

## Molecular characterization of biofilm producing Staphylococci isolated from Central Venous Catheters in a tertiary care hospital

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### Abstract

**Introduction:** Medical device associated infections are a major cause of substantial morbidity, due to wide usage of intravenous catheters in various medical disciplines like renal dialysis units and in cardiology department. Several species of Staphylococcus which usually occur as normal microbial flora on mucosa and skin are a frequent cause of device associated infections due to biofilm production. This polysaccharide slime synthesis is mediated by ica operon.

**Aims and Objectives:** To determine the prevalence of biofilm producing strains of Staphylococci isolated from Central Venous cannulas by phenotypic and genotypic methods

**Materials and Methods:** 220 Central Venous devices were received and processed, of which 30 yielded growth. Staphylococci were identified and biofilm production was determined by the tissue culture plate method and by PCR. Genotyping of biofilm producing Staphylococcal species was done for detection of ica genes (*intercellular adhesion*) by conventional PCR.

**Results:** A total of 220 central venous catheter tips were collected, out of which 30 bacterial organisms were isolated. Among these isolates, 20 (67%) were Staphylococcus species which were considered for phenotyping by tissue culture method and genotyping by conventional PCR. Out of these, 10 (50 %) Staphylococcal species were phenotypically positive for biofilm production and 11 (55%) showed the presence of ica gene by PCR.

### Introduction

Biofilms are colonies of living micro-organisms such as bacteria, fungi, algae, and/or protozoa growing on surfaces like metals, plastics, tissue, soil particles and teeth. Some bacteria are capable of producing biofilms on different surfaces and enclose in a self-produced extracellular matrix polysaccharide (EPS) and several proteins. These substances are encoded by intercellular adhesion (*ica*) locus, ica ABCD. The ica A codes for the enzyme N-acetylglucosamyl transferase, the ica D increases the activity of this enzyme, deacetylation of PIA is regulated by ica B and ica C codes for transmembrane protein<sup>1</sup>. There are about forty six genes responsible for biofilm production. Among the various invasive devices, central venous catheters pose a greater risk in developing infections of 3-5%<sup>2</sup>. Several studies have shown the presence of biofilm formation on central venous catheter tips when they were examined by scanning and transmission electron microscopy<sup>1</sup>.

There is a widespread relation between biofilm formation and disease process, which involves detachment of cells or cell aggregates, production of endotoxins, resistance to host immune system and also a niche for the generation of antimicrobial resistant organisms. These factors lead to slow persistence of device associated infections which pose a major problem in treatment<sup>3</sup>.

Species belonging to genus Staphylococcus are widespread in environment and they are capable of producing a large spectrum of diseases and many of them cause chronic infections and are prone to produce biofilms<sup>4</sup>. Several species of Staphylococcus are

capable of producing polysaccharide intercellular adhesion (PIA) molecule a product of ica gene cluster, which is considered as a virulence factor (*ica1*), for adhesion and colonization on artificial materials<sup>2</sup>. Synthesis of oligomers in vitro by ica A and ica D was demonstrated by using UDP-N-acetylglucosamine as a substrate, this N-acetylglucosamyl transferase activity along with ica C produce a product which is recognized by antibody against PIA. *S.epidermidis* and *S.aureus* along with several other Staphylococcus species possess ica locus implying that adhesion function is conserved within this genus<sup>5</sup>. The present study was carried out to detect the presence of ica gene in Staphylococcus species by using PCR and also to compare the prevalence of this gene in methicillin sensitive and resistant strains.

### Materials and Methods

**Staphylococcus strain isolation:** A total of 220 central venous catheter tips were collected from renal dialysis unit and cardiology department mainly from cathlab at Chettinad Hospital and Research Institute and processed for culture and sensitivity by the semi quantitative method and by a modified quantitative method. The criterion for the quantitative culture method is, more than or equal to 1000 CFU, and for the semi-quantitative method, it was more than or equal to 15 CFU<sup>6</sup>.

These samples were collected for a period of two years from January 2013 to February 2015. Further identification was made by performing the biochemical tests as per the flow chart described by Bailey and Scott's diagnostic microbiology<sup>7</sup>. In our study, the

antimicrobial susceptibility testing was done by using the Kirby Bauer Himedia<sup>8</sup>.

**Detection of Biofilm production by Tissue Culture Plate Method (TCP):** In the present study, we screened all the *Staphylococcus* species for biofilm production by TCP method as described by Christensen et al<sup>9</sup>.

