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Role of Biofilm Production in Bacteria Isolated from Device Related and Non-Device Related Infection in a Tertiary Care Hospital

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A B S T R A C T

The use of indwelling devices both temporary and permanent, in medical and surgical practice has led to the emergence of implant associated infections in the patients admitted in a Tertiary care hospital leading to partial or complete therapeutic failure. The study was conducted to detect and compare biofilm production in bacteria isolated from device related (DR) and non-device related (NDR) infections by Tissue Culture Method (TCP). A total of 200 bacterial isolates from various DR and NDR clinical samples of patients suffering from hospital acquired infections were subjected to biofilm detection and drug susceptibility testing. Of the 200 strains in the present study 121 bacterial strains were isolated from device related and 79 from non-device related clinical samples. Of the DR isolates, 86 (71.1%) were biofilm producers which included 10 (11.6%) strong, 37 (43%) moderate and 39 (45.4%) weak producers whereas of the NDR isolates 66 (83.5%) were biofilm producers including 18 (27.3%) strong, 21 (31.8%) moderate and, 27 (40.9%) weak producers respectively. Device related biofilm producing strains of *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* showed higher rate of drug resistance in comparison to their non-biofilm producing isolates. It is concluded that of bacteria isolates not only in device related infections but is also associated with multi drug resistance. Early detection of biofilm production will be of immense help in changing the modality of treatment with better patient outcome in device related infections.

Introduction

A Device related infection is defined as the host immune response to one or more microbial pathogens on an indwelling medical device. The use of indwelling devices both temporary and permanent, in medical and surgical practice has led to the emergence of implant associated infections. The association of biofilm and medical device related infections was first recognized in 1972.[1]These infections include catheter-associated urinary tract infections (CAUTI), central-line-associated blood stream infections (CLABSI), and ventilator-associated pneumonias (VAP).[2,3]

These infections are caused by bacterial colonization and biofilm formation on devices which help the microorganisms to acquire multiple antibiotic resistance and evade host immune response. Device related infections that unfortunately has received the least amount of attention, but which continues to

contribute to major morbidity and mortality. [4]Biofilm is an association of micro-organisms in which microbial cells adhere to each other on a living or non-living surface within a self-produced matrix of extracellular polymeric substance.[5]

Biofilms on indwelling medical devices may be formed by gram-positive bacteria, gram-negative bacteria and yeasts.[6]The predominant organisms responsible for device related infection are mostly *Staphylococcus aureus* (*S aureus*), *Pseudomonas aeruginosa* (*Ps aeruginosa*), and Enterobacteriaceae, but the etiologic agents differ widely according to the patient population in an intensive care unit, duration of hospital stay and prior antimicrobial therapy.[7-10] These infections in turn lead to prolonged hospital stay along with increased burden of antibiotic usage, thereby leading to an overall increase in the health care cost. [11]

Very few studies have so far been reported in India as regards biofilm production by bacterial isolates in device related infections. In a study by Patel *et al.*, vascular catheters and blood collected through catheter yielded the highest number of biofilm producing Gram negative bacilli.[12] Most common pathogens isolated from device associated HAI patients were *Klebsiella pneumoniae* (*K. pneumoniae*) (24.6%), *Escherichia coli* (*E. coli*) (21.9%), and *Ps aeruginosa* (20.2%). More than 80% of these strains were multi-drug resistant. They were only susceptible to Colistin and Tigecycline.[13]

A prospective study has conducted over a period of one year (from April 2016 to March 2017). As this hospital is a tertiary care set up, indwelling medical devices are widely used in the various medical and surgery specialties. Commonly used medical devices are endotracheal tubes, tracheostomy tube, biliary stents, urinary catheters, CVC lines, peripheral venous catheters and drain tubes. No data is available in our institution regarding biofilm production by bacterial isolates in device related infections.

