Multiple Drug Resistant Bacterial Biofilms on Implanted Catheters - A Reservoir of Infection

SS Pradeep Kumar*, HV Easwer**, A Maya Nandkumar***

Abstract

Background: Medical devices are an essential part of modern health-care, but their usage has led to the emergence of medical device associated infections otherwise known as Foreign–Body related infections (FBRIs). This is caused by bacterial adhesion and biofilm formation on their surfaces which act as a nidus of infection. These biofilms are resistant to antibacterial agents and host immune response. The antibiotics which are useful in treating planktonic forms cannot clear the biofilm and the device must usually be removed to resolve the infection.

Objective: The main objective of the study was to identify the prevalence of bacterial biofilms on retrieved catheters which included vascular catheters (141) and Foley catheters (86) from patients and understand the nature of antibiotic resistant strains in these biofilms.

Materials used: 227 numbers of retrieved catheters which included vascular catheters (141 nos) and Foley catheters (86 nos) were used in the study. These retrieved implants were from patients from neurosurgery ward of SCTIMST who had undergone selective neurosurgical procedures. Patients with pre-existing infections including brain abscess were not included in the study. These patients had no clinical infection or pyrexia. Skin swabs were taken from patients using intravascular devices after retrieval of catheter.

Results and Conclusions: Among vascular catheters Staphylococcus species were the major isolate and among the isolates many of the strains were Methicillin and some were Vancomycin resistant. Among the Foley catheter isolates E. faecalis was the major isolate followed by E. coli, Staphylococcus species, Klebsiella species, Pseudomonas species, Citrobacter etc. Most of the strains were resistant to multiple antibiotics. Although the patients selected did not have any clinical symptoms of infection, the presence of multiple-drug resistant organisms as biofilm points to this niche which can constitute a threat for HAIs and its resultant complications. This suggests that catheters should be removed as early as possible to prevent Biofilm development on them.

Introduction

The use of indwelling devices, both temporary and permanent, in medical and surgical practice has lead to the emergence of implant associated infections (IAIs). Due to their underlying disease conditions, hospitalised patients are highly susceptible to IAIs. About 60 - 70% of nosocomial infections are associated with some type of implanted medical device.1,2 Darouiche et al states that about 30 million Foley catheters are inserted each year with an infection rate of 20 - 30%, the second most common is the vascular catheter and about 6 million of these are used yearly and have an infection rate of 4 to 5% and the complications are serious.3 IAIs are caused by the bacterial colonisation and biofilm formation on the devices which helps the micro-organisms acquire multiple antibiotic resistance and evade the host immune response. This paper opens a window to the Indian scenario and the enormity of the problem. The data discussed here pertains to biofilms on retrieved implants from patients who did not
was conducted to quantify antibiotic usage in the community at New Delhi. This study revealed that the highest consumption of antibiotics was Fluoroquinolones followed by cephalosporins and penicillins. Surveillance conducted at two other sites Vellore and Mumbai also followed this pattern. The data available on antibiotic usage in India is often fragmented and usually is an indicator study and there is little correlation with antibiotic resistance development. It is under these circumstances that this study gains importance. In this paper we are looking into the prevalence of biofilms and antibiotic resistance, on medical devices retrieved from patients who had no clinical infection at the time of device removal and its significance. The aim of the study was to identify and characterise bacterial Biofilms on the retrieved implants and investigate the presence of antibiotic resistance in these organisms.

### Materials and Methods

Medical devices/ implants used in the study: The samples used in the study were temporary implants - vascular catheters which are blood contacting devices and Foley catheters which are mucous membrane contacting devices removed from patients of neurosurgery ward. The total number of retrieved implants was 227. These included vascular catheters (141) and Foley catheter (86). All the retrieved implants were from patients who had no clinical infection at the time of device removal. So, IAIs lead to increased duration of hospital stay, increased cost, increased morbidity and mortality.

