Original Research Article

Study of biofilm producing capacity of pathogens isolated from different catheters

Harsha Vardhana S[1*], P R Sreenivasa Babu[2], M R Sandhya Belawadi[3]

[1]Assistant Professor, Department of Microbiology, Kidwai Cancer Institute, MH Marigowda Road, Bangalore-560029, Karnataka, NDIA.
[2]Former Professor and HOD, [3]Former Principal and HOD, Department of Microbiology, MS Ramaiah Medical College, Bangalore, Karnataka, INDIA.
Email: harshadoc@gmail.com

Abstract

Background: Persistent infection due to biofilm formation is certainly a new and additional burden to clinicians who treat infections. The purpose of this study is to determine the biofilm producing capacity of pathogens isolated from different catheters. **Material and Methods:** A total of 109 catheter samples from 100 adult patients of both sexes admitted to the Intensive Care Units (ICUs) who have been catheterized for more than 48 hrs showing clinical signs of sepsis. Maki's semi-quantitative method was used to culture thecatheters and biofilm detection was done by culture plate assay. **Results:** Out of the 109 catheters studied and cultured, a total of 26 isolates were obtained. Coagulase negative staphylococci and *Escherichia coli* formed the most commonly isolated pathogens at 5 (19.2%) each. Among the 26 isolates cultured, 22 of the organisms produced biofilms as evidenced by the tissue culture plate assay. **Conclusion:** Biofilm formationpose a public health problem for the persons who require indwelling medical devices. Its detection should be mandatory in a laboratory set up. Tisue culture plate method is an accurate and reproducible screening method for biofilm production.

Key words: Catheters, biofilms, Maki's semi-quantitative method, tissue culture plate assay.

[*]**Address for Correspondence:**
Dr. Harsha Vardhana S., Assistant Professor, Department of Microbiology, Kidwai Cancer Institute, MH Marigowda Road, Bangalore-560029, Karnataka, NDIA.
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INTRODUCTION

With the remarkable modern advances in medicine and the concomitant increases in the numbers of elderly and immunocompromised patients, the use of medical biomaterials which are the plastic, rubber and metallic materials that are used to construct the myriad of medical devices and prostheses, has increased exponentially. An increasing number of infections is centered on such implanted devices.[1] Biofilms are the microbial communities of the surface-attached cells which are embedded in a self-produced extracellular polymeric matrix.[2]The role of biofilms in the contamination of medical implants has been well established. Biofilms are now believed to be the most important cause of persistent infections due to catheters and it is also regarded as one of the most significant reasons for development of resistance to antimicrobial agents. Millions of catheters (central line, intravenous, urinary catheters) are inserted into patients every year and these implants serve as a potential surface for biofilm formation. The emergence of resistance to antibiotics in several pathogenic bacteria in the past two decades has gradually rendered antimicrobial treatment less effective. Among the mechanisms evolved by microbes to evade antimicrobial therapy, probably the most important among them is the ability to either form or live within a biofilm.[3] Today, a global concern has emerged that we are entering a post – antibiotic era with a reduced capability to combat microbes. Persistent infection due to biofilm formation is certainly a new and additional burden to clinicians who treat infections. The

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purpose of this study is to highlight and emphasize certain important problems that have emerged recently and which demand serious attention in the management of some important infectious diseases.

MATERIAL AND METHODS

The present study was conducted in M.S. Ramaiah Teaching Hospital over a period of two years.

Inclusion Criteria: A total of 109 catheter samplesfrom 100 adult patients of both sexes admitted to the Intensive Care Units (ICUs) who have been catheterized for more than 48 hrs showing clinical signs of sepsis.[4]

Exclusion Criteria: All catheterized patients in ICUs without any signs of sepsis.

Catheters collected: Both vascular (central arterial, central venous and peripheral venous) and urinary (Foley's catheter) catheters were included as part of the study.

Collection of vascular catheters[4](Fig.1): The catheter was withdrawn with sterile forceps, the externalized portion being directed upward and away from the skin surface. For 5.7 cm catheters, the entire length, beginning several millimeters inside the former skin - surface - catheter interface, was aseptically amputated and cultured. With longer catheters(20.3 cm and 60.9 cm in length) two 5 cm to 7 cm segments were cultured: a proximal one beginning several millimeters inside the former skin catheter interface, and the tip. Catheter segments were transported to the laboratory in a sterile tube.