Materials and Methods

Various clinical samples from patients with device related hospital acquired infection, collected and processed as per Standard technique. Antimicrobial susceptibility testing has done by Kirby-Bauer disc diffusion technique as per CLSI guidelines 2016. [14]The drug susceptibility was done for the following: Cephalosporins, Aminoglycosides, Fluoroquinolones, Penicillins, Macrolides, Glycopeptides, Oxazolidones, Cefoxitin and Carbapenams (Hi-media) were used for drug susceptibility testing. The control strains used were *Esch coli* ATCC 25922, *Ps. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213. Identification and drug susceptibility of the bacterial isolates was confirmed by the automated Vitek II Compact system. All these bacterial isolates were preserved for biofilm detection.

Biofilm detection

Biofilm detection was performed by the Tissue Culture Plate (TCP) method as described by Christensen *et al.*, (1995), which is considered as the gold standard method.[15]The bacteria were grown in polystyrene tissue culture plates for 24 hours. After washing fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Biofilm formation was detected by measuring the optical density (OD) using ELISA reader. The experiment was performed in triplicate and repeated three times. The interpretation of biofilm production was done according to the criteria of Stepanovic *et al.*,[16]

Data analysis was performed by Pearson Chi-Square test using SPSS software version 18.

Results and Discussion

During the study period 219 bacterial strains were isolated from various clinical samples. Of these 200 isolates associated with hospital acquired infection (HAI) were included in the study. One hundred twenty one isolates (60.5%) were device related (DR) and 79 (39.5%) isolates were non-device related (NDR).

Details of device related and non-device related clinical sample is shown in table 1.

Maximum number of DR isolates were from respiratory samples (64) followed by drain fluid (42). While maximum NDR isolates were from pus (36) followed by blood samples (29).

Of the 121 bacterial strains were isolated from device related clinical samples maximum were *K. pneumoniae* 57(47.1%) followed by *Escherichia coli* 28(23.1%), *Psaeruginosa* 19(15.7%), *Acinetobacter baumannii* (*A baumannii*) 11(9.1%), *S aureus* 3(2.5%), *Proteus mirabilis* (*P mirabilis*) 2 (1.7%) and *Providencia species* 1(0.8%). Of the 79 isolates from non-device related samples, highest were *E. coli* 22(27.9%) followed by *K.pneumoniae* 21(26.6%), *S. aureus* 12(15.2%), *A. baumannii* 12(15.2%), *Ps. Aeruginosa* 9(11.4%), *P. mirabilis* 1(1.3%), *Burkholderia cepacia* (*B. cepacia*) 1(1.3%) and *Enterobacter cloacae* (*E. cloacae*) 1(1.3%).

Out of 200 MDROs, 152 isolates were biofilm producers and 48 were non-biofilm producers. Of 152 biofilm producing strains, 86wereDR while 66 were NDR isolates. Statistically significant difference was observed between DR and NDR biofilm production (*p* value is 0.04).

Of the 121 isolates from device related infections, 86 (71.1%) strains were biofilm producers with maximum number being *A. baumannii*, *S. aureus*, *P. mirabilis* and *Providencia spp.* (100%) followed by *K. pneumoniae* (77.2%), *P. saeruginosa* (73.68%) and *E. coli* (39.28%).

55.8% of the biofilm producers were associated with VAP followed by 32.6% Pyogenic infection, 8.1% CRI and 3.5% with CAUTI.

Of the 79 isolates from non-device related infections, 66 (83.54%) strains were biofilm producers. Of these isolates all strains of *A.baumannii*, *Ps. aeruginosa*, *P. mirabilis*, *B. cepacia* and *Enterobacter cloacae* were biofilm producers (100%) whereas 91.7% of *S. aureus*, 90.5% of *K. pneumoniae* and 54.5% of *E. coli* were found to be biofilm producers respectively. Among non-device related isolates 42.4% of biofilm producers were associated with pus followed by 39.4% blood stream infection (BSI), 12.1% respiratory and 6% body fluids.

Pus samples have the maximum number of biofilm producers 28(42.4%) followed by Blood stream samples 26(39.4%), respiratory samples 8(12.1%) and body fluids with the least number 4(6.1%).

Biofilm production in (DR)/(NDR) isolates is shown in table 2.

Among Gram negative isolates from both DR and NDR infections maximum of the biofilm producing strains isolated were *K. pneumoniae* (44 and 19 respectively).

Grading of biofilm producing isolates from device related /non- device related samples are shown in table 3.

