A STUDY OF BIOFILM PRODUCTION IN STAPHYLOCCUS AUREUS

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Aims and objectives: (1) To detect biofilm formation in clinical isolates of \textit{Staphylococcus aureus}. (2) To know the antimicrobial resistance pattern of the isolates and correlation with biofilm production.

Methodology: The present study was done to detect biofilm formation in 50 clinical isolates of \textit{Staphylococcus aureus} from blood and indwelling devices. For detection of biofilm formation, the clinical isolates were screened by Modified Tissue Culture Plate method (MTCP) and Tube Method (TM). Antibiotic susceptibility of the isolates was determined by using Kirby-Bauer disc diffusion method. Results: Out of 50 isolates of \textit{Staphylococcus aureus}, 46% were biofilm positive by MTCP and 38% by TM. The biofilm producers were multi drug resistant as compared to the non producers. Interpretation: The effective control for staphylococcal infections will require a concerted effort to develop therapeutic agents that target the biofilm phenotype.

Keywords: \textit{S. aureus}, Biofilms, Multidrug resistance, Antibiotics

INTRODUCTION

Biofilms are complex communities of single or multiple species of micro-organisms that develop on biotic and abiotic surfaces. Since biofilms contaminate catheters, ventilators and medical implants, they act as a source of diseases for humans. \textit{Staphylococcus aureus} and \textit{Staphylococcus epidermidis} are the predominant species forming biofilms on polymeric surfaces (Kloos and Bannerman, 1994). The genetic and molecular basis of biofilm formation in staphylococci is multifaceted. The ability to form a biofilm affords at least two properties: the adherence of cells to a surface and accumulation to form multilayered cell clusters. A trademark is the production of slime substance PIA, a polysaccharide composed of \(\beta\)-1,6-linked N-acetyl glucosamines with partly deacetylated residues in which the cells are embedded and protected against the host’s immune defence and antibiotic treatment (Gotz, 2002).

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Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates in hospitals/communities have been recognized as the major challenges as they are becoming Multi Drug Resistant (MDR) (Mehta *et al*., 1998). Therefore, keeping this in view, the present study was designed to detect the production of biofilm by *Staphylococcus aureus* and its correlation with antibiotic resistance.

### METHODOLOGY

A total of 50 non repetitive, clinical isolates of *Staphylococcus aureus* isolated from blood and infected indwelling devices received in Microbiology Department, Pt.BDS, PGIMS, Rohtak, Haryana were investigated. Organisms were identified by standard microbiological techniques (Collee *et al*., 1996). Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method (CLSI). Biofilm production was detected by using two methods: (1) Modified Tissue Culture Plate (MTCP) method (Mathur *et al*., 2006) by using crystal violet binding assay in a 96-well flat bottomed tissue culture plate and quantitated spectrophotometrically using ELISA reader at 600 nm; and (2) Tube Method (TM) (Christensen *et al*., 1982) in which biofilm formation was taken as positive when a visible film lined the wall and bottom of the tube.

### RESULTS

In our study, by MTCP method we could detect biofilm formation in 23 isolates (46.0%). By tube method, 19 isolates (38%) were positive for biofilm formation.

Out of 50 isolates of *Staphylococcus aureus*, 33 (66.0%) were MRSA, of which 20 (60.6%) were positive for biofilm production. Also MDR was more frequent in biofilm producers in comparison to non-biofilm producers.

### DISCUSSION

Infections by *Staphylococcus aureus* are a major problem in hospital settings, especially among patients with indwelling devices. Most serious infections such as endocarditis, osteomyelitis and catheter-related infections are caused by biofilm producing strains. Such infections are difficult to manage and costlier to treat (Gara and Humphreys, 2001). Over the last few years, several studies have been performed to demonstrate the biofilm formation by staphylococci (Ammendolia *et al*., 1999; Ruzicka *et al*., 2004).

We tested 50 clinical isolates of *Staphylococcus aureus* by two in-vitro screening procedures for their ability to form biofilms. In the MTCP method, biofilm production was seen in 46.0% which is less than that by other workers who demonstrated biofilm formation in 53.9% and 57.1% of isolates respectively (Mathur *et al*., 2006; Ammendolia *et al*., 1999). By tube method, we could detect biofilm formation in 38.0% isolates, while other studies have demonstrated 41.4% and 53.7% positivity rate respectively (Mathur *et al*., 2006; Ruzicka *et al*., 2004).

In the present study, biofilm was more common in MRSA isolates. The biofilm producers exhibited significantly higher resistance to all the antibiotics which is in concordance with other studies (Smith and Hunter, 2008; Campoccia *et al*., 2006). We did not observe resistance to linezolid. Linezolid have been evaluated in many *in-vitro* biofilm models and it has been demonstrated that there is suppression of bacterial growth in a biofilm, but it does not eradicate bacterial colonization.

Biofilm is one of the known virulence factors of staphylococci. So a greater understanding of
the nature of intracellular bacterial communities in infections, their early detection and management will aid in the development of new and more effective treatments.

CONCLUSION
Microbial biofilms pose a public health problem especially in persons with indwelling devices. Therefore, an effectual control will need an intensive effort to build up newer therapeutic agents that aim to prevent the formation of biofilms or encourage the biofilm detachment.

REFERENCES