

# A Study on Device-Related Infections with Special Reference to Biofilm Production and Antibiotic Resistance

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## ABSTRACT

**Background:** Indwelling medical devices (IMDs) in critical patients are vulnerable to colonization by biofilm producing bacteria. Complex characteristics of bacterial biofilms promote antibiotic resistance, leading to the emergence of resistant device-related infections (DRI), which pose new challenges in their management. **Materials and Methods:** The study was done on 135 hospitalized (Intensive care units) pediatric patients with IMDs (intravascular catheter, urinary catheter, and endotracheal tube) to determine the device-specific infection rates. Biofilm formations were demonstrated by the tube method and by scanning electron microscopy (SEM). Bacteria in biofilms were identified by the standard conventional methods and tested for antibiotic resistance. We also detected the presence of extended spectrum  $\beta$ -lactamases (ES $\beta$ Ls), particularly, bla<sub>CTX-M</sub>, in gram-negative isolates. **Results:** The rates of biofilm-based catheter-related blood stream infections (CRBSI), catheter-associated urinary tract infections (CAUTI), and Ventilator Associated Pneumonia (VAP), in our study, were 10.4, 26.6, and 20%. Biofilm formation by the tube method correlated well with the SEM findings. A majority of infections were caused by *Klebsiella pneumoniae* followed by *Staphylococcal* biofilms. A high percentage (85.7%, 95% confidence interval 64.5 to 95.8%) of biofilm producing bacterial isolates, causing infection, were multidrug resistant. Many biofilm producing gram-negative isolates were ES $\beta$ Ls producers, and a majority particularly harbored bla<sub>CTX-M</sub>, among the ES $\beta$ Ls genotypes. **Conclusion:** The incidence of resistant device-related infections, predominantly caused by biofilm producing bacteria, is rising. The tube method is an effective screening method to test biofilm production, where sophisticated microscopy facilities are not available. The varying resistance pattern of organisms isolated in our setup, emphasizes the importance of studying the pattern of infection in every setting and providing antibiotic guidelines in the management of such infections.

**Key words:** Biofilms, Device-related infection, ES $\beta$ L, MRSA, Scanning electron microscopy

## INTRODUCTION

Indwelling Medical Devices (IMDs) are significant in patient care and due to their routine use in hospitals, critical patients easily become vulnerable to microbial colonization. Colonization of IMDs by biofilm producing bacteria is a universal phenomenon<sup>[1]</sup> and should not be ignored as it leads to resistant infections. Costerton *et al.* provided a partial listing of IMDs<sup>[2]</sup> that have been colonized by biofilms and are a source of persistent infection, unless removed from patient. A device-related infection (DRI) is an infection in a patient with a device (intravascular catheter, endotracheal tube or indwelling

urinary catheter) that was in use for at least 48 hours before the onset of infection.<sup>[3]</sup> The extensive use of indwelling devices in hospitalized patient has increased the incidence of DRI, especially blood stream infections, originating from the microbial colonization of the intravascular catheter. Among the DRI, a majority of infections are catheter-related blood stream infections (CRBSI), followed by catheter associated urinary tract infections (CAUTI), and ventilator associated pneumonia (VAP), as seen in developed countries.<sup>[4]</sup> However, we have scant literature available in developing countries such as ours, on the infection rates and their resistance pattern. Empirical use of antimicrobial agents and the superimposed complex nature of bacteria in biofilms colonizing IMDs have resulted in resistant DRIs. Management of such infections is now a huge challenge, as they lead to persistent and resistant infections. Although biofilms on various medical devices have been studied extensively, much of the

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published research has used specialized microscopy such as confocal laser scanning microscopy and scanning electron microscopy (SEM), to visualize the biofilms. However, newer methods like the tube method, tissue culture plate method, and so on, have been recently used to demonstrate the biofilm production ability of microbes and are basic screening techniques, which can be incorporated in the diagnostic clinical laboratory.<sup>[5,6]</sup>

The aim of this study is to determine the overall rates of DRI (device specific rates), bacteriological profile of the biofilms colonizing the devices, and the associated resistance, with special reference to the prevalent genotypes in our hospital.

## MATERIALS AND METHODS

In all, 135 hospitalized (Intensive Care Unit) pediatric patients with IMDs (105 with intravascular catheters, 15 with urinary catheters, and 15 with endotracheal tubes) and suspicion of DRI were studied. Complete blood count, Chest X-rays, and urine culture were done to rule out any infective etiology at the time of admission. The IMDs were immediately removed on the clinical suspicion of DRI and their tip was cut with a sterile blade and sent to the microbiology laboratory in a sterile container. An intravascular catheter tip culture by the roll plate method was done in patients with suspicion of CRBSI.<sup>[7]</sup> The clinical isolates obtained from various specimens (Blood, urine, and bronchoalveolar lavage (BAL)) of the patient with suspected CRBSI, CAUTI, and VAP, respectively, were processed and identified according to the standard protocol.<sup>[8]</sup>

Tips of removed IMDs were also fixed in 1% glutaraldehyde to be used later on for examining biofilms on them.

### Establishment of device-specific infection

The device-specific infection was established according to a surveillance definition by the Centers for Disease Control (CDC) and Prevention for healthcare-associated infections:<sup>[4]</sup>

#### *Catheter-related blood stream infection*

Is defined as, bacteremia in a patient with an intravascular device, developing after 48 hours of admission, with two positive blood cultures obtained by peripheral vein and clinical manifestations of sepsis in the absence of any source of sepsis apart from the device. The semi-quantitative culture from a catheter segment should have  $\geq 15$  bacterial colony forming units (CFU), and the same organism should be obtained by peripheral culture.