Of the 86 biofilm producing DR isolates 10 were strong, 37 moderate and 39weak biofilm producers whereas among 66 biofilm

producing NDR isolates 18, 21 and 27 were strong, moderate and weak biofilm producers respectively. Statistically significant difference was observed in grading of biofilm produced by DR and NDR isolates (*p* value is 0.04).

Drug resistance pattern (in percentage) of biofilm (BF) producers and non-biofilm (NBF) producers of DR isolates is depicted in table 4.

All strains of *A. baumannii*, *P mirabilis*, *Providencia* spp. and *S. aureus* isolated from device related clinical samples were biofilm producers and were multi drug resistant (MDR). Device related biofilm producing strains of *K.pneumoniae* showed higher rate of drug resistance in comparison to the non-biofilm producing isolates against Piperacillin/Tazobactum (93% and 77%), Ciprofloxacin (95% and 77%), Levofloxacin (79% and 50%), Imipenem (89% and 69%) Meropenem (91% and 77%) and Ertapenem (93% and 58%) respectively. Similar resistant pattern was observed in device related biofilm producing strains of *E. coli* and *Ps.aeruginosa*. NDR biofilm producing isolates showed almost similar resistance pattern as DR isolates. Biofilm producing (DR) *Ps aeruginosa* also showed 50% resistance against Tigecycline.

Table.1 Clinical sample vs Device related (DR)/Non device related (NDR) isolates

Clinical Samples	DR	NDR
Respiratory sample	64	9
Drain Fluid	42	5
Tip	10	0
Urine (Catheterized)	5	0
Pus	0	36
Blood	0	29
Total	121	79

Table.2 Biofilm production in (DR)/(NDR) isolates

BacterialIsolate (DR+NDR)	DR	NDR
***Klebsiellapneumoniae*(57+21)**	44 (77.2%)	19 (90.48%)
***Pseuodomonasaeruginosa*(19+9)**	14 (73.68%)	09 (100%)
***Escherichia coli*(28+22)**	11 (39.28%)	12 (54.55%)
***Acinetobacterbaumannii*(11+12)**	11 (100%)	12 (100)
***Staphylococcus aureus*(3+12)**	3 (100%)	11 (91.67%)
***Proteus mirabilis*(2+1)**	2 (100%)	1(100%)
***Providenciaspp.*(1+0)**	1 (100%)	0
***Enterobacter Cloacae*(0+1)**	0	1(100%)
***Burkholeriacepacia*(0+1)**	0	1(100%)
Total	86 (71.1%)	66 (83.54%)

Table.3 Grading of biofilm producing isolates from device related /non- device related samples

Bacterial Isolate name & no.	Device related					Non- device related				
	S	M	W	Total	N	S	M	W	Total	N
***Acinetobacterbaumannii*(23)**	2	6	3	11	0	9	2	1	12	0
***Pseudomonas aeruginosa* (28)**	1	7	6	14	5	2	1	6	9	0
***Escherichia coli* (50)**	1	2	8	11	17	2	4	6	12	10
***Klebsiella pneumoniae*(78)**	6	18	20	44	13	3	8	8	19	2
***Staphylococcus aureus*(15)**	0	3	0	3	0	1	5	5	11	1
***Proteus mirabilis* (03)**	0	1	1	2	0	0	0	1	1	0
***Providencia spp.* (01)**	0	0	1	1	0	0	0	0	0	0
***Enterobacter cloacae* (01)**	0	0	0	0	0	1	0	0	1	0
***Burkholderia cepacia*(01)**	0	0	0	0	0	0	1	0	1	0
Total	10	37	39	86	35	18	21	27	66	13

(*Abbreviations used for biofilm production: S= Strong, M= Moderate, W= Weak, P= Positive, N= Negative)

Table.4 Drug resistance pattern (in percentage) of biofilm (BF) producers and non-biofilm (NBF) producers of DR isolates