Today in India, indiscriminate antibiotic usage is widely prevalent. This has lead to emergence of antibiotic resistant micro-organisms, the latest incidence being the discovery of NDM-1 (New Delhi mettalo-β lactamase) in Enterobacteriaceae. Deshpande et al reported the prevalence of NDM-1 in 22 out of 24 isolates of Enterobacteriaceae at the PD Hinduja Hospital and Medical Research Center, Mumbai. In collaboration with WHO, surveillance

<table>
<thead>
<tr>
<th>Name of implant</th>
<th>Foley Catheter</th>
<th>Vascular catheters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms isolated</td>
<td>Staphylococcus species</td>
<td>E. coli</td>
</tr>
<tr>
<td>Enterococcus species</td>
<td>Enterococcus species, Streptococcus species</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus species</td>
<td>Klebsiella species</td>
<td></td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>Pseudomonas species</td>
<td></td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>Micrococcus species</td>
<td></td>
</tr>
<tr>
<td>Citrobacter species</td>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>Yeast</td>
<td></td>
</tr>
</tbody>
</table>

---

**Biofilms** play a central role in health care associated infections (HAI) or medical device associated infections. Biofilms are structured microbial communities in which the microbial cells irreversibly attach to a surface or interface and are embedded in a matrix of extra-cellular polymeric slime produced by these cells. Most of the biofilms grow slowly, adopt many different metabolic strategies and they detach planktonic cells and biofilm fragments in a programmed manner. The clinical consequence is that such biofilms on implanted medical devices act as a nidus for chronic infections which are recalcitrant to antibiotic therapy. It has been reported that urinary tract infection (UTI) is the most common nosocomial infection and in about 75 – 80%, this follows catheterisation. Between 5 - 25% of all intravascular devices are colonised at the time of removal. So, IAIs lead to increased duration of hospital stay, increased morbidity and mortality.

Fig. 1: Percentage prevalence of Micro-organisms isolated from Foley catheter retrieved from patients

Fig. 2: Nature of biofilms present on retrieved Foley catheter

have any infection at the time of their hospitalisation and surgery and also at the time of implant retrieval and its significance.

Today in India, indiscriminate antibiotic usage is widely prevalent. This has lead to emergence of antibiotic resistant micro-organisms, the latest incidence being the discovery of NDM-1 (New Delhi mettalo-β lactamase) in Enterobacteriaceae. Deshpande et al reported the prevalence of NDM-1 in 22 out of 24 isolates of Enterobacteriaceae at the PD Hinduja Hospital and Medical Research Center, Mumbai. In collaboration with WHO, surveillance
abscess were excluded (head and spine injuries are not routinely operated in our Centre (Sree Chitra Tirunal Institute for Medical Sciences and Technology, a tertiary referral centre) and hence such cases were not included in this series). In cases of active infection the retrieved catheters were subjected to clinical Microbiological profiling for treatment. This forms a group where infection is overt and therefore identified. So these are not included in this study and this data forms part of patient records.

**Microbiological culture methods:** The tips of various catheters (vascular and Foley) withdrawn from patients were collected into sterile containers containing Fluid D. Bacterial biofilm on the catheters were removed by sonication (Ultrasonic cleaner-

![Fig. 3: Percentage prevalence of organisms isolated from vascular catheters](image)

- **E. coli**: 2%
- **Enterococcus species**: 2%
- **Staphylococcus species**: 65%
- **Micrococcus species**: 2%
- **Streptococcus species**: 5%
- **Pseudomonas species**: 5%
- **Klebsiella species**: 5%
- **Yeast**: 14%

![Fig. 4: Nature of biofilms isolated from vascular catheters retrieved from patients](image)

![Fig. 5: Prevalence of Bacteria present in the skin swab taken from around the insertion site of intravascular catheters](image)

**Fig. 6:** Antibiotic resistance pattern of E.coli isolated from Foley catheters retrieved from patients

- **Penicillin group**: 61%
- **Quinolones**: 74%
- **Gentamycin**: 92%
- **Vancomycin**: 8%

**Fig. 7:** Antibiotic resistance pattern of E. faecalis isolates from retrieved Foley catheters

- **Methicillin**: 83%
- **Vancomycin**: 62%
- **Aminoglycosides**: 62%
- **Quinolones**: 14%

**Fig. 8:** Antibiotic resistance pattern of Staphylococcus species isolates from vascular catheters. 83% of the *Staphylococcus* species isolates were methicillin resistant and 14% was vancomycin resistant

![Fig. 9: Scanning electron micrographs of biofilms on retrieved Foley catheters. A: Staphylococcus biofilm B: E.coli biofilm C: Crystal formation as a result of biofilm and this is responsible for encrustation and blockage of the catheter.](image)

A: Staphylococcus biofilm  
B: E.coli biofilm  
C: Crystal formation as a result of biofilm and this is responsible for encrustation and blockage of the catheter.
Cole – Palmer 8891) and vortexing. The bacteria removed by sonication and vortexing were isolated and identified by standard microbiological techniques and their antibiogram patterns were studied by disc diffusion assay. 5

Scanning electron Microscopic examination of retrieved implants: Retrieved implants from patients were fixed with 2% gluteraldehyde and dehydrated in graded series of ethanol and gold sputter coated and processed for SEM and viewed using Hitachi Scanning electron microscope S2400.

