MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2024.6.250.254

Catheter Related Infection: Biofilm Forming Capacity of Micro Organisms

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Abstract
Biofilms pose a number of clinical challenges to diseases involving uncultivable species, chronic, inflammation, impaired wound healing, recalcitrance to host immune defense mechanisms. Also, rapidly acquired antimicrobial resistance and the spread of infectous emboli. Regardless of the sophistications of the implant all medical devices are susceptible to microbial colonization and infection. Catheter associated infections due to microbial colonization and biofilm formation has gained more attention, increasing the incidence of nosocomial infections as a result of catheter use. The present study was carried out in the Department of Microbiology, to look for the biofilm forming ability of the organisms causing catheter related infection. Samples analyzed were intravascular catheters, Foley's catheters, blood and urine samples. Samples were processed as per standard protocol and methods. A total of 106 catheter samples obtained from 105 patients were studied. Of the 106 catheter cultures, 11 isolates were coagulase negative staphylococci which were all 100% biofilm producers. All were 100% resistant to Ampicillin, 91.6% sensitive to Vancomyan and 100% sensitive to Linezolid. Out of 12 gram negative isolates, 11 were biofilm producers, which were 100% resistant to Ampicillin and Amoxycillin-clavulanic acid, 100% sensitive to Imipenem. Therefore, it is necessary to detect the biofilm production in catheter-related infections as they lead to persistent infections, show high antimicrobial resistance and are difficult to eradicate.

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Key Words
Catheter related infection, biofilm forming capacity of organisms, intravascular catheters, foley's catheters

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Received: 10 March 2024
Accepted: 28 April 2024
Published: 6 May 2024

Citation: H. Kavitha, Pooja Mansabdar, B. Amrutha Kumari and K.R. Shobha Medegar, 2024. Catheter Related Infection: Biofilm Forming Capacity of Micro Organisms. Res. J. Med. Sci., 18: 250-254, doi: 10.59218/makrjms.2024.6.250.254

INTRODUCTION

Biofilms are complex assemblages anchored to abiotic or biotic surfaces. The presence of indwelling catheters introduce an artificial substation into the body leading to biofilm formation.

Biofilm enhance the virulence of the pathogen and cause a significant amount of all human microbial infections. Nosocomial infections are the fourth leading cause of death. About 60-70% of Nosocomial infections are associated with some type of implanted medical device[1].

It has been estimated that the frequency of infections caused by biofilms is between 65% and 80% as per reports from CDC and NIH respectively.

According to CDC report 2007, Health-care associated infections account for an estimated 1.7 million infections. Of these, 32% are UTIs, 22% are surgical site infections, 15% are pneumonia and 14% are bloodstream infections[2].

Biofilm formation has been observed in organisms such as CONS, *S. aureus, Enterococus spp, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, Enterobacter spp, Candida, Acinetobaetic spp, Citrobacter freundi, S. marcescens, Streptococi, etc*[3].

Biofilms pose a number of clinical challenges to diseases involving uncultivable species, chronic, inflammation, impaired wound healing, recalcitrance to host immune defense mechanisms. Also rapidly acquired antimicrobial resistance and the spread of infectous emboli. Regardless of the sophistications of the implant all medical devices are susceptible to microbial colonization and infection. Research efforts are currently directed towards eliminating or reducing infection of medical devices[4].

The present study was carried out in the Department of Microbiology, to look for the biofilm forming ability of the organisms causing catheter related infection. Samples analyzed were intravascular catheters, Foley's catheters, blood and urine samples.

MATERIALS AND METHODS

Clinical samples were collected from the catheterized patients of both sex. Specimens included catheter samples (intravascular and Foley's urinary catheters), blood and urine samples from 110 patients. An informed consent was taken from all the catheterized patients under study.

Detailed clinical history such as fever with or without chills, burning micturition, frequency of micturition, lower abdominal pain, swelling, pain at catheter site and duration of catheterization were recorded in the proforma.

All the in-patients who have been catheterized for more than 48 hours showing clinical signs of sepsis were included. All the catheterized patients without any signs of sepsis and all the catheterized patients <48 hours were excluded from the study.