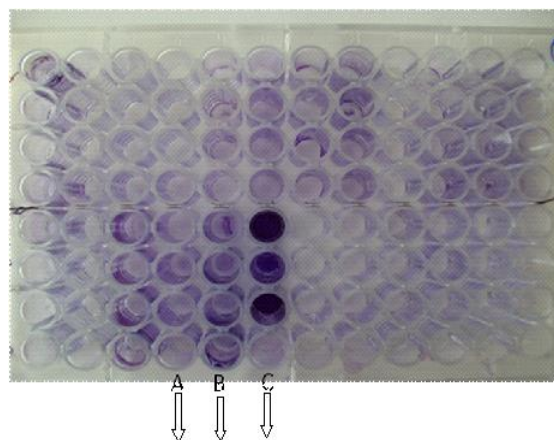
Isolates from fresh agar plates were inoculated in trypticase soy broth with 1% glucose (TSBglu) media and incubated for 18 hours at 37°C in stationary condition and diluted 1in100 with fresh medium. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates (Tarson, Kolkata, India) were filled with 0.2 ml aliquots of the diluted cultures. Aliquots containing only broth served as control to check sterility and non-specific binding of media.

The tissue culture plates were incubated for 24 hours at 37°C. After incubation, the content from each well was gently removed by tapping the plates. The wells were washed four times with 0.2 mL of phosphate buffer saline (PBS pH 7.2) to remove free-floating planktonic bacteria. Biofilms formed by adherent 'sessile' organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent cells usually form biofilm on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria were determined with a micro ELISA auto reader (model PR 601, Qualigens) at wavelength of 570 nm (OD570 nm). These OD values were considered as an index of bacteria adhering to surface and forming biofilms<sup>10</sup>.

Experiment was performed in triplicate and repeated three times, the data was then averaged. To compensate for background absorbance, OD readings from sterile medium, fixative and dye were averaged and subtracted from all test values. The mean OD value obtained from media control well was deducted from all the test OD values.

### Interpretation of Results

Mean OD values	Biofilm formation
<0.120	Non/ weak
0.120-0.240	Moderate
>0.240	High



**Fig. 1: Tissue culture plate method A. No biofilm production B. Moderate biofilm production C. Biofilm production**

**'ICA' Gene Identification:** The chromosomal DNA from the *Staphylococcal* isolates are amplified using the following provided by the DNA purification kit (PureFast® Bacterial Genomic DNA purification kit), PCR Master Mix, Agarose gel electrophoresis consumables and the primers were used for the present study from HELINI Biomolecules, Chennai, India. The primers included were ICA Primers with Forward-5'-TCCAGAAACATTGGGAGGTC-3' and Reverse-5'-TGGGTATTCCCTCTGTCTGG-3', the PCR product size was 500bp.

The PCR reaction was setup as follows

**Initial Denaturation:** 94°C for 5 min followed by 35 cycles each of denaturation at 94°C for 30sec, Primer annealing at 58°C for 30sec and then by Extension: at 72°C for 30sec A Final extension process was carried out at 72° C for 5 min.

PCR samples were loaded after mixing with gel loading dye along with 10µl HELINI 100bp DNA Ladder. The electrophoresis was run at 50V till the dye reached three fourth distance of the gel and viewed in UV transilluminator and the bands pattern was observed.