Collection of Foley's Catheters: Using another syringe without needle, the water or saline injected initially during catheter insertion was drained out. Initially, one or two gentle tugs were given on the catheter and it was slowly withdrawn. With the help of the sterile scissor, a 5 cm portion of the catheter tip was cut off and placed in a sterile test tube and plugged. It was then taken to the lab and processed.

Processing of Urinary Catheters[5]: The catheters were placed in 10 ml of 0.15m phosphate buffered saline that contained 0.1% Tween-80 and sonicated for 30 minutes at room temperature to detach adherent microorganisms. The microbial suspension was vortexed vigorously for 15 seconds to break up clumps. Ten-fold serial dilutions of each suspension were plated on 5% sheep blood agar using spread plate technique, incubated at 30°C for 18 hours and the mean number of colony forming units was determined. All microbial strains so obtained were maintained on Nutrient Agar deeps and sub-cultured every 2-3 months.

Maki's Semi-Quantitative Technique[4](Fig. 2): In the laboratory, each catheter segment was transferred to the surface of a 100 mm 5% sheep blood agar plate for semiquantitative culturing. While downward pressure was exerted with a flamed forceps, the catheter was rolled (or if bent, smeared) back and forth across the surface atleast four times. Plates were incubated at 37°C. All colony types appearing on the primary plates were enumerated and all organisms recovered from the plates were fully identified. All isolates so obtained were identified by standard biochemical reactions.

Monitoring of Biofilm formation by Microtitre Plate Assay[6](Fig. 3 and 4): This method is also known as 'Tissue culture plate assay' (TCP). Isolates from Nutrient Agar deeps were inoculated in respective broths and incubated for 18 hours at 37°C and diluted 1 in 100 in fresh medium. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates were filled with 0.2ml aliquots of the diluted cultures and only broth served as control to check sterility. The tissue culture plates were incubated for 18 hours at 37°C. After that, the incubation content of each well was gently removed by tapping the plates. The wells were washed four times with 0.2ml of phosphate buffered saline to remove free-floating organisms. Biofilms formed in the plate were fixed with sodium acetate 2% and stained with crystal violet 0.1%. Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent cells usually formed on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria were determined with a spectrophotometer at a wavelength of 570nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms.[5,6]


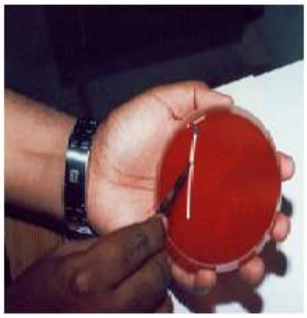
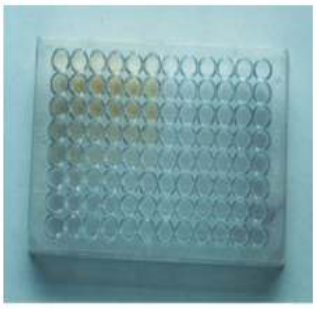
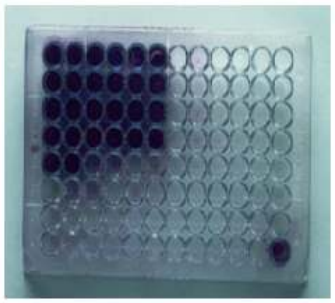

Figure 1: Different types of catheters collected
Figure 2: Maki's technique
Figure 3: TCP- after incubation
Figure 4: TCP- after crystal violet staining

RESULTS

In the present study a total number of 109 catheters obtained from 100 patients were examined and studied. Of the 109 catheters, 71 were central venous catheters, 36 were Foley's catheters, 1 was a peripheral venous catheter and 1 was a Hickman catheter. Central arterial catheters were not obtained. Out of the 109 catheters studied and cultured, a total of 26 isolates were obtained. Coagulase negative staphylococci and *Escherichia coli* formed the most commonly isolated pathogens at 5 (19.2%) each. *Pseudomonas aeruginosa* formed the next most common isolate at 4 (15.4%). Non fermenting gram negative bacilli (NFGNB), *Enterobacter*spp. and *Klebsiella*spp. came third most frequently forming 3 (11.5%) isolates each. *Candida albicans* was isolated on 2 (7.7%) occasions while *Staph. aureus* was cultured once (3.8%) (Table 1).