Results

Biofilm based infections are associated with antibiotic resistance development in pathogenic bacteria and fungi. Biofilms are an ideal site for plasmid exchange in bacteria and provide the necessary environment for induced antibiotic resistance development, specifically, when we consider that many of the catheterised patients may be receiving antibiotics. Here we have specifically analysed catheters collected from patients who had no clinical infection in order to understand the threat constituted to patients in general due to the presence of multiple-drug resistance properties in this group of biofilm isolates from these temporarily implanted medical devices.

The total numbers of retrieved implants were 227. Out of them, 141 were vascular catheters (peripheral venous catheters/ central venous catheter) and 86 were Foley catheters. The implants remained in situ for variable periods of time (1 day-14 days). Table 1 shows the microorganisms isolated from these two classes of temporary medical devices and Figure 9 is a representation of the biofilms on these catheters. About 80% of the retrieved Foley catheters showed microbial biofilms. Figure 1 shows that Enterococcus species was the major isolate, forming about 28%, followed by E.coli and Staphylococcus species which were isolated from approximately 16% of catheters. Figure 2 shows that in about 69% of the retrieved Foley catheter, biofilm was formed by a single genus. About 21% of the retrieved Foley catheters had polymicrobial biofilm formed from two or more types of microorganisms. Among the isolates most of the strains were resistant to multiple antibiotics. About 28% of the retrieved vascular catheters show microbial biofilms. Figure 3 details the percentage prevalence of microorganisms isolated from vascular catheters. Biofilm present in about 93% of the retrieved vascular catheters contained single genus and about 7% biofilms contained multiple genus (Figure 4). In single genus biofilms Staphylococcus species dominated and accounted for about 65% of single genus biofilms. Majority of Staphylococcus strains were resistant to multiple antibiotics including methicillin. When comparing the isolates from skin at insertion point Figure 5 and Figure 10 it was observed that 80% of isolates were of the same genera. Only 20% of isolates from the biofilm on vascular catheters were not part of the skin flora. Figures 6, 7 and 8 show the prevalence of antibiotic resistance profile in these isolates. These finding are an indication of this
niches which can give rise to multiple drug resistant infection in patients which may be recalcitrant to antibiotic therapy. Figure 9 shows SEM of biofilms and crystal on retrieved Foley catheters. The crystal formation leads to blockage of the catheter preventing easy deflation of the balloon and catheter removal. Figures 11 and 12 give an indication of the duration of catheterisation required for biofilm development. In Foley catheters when duration of catheterisation was more than 3 days, 80% of time there was biofilm formation. In vascular catheters 33% of time biofilm was detected when duration exceeded three days.

**Discussion**

Biofilm is the predominant mode of growth of bacteria and this plays a central role in pathogenesis of catheter associated Urinary tract infection (CAUTI) and vascular catheter associated blood stream infections (BSI). Foley catheters are mucosal surface contacting medical devices and CAUTI accounts for about 40% of all nosocomial infections. In our study of biofilms in Foley catheters it has been found that about 80% of the retrieved Foley catheters showed microbial biofilms. The patients from whom the Foley catheters were retrieved were on antibiotic cover of Cephalosporin / Chloramphenicol and aminoglycosides as prophylactic therapy. *Enterococcus* species was the major isolate and was isolated from about 36% of all catheters. As per previous reports in intensive care units, *Enterococcus* was the major causative agent for CAUTI although generally *E. coli* is predominant pathogen for UTI. A similar situation was noticed in our study also with increased isolation of *Enterococcus* from Foley catheter biofilms. This may be due to the presence of prophylactic antibiotic cover in these patients resulting in increased survival rate due to multiple drug resistance. Biofilm formed in about 69% of the retrieved Foley catheters contained single genus. About 31% of the retrieved Foley catheters contained multiple genera. Among the isolates most of the strains were resistant to multiple antibiotics.