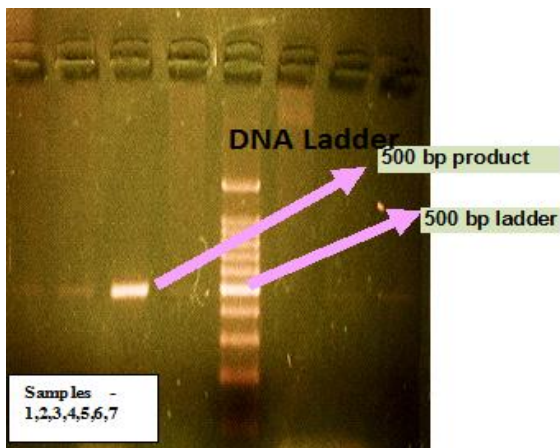


Fig. 2

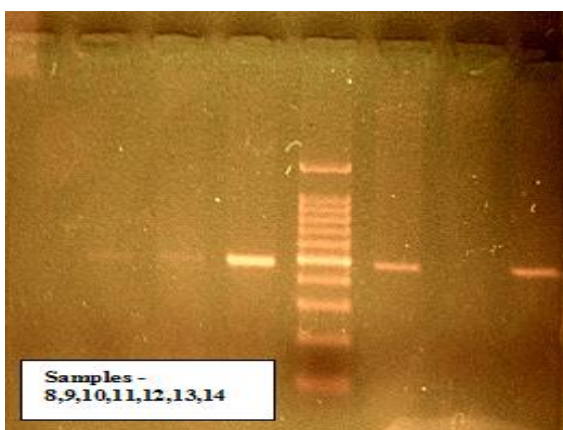


Fig. 3

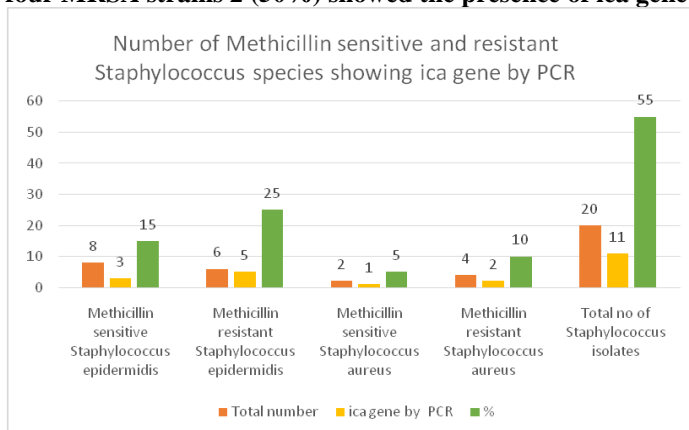
**Result**

Of the 220 CVC tip samples collected from the ICU, 30 samples showed bacterial growth out of which 20 isolates belonged to the genus *Staphylococcus*. 14 (70%) were *Staphylococcus epidermidis* and 6 (30%) were *Staphylococcus aureus*. 10 (50%) *Staphylococcal* isolates were positive for biofilm production by the phenotypic method and 11(55%) were positive by PCR. 7 *Staphylococcus epidermidis* isolates were positive for biofilm production by the phenotypic tissue culture plate method and 8 isolates showed the presence of ‘ica’ gene by PCR. All the 7 *Staphylococcus epidermidis* isolates which were positive by phenotypic method were also positive by PCR. One isolate showed the presence of the ica gene but biofilm production was not detected by the phenotypic method. Of the total 6 *Staphylococcus aureus* isolates, 3 were positive for biofilm production by the phenotypic method and PCR.

**Table 1: Shows the distribution of *Staphylococcus species* producing biofilms by both phenotypic and genotypic methods from the central venous catheter tips**

Name of the organism	Number of isolates	Biofilm producers	
		TCP	PCR
<i>Staphylococcus epidermidis</i>	14 (70%)	7(35%)	8 (40%)
<i>Staphylococcus aureus</i>	6 (30%)	3(15%)	3(15%)
Total	20(100%)	10(50%)	11(55%)

**Graph 1: The ica gene detection was compared between the methicillin resistant and sensitive *Staphylococcus* species, and it was found that out of six resistant strains of *S. epidermidis* 5(83%) showed ica gene, and among four MRSA strains 2 (50%) showed the presence of ica gene by PCR**



## Discussion

Catheter related septicaemia are more common in biofilm producing organisms as they account for 82% of nosocomial infections<sup>1</sup>. In the present study 220 central venous catheter tips were processed, out of which 30 samples yielded growth. Majority of isolates (67%) were gram positive cocci. The organisms most commonly isolated from central venous catheter biofilms were *Coagulase negative Staphylococcus*, *S. aureus*, *Pseudomonas species*, *Klebsiella species*, and *Enterococcus species*<sup>11</sup>.

*Staphylococcus aureus* and *S.epidermidis* are capable of exhibiting rapid phenotypic switching of properties like growth rate, colony morphology, antibiotic susceptibility testing and also biofilm formation. Close proximity to catheter insertion sites and transmission via skin and mucosa of patients and health care personnel may lead to increased prevalence of *Staphylococcus* species in causing catheter related infections<sup>5</sup>.

*Staphylococcus* species which show resistance to methicillin are more prone to develop biofilms and contribute to increased morbidity and mortality in catheter related nosocomial infections. In our study 83% of *Methicillin resistance Staphylococcus epidermidis* showed the presence ica gene by PCR whereas only 50% in *MRSA* strains were positive for ica gene PCR.<sup>12</sup> Development of quorum sensing for virulence and survival by several *Staphylococci* is the major advantage in biofilm producers via accessory gene regulator (agr). There is significant correlation between this agr gene and other plethora of virulence factors produced by *Staphylococcus* species. Carriers of *MRSA* in both community and nosocomial occurrence had shown significance in the development of biofilm formation<sup>13</sup>.

## Conclusion

Most of the biofilm producing strains of *MRSA* are prone to cause blood stream infections in patients with various invasive devices, than non-biofilm producing strains as they possess virulence associated genes which was proved by statistical analysis. Thus epidemiological and molecular analyses of biofilm producing *Staphylococcus* species is gaining significance in the prevention of health care associated infections

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