Table 1: Different types of isolates obtained

Type of isolate	Frequency (Percentage)
CONS	5 (19.2%)
Escherichia coli	5 (19.2%)
Pseudomonas aeruginosa	4 (15.4%)
NFGNB	3 (11.5%)
Enterobacter spp.	3 (11.5%)
*Klebsiella*spp.	3 (11.5%)
Staphylococcus aureus	1 (3.8%)
Candida albicans	2 (7.7%)
Total	**26 (100%)**

Among the 26 isolates, 20 isolates were obtained from the central venous catheters, 5 were obtained from the Foley's catheters and 1 was cultured from the lone Hickman catheter. Among the 26 isolates cultured, 22 of the organisms produced biofilms as evidenced by the tissue culture plate assay. It was seen that 12 (54.6%) were positive for biofilm formation in the 18-40 yrs age group. 6 (27.3%) of the biofilms were formed by the patients in the 41-60yrs group while 4 (18.2%) showed biofilm formation in the above 60yrs category. The males accounted for 14 (63.6%) of the biofilms whereas, the female sex accounted for 8 (36.4%) of the biofilms formed.

Table 2: Biofilm formed on different types of catheters

Type of catheter	No. forming biofilms	Percentage
Central venous catheter	16	72.7
Foley's catheter	5	22.7
Hickman catheter	1	4.6
Total	**22**	**100**

The table 2 depicts the different types of catheters from which biofilm producing organisms were cultured. The central venous catheters showed 16 (72.7%) of biofilm producing organisms while the Foley's catheters cultured 5 (22.7%) of the biofilm forming isolates. The lone isolate from the Hickman catheter, was also a biofilm producer 1 (4.6%) thereby completing the picture.

DISCUSSION

In the present study, a total of 26 isolates were obtained from 109 catheters. These 109 catheters were obtained from 100 patients some of whom provided more than one catheter sample. Only those catheters that grew more than 15 colonies by Maki's technique were regarded as culture-positive (colonized). The rest (<15 colonies) were ruled out as possible contamination. In present study, coagulase negative Staphylococci and *Escherichia coli* formed the most commonly isolated pathogens at 5 (19.2%) each. Seifert *et al*. showed coagulase-negative staphylococci were present in 50% cases of CRBSI in their study.[7]The incidence of CoNS CRBSIs is increasing with the increased use of implanted devices, such as central venous catheters and temporary dialysis catheters, which are especially common in the ICU.[8] In the study of Parameswaran R*et al*., 64% of the pathogens causing CRBSI were Gram-positive and 36% were Gram-negative.[9] According to Krishnan *et al*., Gram-positive cocci constituted 27% of isolates and gram-negative

bacilli were 56%.[10]*E. coli* was the other commonest organism causing urinary catheter related infection in our study. This is similar to the observation by Tullu MS *et al*, Schaeffer *et al* and Igra*et al*.[11-13] There was no particular age or sex incidence with regard to catheter infection or biofilm formation. In present study, the central venous catheters showed 16 (72.7%) of biofilm producing organisms. Vascular catheters that had been*in situ* forover 30 days showed evidence of predominantly luminalcolonization and biofilm formation.[14] It has also been noted that catheter colonization and biofilm formation on centralvenous catheters occurs early. Anaissie and colleagues found that microbial colonization and biofilm formation occurredas early as 1 day after catheter insertion in a cohort of adultcancer patients whose central venous catheters wereremoved.[15] The Foley's catheters cultured showed 5 (22.7%) of the biofilm forming isolates. Biofilms can readily develop on both the inner andouter surfaces of urinary catheters,[16] and theascending colonization cannot be avoided solely through hygiene measures. Therefore, in order to prevent suchinfections, it is important for clinicians to utilize cathetersonly when necessary and to avoid catheterization forextended periods of time.[17] Among the 26 isolates cultured, 22 of the organisms produced biofilms as evidenced by the tissue culture plate assay. Hassan *et al*. have considered TCP a reliable method and the gold standard for biofilm detection.[18] To conclude, biofilm formation has an important role in pathogenicity of infections, and they pose a public health problem for the persons who require indwelling medical devices. As the microorganisms in the biofilms are difficult to treat with antimicrobial agents, its detection should be mandatory in a laboratory set up. TCP method is an accurate and reproducible screening method for biofilm production.

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