Vascular catheters are externally communicating devices and biofilms associated with them were an important cause of BSI. In our study it was found that 28% of retrieved vascular catheters had bacterial biofilms. Biofilm found in about 92% of the retrieved vascular catheters were single genus and mixed genera was found only in 8% of the retrieved vascular catheters. Among the different genera *Staphylococcus* species was the major isolate and most of them were methicillin resistant and a proportion of them were Vancomycin resistant. The 80% bacteria isolated from the skin near the point of insertion of vascular catheters and vascular biofilm constituents were of similar genera and included *Staphylococcus* species (Both coagulase positive (38%) and coagulase negative (14%). Many of the isolates were resistant to multiple antibiotics. 82% of the *Staphylococcus* species isolates were methicillin resistant and 14% was vancomycin resistant.

The device provided a safe haven for microbial colonisation and also for such colonies to develop multiple drug resistance for which the antibiotic cover provided sufficient stimulus. Our hospital policy is that when there is any clinical symptoms of the infection either UTI or BSI the catheters and blood or urine samples are sent to clinical microbiology laboratory for microbiological profiling and treatment. Such patients had an overt infection and were treated and therefore are not included here. The samples that were analysed here were from patients who did not have clinical symptoms of infection clearly pointing to the potential for this group to give rise to antibiotic resistant infections at any point of time till the device is in place. As the devices were removed before development of clinical symptoms we believe that the threat of infection to patient was aborted.

Catheters and medical devices have become an indispensable part of modern day medical practice and device associated infections are adverse outcome of it. Most developing countries do not have laws mandating health-care associated infection control programmes and this is true of India also. Here funds and resources are limited and nurse to patient ratio is lower than in developed nations. But the recent document “National Policy on containment of antimicrobial resistance in India” released by Directorate General of Health Services, India is an indication that Government of India is serious about the prevention and spread of anti-microbial resistance. Prevention of HAIs is the responsibility of all those associated with the development of the medical devices and the physicians and surgeons who use it. Surveillance of health-care associated infections – defining magnitude and nature of the problem is the first step towards reducing risk of infection. The second step is to implement targeted basic infection control practices that have been shown to prevent HAIs. Then technology and a dynamic antibiotic usage policy will help in an effective nosocomial infection control programme.

Some of the technological advancements in this area specifically to prevent catheter related blood stream infection (CRBSI) are antibiotic lock prophylaxis by flushing and filling the lumen of the catheter with an antibiotic solution and leaving the solution to dwell in the lumen of the catheter. For CAUTI usage of antimicrobial ointments and lubricants, bladder instillation/ irrigation, antimicrobial agents in collection bags, impregnation of catheter with
antimicrobial agents such as silver oxide or use of synthetic antibiotics. The most promising development in this area has been catheter with antiseptic Chlorhexidine and silver sulphadiazine (CHSS catheter - ArrowGard) which became available 10 years ago. Since its inception more than 8 million catheters have been sold world-wide and has shown two-fold reduction in the incidence of catheter colonisation and 5 fold reduction in CRBI. Silver has been used extensively to develop infection resistant urinary catheters. Today silver nanoparticles are being used for infection resistant catheters specifically urinary catheters.

Other technologies like more potent anti-infective materials, microbe-impervious anti-reflux valves, conformable (collapsible) urethral catheters and vaccines for bacteria in Biofilms are all at various stages of research and development. So strategies have to be devised to control and prevent nosocomial infections associated with the use of implants in clinical practice. The antibiotic policy need to be changed at regular intervals to prevent the development of resistant pathogens that leads to medical device related complications. Also newer microbiological techniques need to be developed to identify biofilm based infection. Current antibiotic sensitivity assays are performed on planktonic forms of pathogens while in biofilms they are resistant to more than 1000 times the pharmacologically effective concentrations.

**Conclusion**

In conclusion it can be seen that bacteria colonise and develop biofilms on temporary indwelling devices often by 75 hours and hence these devices should be removed as early as possible. As duration of catheterisation increased the biofilms consisted of multiple genera and majority of them were resistant to a number of antibiotics. Vascular catheter biofilms in 80% of cases were of Staphylococcus species a major skin isolate. This study underscores the pressing need for development of antimicrobial vascular and urinary catheters and their deployment when longer durations of catheter access is required.

**Acknowledgements**

The authors wish to acknowledge the financial assistance from KSCSTE (Kerala State Council for Science Technology Environment), Director SCTIMST and Head BMT Wing for encouragement and support with infrastructure also “Mr. R. Sreekumar’s help with SEM imaging is acknowledged”.

**References**