Collection of Intravenous Catheters: At the time of catheter removal the site was examined for the presence of swelling, erythema, local rise in temperature and tenderness. The site was cleaned with an alcohol pledget and the catheter was withdrawn with sterile forceps, the externalized portion being directed upward and away from the skin surface. After removal, the site was examined and milked to express any exudate.

For short catheters (<6cm), the entire length of the cannula was cut 1cm below the surface/catheter junction aseptically. For long catheters, two 5cm segments were collected: the tip and the intracutaneous segment. The catheter segments were transported to the laboratory in sterile, dry containers.

Collection of Blood Sample: The venepuncture site was disinfected and with standard aseptic precautions, 5ml of blood was drawn. The sampling needle was safely detached and discarded., then a fresh needle was fitted and the drawn blood was inoculated into the blood culture bottle.

Gram Staining: Catheter segments were air dried and clotted blood if present was removed with sterile wire. Sterile forceps was used to handle the segment. Opaque catheters were cut in half longitudinally. The staining procedure was done in a series of different sterile petri dishes, each containing Crystal violet, Lugol's iodine solution and dilute carbol fuchsin. It was then air dried and examined under oil immersion at 1000x after being taped firmly on a glass slide.

Culture of Catheter Sample: Catheters were cultured by using the semiquantitative method described by Maki *et al.* Flamed forceps were used to transfer the entire catheter segment onto the surface of a 5% sheep blood agar plate and the catheter was rolled back and forth four times across the agar surface. Plates were incubated at 37^0 C for 48 hours, inspected for microbial growth and colonies were enumerated.

Growth >15 colonies on agar plate indicates infection, 1-14 colonies on agar plate indicates contamination. Samples which grew >15 colonies on plate were considered for the study. All the colony types were identified by standard microbiological methods.

Catheter segment was inoculated into 5ml trypticase soy broth (TSB) and incubated overnight at 37^0 C. Subculture was done from the broth onto Blood agar and Mac Conkey agar, incubated for 24 hours and colonies were enumerated and identified.

Collection of Urine from Catheterized Patients: Urinary catheterization will allow collection of bladder urine with less urethral contamination. Specimen collection from such patients was done with strict

aseptic techniques. A pair of gloves was worn while handling urinary catheter. The catheter tubing was clamped off above the port to allow collection of freshly voided urine. The catheter port or the wall of the tubing was then cleaned vigorously with 70% ethanol and urine aspirated with a sterile needle and syringe, the integrity of closed system was maintained to prevent introduction of organisms into the bladder.

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

Urine Culture: A 5% sheep blood agar and a Mac Conkey agar were used for plating. Before inoculation, urine was mixed thoroughly and the top of the container was then removed. The calibrated loop was inserted vertically into the urine in the container. The loop is touched to the centre of the plate. Without flaming or re-entering urine, the loop is drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.

A colony count of $>10^3$ CFU/ml was taken as indicative of a positive culture as all urine samples collected were catheterized urine samples.

Processing of Urine Catheters: The catheters were placed in 10ml of 0.15M phosphate buffer saline with 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. Tenfold serial dilutions of each suspension were plated on 5% blood agar, incubated at 30^0 C for 18 hours and the mean number of colony forming units was determined.

Identification of the Organisms by Biochemical Reactions: All isolates so obtained were identified by biochemical reactions as per standard protocol.

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disc diffusion method.

Demonstration of Biofilm Formation by Microtitre Plate Assay: This is also called as the Tissue Culture Plate Assay. Isolates from fresh agar plates were inoculated in 5ml trypticase soy broth with 1% glucose and incubated at 37^0C for 18 hours and then 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 37^0 C. After incubation, content of each well was gently removed by tapping the plates. The wells were washed four-times with 0.2ml of phosphate buffer saline (PBS pH 7.2) to remove free-floating 'planktonic' organisms. Biofilms formed by adherent sessile organisms in plate were fixed with sodium acetate 2% and stained with 0.1% crystal violet. 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 was 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.