Antibiotics	*K.pnemoniae* BF (n=44)	*K.pnemoniae* NBF (n= 13)	*E.coli* BF (n=11)	*E.coli* NBF (n=17)	*A.baumannii* BF (n=11)	*P.aeruginosa* BF (n=14)	*P.aeruginosa* NBF (n=5)	*P.mirabilis* BF (n=2)	*Providencia* Spp. BF (n=1)
Ac	98	92	94	72	100	100	86	100	100
Tz	93	77	73	24	100	79	40	100	100
Co	95	92	100	12	100	100	86	100	100
Cpm	100	92	100	100	100	100	93	100	100
Ca	100	92	100	91	100	100	86	100	100
Cf	95	77	94	82	100	100	86	100	100
Of	100	95	94	80	100	100	86	100	100
Le	79	50	90	83	100	100	100	100	100
Akj	100	75	41	25	91	75	71	100	100
Nt	100	83	71	63	100	100	86	100	100
Gm	98	85	73	71	100	100	79	100	100
Tb	98	92	82	71	100	83	80	100	100
Imp	89	69	53	27	100	57	20	-	100
Mr	91	77	59	55	100	80	77	0	100
Etp	93	58	59	55	100	75	75	50	100
Tg	25	23	6	0	18	50	30	0	0
CL	30	23	14	12	-	23	0	0	100

*Abbreviations:
BF= Biofilm producer, NBF=non-biofilm producers,
n=total no. of isolates,
AC= Amoxycilline + Clavulanic acid, TZ= Piperacillin+tazobactum, CO= Cotrimoxazole, CPM= Cefpirome, CA=Ceftazidime, CF=Ciprofloxacin, OF=Ofloxacin, Le= Levofloxacin, AK=Amikacin, NT=Netillmycin, GM=Gentamycin, Tb=Tobramycin, I=Imipenem, Mr=Meropenem, Etp=Ertapenem, Tg=Tigecycline, Cl=Colistin.*

Use of indwelling medical devices in patients admitted in the health care setup is colonized by biofilm producing organisms which are often multidrug resistant, thereby promoting drug resistant device-related infections. Medical device related infections not only pose huge financial burdens on the health care services but also prolong the course of the treatment thereby increasing morbidity and mortality.

The microorganisms are thus able to survive in the hospital environment despite unfavorable conditions such as desiccation, nutrient starvation and antimicrobial treatment. It is hypothesized that the microbes can persist in the environments and show high degree of virulence as a result of their capacity to colonize medical devices.[17]

Out of 200 strains in the present study 121 bacterial strains were isolated from device related and 79 from non-device related clinical samples. Of the device related isolates, 86 (71.1%) were biofilm producers. Majority of biofilm producing bacteria included *P. mirabilis*, *Providencia spp*., *A.baumannii* and *S aureus*, (100%) followed by *K. pneumoniae* (77.2%), *P. saeruginosa* (73.68) and *E. coli* (39.28%). In the study maximum number of biofilm producers were isolated from VAP (55.8%) followed by Pyogenic infections (44.2%), CRI (8.1%) and CAUTI (3.5%). Of the 86 biofilm producing device related bacterial isolates, 10 (11.6%) were strong, 37(43%) were moderate and 39 (45.4%) were weak biofilm producers respectively.

Christensen *et al.*, [15] reported that 30% of biofilm forming bacteria were isolated from various indwelling medical devices, which is lower than the finding of the present study (71.1%). The association of biofilm producing bacteria in urinary catheters was reported by Donalan (2001) in his study.[5]According to a study by Hassan *et al.*,[18] the majority of the organisms associated with biofilm production were *S. epidermidis* (37.1%) followed by *E. coli* (27.1%), *K. pneumoniae* (15.7%), *S. aureus* (11.4%), *E. faecalis* (4.2%) and *P. aeruginosa* (4.2%). Of these biofilm producing bacteria 25.7% were isolated from urinary catheter tips followed by intravenous catheter tips (10%). The findings of the present study is much higher than that of Hassan *et al.*, but in both studies similar organisms were associated with device related infections. In a study conducted by Mulla *et al.*,[19] the overall biofilm production by bacterial isolates from patients with medical devices was 88% which is slightly higher in comparison to the results of the present study.

