical density values were found to be more from IV catheter associated strains (>. 2, 8 isolates). Post-operative wound infections (stitch abscesses) revealed least optical density. Therefore it is concluded that the device associated infections caused by *Staphylococcus epidermidis* are mainly due to biofilm formation which ultimately makes treatment difficult.

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