O.D. value <0.12 were weak/no biofilm producers, 0.12-0.24 were moderate and >0.24 were strong biofilm producers.

RESULTS AND DISCUSSIONS

A total of 106 catheter samples obtained from 105 patients were studied. Of the 106 catheters, 83 were peripheral intravascular catheters (IVC), 22 were Foley's catheters and 1 was a central catheter. Central arterial catheters were not obtained.

Out of 105 cases, maximum number of samples were from neonates. 65 were males and 40 were females. The male: female ratio was 1:6:1.

Positive tip culture was found in 46.98% peripheral intravascular catheters (IVC) and 31.8% urinary catheters. A total of 53 isolates were obtained. Of these, 41 isolates were obtained from IVC and 12 isolates were from Foley's catheter. 4 isolates were obtained from blood culture only, indicating the primary site of infection other than vascular catheter (not included for the study).

A total of 53 isolates were obtained. Of these, 41 isolates were obtained from IVC and 12 isolates were from urinary catheter.

The commonest organisms colonizing IVC were *Candida spp*. 30 (73.1%), CONS 8 (19.5%) and *Klebsiella spp*. 2 (4.8%). Out of 30 (73.1%) Candida spp., 4 (13.3%) were *C. albicans* and 26 (86.7%) non-C. albicans. Urinary catheter samples were commonly colonized with CONS 3 (25%), *P. aeruginosa* and *Acinetobacter spp*. 2 (16.6%).

Out of 53 clinical isolates, 51 (96.2%) were found to produce biofilm. Based on Optical density values, bacterial adherence were grouped as weak (<0.12), moderate (0.12-0.24) and strong (>0.24) biofilm producers. In the present study, 51 (96.2%) were strong biofilm producers.

Biofilm Formation among the Clinical Isolates: Out of 53 clinical isolates. 51 (96.2%) were found to produce biofilm.

Table 1:Distribution of organisms associated with Catheter colonization

Isolates	IVC (41) No. (%)	Urinary Catheter (12) No. (%)	Total (53) No. (%)
Coagulase negative staphylococci	8 (19.5)	3 (25)	11 (20.7)
E. coli	1 (2.4)	1 (8.3)	2 (3.7)
Klebsiella spp.	2 (4.8)	1 (8.3)	3 (5.6)
Enterobacter spp.	-	1 (8.3)	1 (1.8)
P. aeruginosa	-	2 (16.6)	2 (3.7)
Acinetobacter spp.	-	2 (16.6)	2 (3.7)
NF-GNB	-	2 (16.6)	2 (3.7)
Candida	30 (73.1)	-	30 (56.6)

Table 2: Biofilm formation among the clinical isolates

Organisms	Total	Biofilm producers No. (%)
CONS	11	11 (100)
Gram negative bacteria	12	11 (93.3)
Candida	30	29 (96.6)
Total	53	51 (96.2)

Table 3: Antibiotic susceptibility pattern of biofilm producing organisms

Organisms (n)	A	CN	G	E	CD	CF	CO	VA	LZ	AC	CE	CA	I
Coagulase negative staphylococci (11)	0	1	4	7	9	3	5	10	11	-	-	-	-
E. coli (1)	0	0	1	-	-	1	0	-	-	0	1	0	1
Klebsiella spp. (3)	0	0	1	-	-	1	1	-	-	0	2	2	3
Enterobacter spp. (1)	0	0	1	-	-	1	1	-	-	0	0	0	1
P. aeruginosa (2)	0	0	1	-	-	1	2	-	-	0	0	1	2
Acinetobacter spp. (2)	0	0	0	-	-	1	1	-	-	0	0	0	2
NFGNB (2)	0	0	1	-	-	2	1	-	-	0	1	1	2
Total	0	1	9	7	9	10	11	10	11	0	4	4	11

All the 11 coagulase negative staphylococci were biofilm producers. All were 100% resistant to Ampicillin, 91.6% sensitive to Vancomycin and 100% sensitive to Linezolid.

Out of 12 gram negative isolates, 11 were biofilm producers, which were 100% resistant to Ampicillin and Amoxycillin-clavulanic acid, 100% sensitive to Imipenem.