Pradeep Kumar *et al.*,[20] studied biofilm formation on 141 vascular catheters and 86 Foley catheters. They reported that 28% of the vascular catheters showed the presence of microbial biofilms and 80% of the Foley's catheter had microbial biofilms. Sayal *et al.*,[21] found that *Escherichia coli* was responsible for more than 80% of the UTIs. 71.23% of these isolates were found to be biofilm producers. Shyam *et al.*,[22] reported in a study that of the 67 clinical isolates from Indwelling Medical devices, 46.3% of the isolates were biofilm producers. According to a study of Patel *et al.*,[12]blood collected from Catheter showed the highest number of biofilm producers which includes *Acinetobacter* spp. (30%), *K.pneumoniae* (22%), *Ps. aeruginosa* (16%), *S. aureus* (14%) and *E. coli* (12%). Most of the results are in agreement with the results of present study.

In a study by Singhai *et al.*,[23] the rates of biofilm-based catheter-related BSI, CAUTI, and VAP were 10.4%, 26.6%, and 20% respectively. Majority of infections were due to *K. pneumoniae* followed by *Staphylococcal* biofilms. A high percentage of the biofilm

producing bacterial isolates, were multidrug resistant and produced infections. Device related *K.pneumoniae* (73.1%) were found to be the highest biofilm producers among device related isolates.[23] Even in our study *K.pneumoniae* (77.2%) was also one of the highest biofilm producers and drug resistant among DR isolates. Most of the studies mentioned above have findings similar to the present study.

Significant difference was observed in the production of biofilm and it's grading by bacterial isolates from device related and non-device related clinical samples (p-value 0.014).

According to a study by Hassan *et al.,*[18] the majority of the organisms isolated from NDR clinical samples associated with biofilm production were *S.epidermidis* (37.1%) followed by *E. coli* (27.1%), *K.pneumoniae* (15.7%), *S. aureus* (11.4%), *E. faecalis* (4.2%) and *P. aeruginosa* (4.2%). Which is lower than the findings of the present study where *Ps aeruginosa* and *A.baumannii* showed maximum number of biofilm producers (100%) followed by *S aureus* (91.67%) and *K.pneumoniae* (90.48%). Maximum biofilm producing bacteria were isolated from urine (30%) followed by, pus (12.8%), sputum (11.4%) and nasobronchial lavage specimens (10%), whereas in the present study maximum of the isolates were from pus (42.4%) followed by blood stream infection (39.4%), respiratory samples (12.1%) and body fluids 6.1%).

In this study all strains of *A. baumannii*, *P mirabilis*, *Providencia* spp. and *S. aureus* isolated from device related clinical samples were biofilm producers and multi drug resistant (MDR). Device related biofilm producing strains of *K. pneumoniae* showed higher rate of drug resistance in comparison to non-biofilm producing isolates. Similar resistant pattern was observed in device related biofilm producing strains of *E. coli* and *Ps aeruginosa*. Fifty percentages (50%) of biofilm producing (DR) *Ps. aeruginosa* also showed resistance against Tigecycline. Biofilm producing isolates from non-device related infections showed almost similar drug resistance pattern as of DR isolates.

Singhai *et al.,*[23] reported in their study that a high percentage of biofilm producing bacterial isolates causing infection were multidrug resistant. Similar results were observed by Subramanian *et al.,*[24] in their study. Approximately 80% of the biofilm producing strains showed multidrug resistance. Shahidul *et al.,*[25] found that, 91.6% of the biofilm producing isolates were Multidrug resistant. All these studies are in agreement with present study.

The rate of drug resistance as seen in by biofilm producing isolates from device related samples is higher than that of the non-biofilm producing isolates in the present study.

In conclusion, the indwelling medical devices provide an ideal condition for the development of bacterial biofilms. These biofilms hinder the entry of antimicrobials and protecting the bacteria from their bactericidal effects thereby leading to increased morbidity and mortality. Thus these biofilm producers becomes MDR pathogens causing device related infections which often leads to partial or complete therapeutic failure.

Hence, biofilm production is an important virulence marker of bacteria isolates not only in device related infections but also associated with multi drug resistance. Biofilm associated bacteria from device related infection are more often MDR and this results in increased morbidity and mortality among the hospitalized patients. Early detection of

biofilm production will be of immense help in changing the modality of treatment with better patient outcome in device related infections

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