In our study, out of 53 isolates, 96.2% were found to be biofilm producers, in accordance with the studies of Sangita *et al.*[5] (88.8%) and Singhai *et al.* (81.5%)[6].

Antibiotic Susceptibility among Biofilm Producers: In the present study, Coagulase negative staphylococci were 91.6% resistant to methicillin and 100% resistant to Ampicillin, 91.6% isolates were sensitive to vancomycin and 100% sensitive to linezolid. This is similar to studies of Harsha *et al.*[7]. and Singhai *et al.*[6] In our study all gram negative bacteria were 100% resistant to Ampicillin and Amoxy-clavulanic acid, similar to study of Harsha *et al.*[7]. All were susceptible to Imipenem, correlating the study of Singhai *et al.*[6]. and Srinivasa *et al.*[8]. In the present study, out of 83 peripheral intravascular catheters (IVC) studied, 46.98% catheters were positive on semi quantitative culture (SQC). This finding correlates with studied of Subha Rao *et al*. (52.5%) and Shaimaa *et al*. (43.3%). The rate of colonization of urinary catheters in the present study was 31.8%.

In the present study, the Gram's stain was positive in 51.2% of the culture positive cases which compares with the study of Francois *et al.* who showed sensitivity of 44%.In the present study, out of 39 positive IVC-tip cultures, 35.8% samples had similar organisms grown from both the tip culture and simultaneous blood culture indicating catheter-related infection. This study correlates with the studies of Akash *et al*. (43.1%) and Harsha *et al.*[7]. (28.5%). 28.5% of the patients in the present study with Foley's catheter had the same growth on catheter tip as urine, similar to the study of Carlos *et al*. (20.3%), but less compared to the study of Akash *et al*. (56.5%).

CONCLUSION

- Biofilm production was done by Microlitre plate assay. Biofilm production was observed in 96.2% isolates
- CONS were 91.6% resistant to methicillin and 100% resistant to Ampicillin. They were 91.6% sensitive to vancomycin and 100% to Linezolid
- GNB showed 100% resistance to Ampicillin and Amoxicillin clavulanic acid and 100% sensitivity to Imipenem
- Catheter associated infections due to microbial colonization and biofilm formation has gained more attention increasing the incidence of nosocomial infections as a result of catheter use. Therefore, it is necessary to detect the biofilm production in catheter-related infections

REFERENCES

1. Rajesh, S. and V. Berry, 2009. Bacterial biofilm formation, pathogenicity, diagnostics and control: An overview Indian J. Med. Sci. 63: 313-321.
2. Bishop, J.Y., M. Sprague, J. Gelber, M. Krol, M.A. Rosenblatt, J. Gladstone and E.L. Flatow, 2005. Interscalene regional anesthesia for shoulder surgery. J. Bone Joint Surg., 87: 974-979.
3. James, D. and Bryers, 2009. Medical Biofilms. Biotechnology and Bioengineering 100: 1-18.
4. Gerald, L., Mandell, E. John, Bennett and D. Raphael, 2005. Mandell, Douglas and Bennett's

principles and practice of infectious diseases. 6th Edn., Elsevier Churchill Livingstone, London, U.K,
5. El, S., S. Koraichi, H. Latrache and F. Hamadi, 2012. Scanning Electron Microscopy (SEM) and Environmental SEM: Suitable Tools for Study of Adhesion Stage and Biofilm Formation. InTech, Houston, USA., ISBN-13: 9789535100928,
6. Pamp, S.J., C. Sternberg and T. Tolker-Nielsen, 2008. Insight into the microbial multicellular lifestyle via flow-cell technology and confocal microscopy. Cytometry Part A, 75: 90-103.
7. Hense, B.A., C. Kuttler, J. Müller, M. Rothballer, A. Hartmann and J.U. Kreft, 2007. Does efficiency sensing unify diffusion and quorum sensing Nat. Rev. Microbiol., 5: 230-239.
8. Schierholz, J.M. and J. Beuth, 2001. Implant infections: A haven for opportunistic bacteria. J. Hosp. Infec., 49: 87-93