#### *Catheter-associated urinary tract infection*

Is found in patients who have had an indwelling urinary catheter for at least 48 hours before the culture is sent and the clean catch catheterized urine culture shows  $>10^5$  bacterial CFU, with no more than two species of bacteria by the semi-quantitative method.

#### *Ventilator associated pneumonia*

Is said to occur when a patient on mechanical ventilation, for at least 48 hours, exhibits increasing Total leucocyte count (TLC), and new shadows (infiltrates) on a chest x-ray, and the BAL culture shows no more than two species of bacteria.

Device Specific Infection rates were calculated as follows

$$= \frac{\text{Number of device-related infections} \times 100}{\text{Number of patients on device}}$$

### Antibacterial susceptibility disk diffusion method

Antibacterial susceptibility testing for various drugs (Himedia) was performed by the Kirby-Bauer disk diffusion method on Muller Hinton Agar as per the *Clinical and Laboratory Standards Institute* (CLSI) guidelines.<sup>[9]</sup>

#### *Extended spectrum beta lactamase detection*

Phenotypic detection: The gram-negative isolates were screened and confirmed (by the double disk synergy and double disk combination methods) for Extended Spectrum Beta Lactamase (ES $\beta$ L) production, as per the CLSI guidelines.<sup>[10]</sup>

#### *bla<sub>CTX-M</sub> detection*

Polymerase chain reaction (PCR) was performed to detect the presence of *bla<sub>CTX-M</sub>*, as done in our earlier study.<sup>[11]</sup> Bacterial plasmid deoxyribonucleic acid (DNA) was extracted by the phenol chloroform method.<sup>[12]</sup> PCR was carried out with a first cycle of denaturation at 94°C for four minutes, and then 35 cycles of annealing at 52°C for two minutes, elongation at 72°C for two minutes, and denaturation at 94°C for 30 seconds. The PCR was completed by a final elongation step at 72°C for 10 minutes. The primers designed were from Operon Biotechnologies Nattermannallee 1, 50829 Cologne Germany, which spanned the universal *bla<sub>CTX-M</sub>* F-ATG TGC AGY ACC AGT AAR GT (CTX-MU1) and R-TGG GTR AAR TAR GTS ACC AGA (CTX-MU-2). The PCR products were analyzed by gel electrophoresis.

#### *Detection of methicillin resistance*

Methicillin resistance in the strains of *Staphylococci* was tested by the ceftioxin disk (30  $\mu$ g) for prediction of mec

A gene-mediated methicillin resistance in the *Staphylococcus* species, as documented in CLSI.<sup>[13]</sup>

## Detection of biofilms

### Tube method

Biofilm production of isolates causing DRI was demonstrated with a slight modification of the method, as described by others.<sup>[5,6]</sup> Briefly 0.5 ml of suspension (0.5 McFarland turbidity standard of saline-washed 24-hour culture isolate) was inoculated into a polystyrene tube containing 4.5 ml of Luria Bertani Broth. The tube was incubated at 37°C for 48 hours without agitation. The culture broth from the tube was then aspirated, washed twice with distilled water, and stained with Crystal violet. Biofilm formation was positive when a blue visibly adherent layer lined the wall and bottom of the tube and was scored as weak (1+), moderate (2+ or 3+) or strong (4+), based on amount of biofilm production. Each isolate was tested at least three times and read independently by two different observers. Biofilm producer *Staphylococcus epidermidis* ATCC 35984 and non-biofilm producer *S. epidermidis* ATCC 12228 were used as the positive and negative controls, respectively.

### Scanning electron microscopy

Device tips fixed in 1% glutaraldehyde were used to examine the biofilm formation by SEM, only if device-related infection was established. The device segments were rinsed in 0.1 M phosphate buffer (twice, three minutes each) and then placed in 1% Zetterquist's osmium for 30 minutes. The samples were subsequently dehydrated in a series of ethanol washes (70% for 10 minutes, 95% for 10 minutes, and 100% for 20 minutes), treated (twice, five minutes each) with hexamethyldisilazane (Polysciences Inc., Warrington, Pa.), and finally air-dried in a desiccator. The specimens were coated with gold-palladium (40% / 60%). After processing, the samples were observed with a scanning electron microscope (Leo 435 VP) in high vacuum mode at 15 kV. The images were processed for display using Photoshop software (Adobe Systems Inc., Mountain View, Calif.).

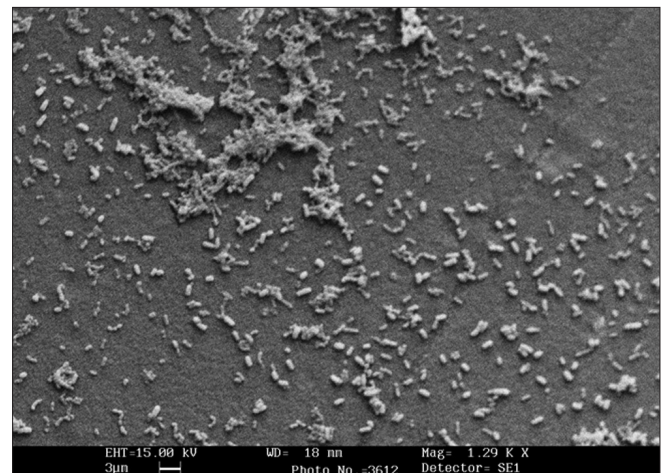
## RESULTS

The rates of biofilm-based CRBSI, CAUTI, and VAP in our study were 10.4, 26.6, and 20%, respectively [Table 1]. The patients who developed DRI also had biofilms on their IMDs, as visualized on scanning electron microscopy, which were seen as bacterial cells embedded in an extracellular matrix [Figure 1]. The bacterial isolates causing DRI showed grade 3 (moderate) or 4 (strong) adherences, confirming their moderate-to-strong biofilm producing ability. All

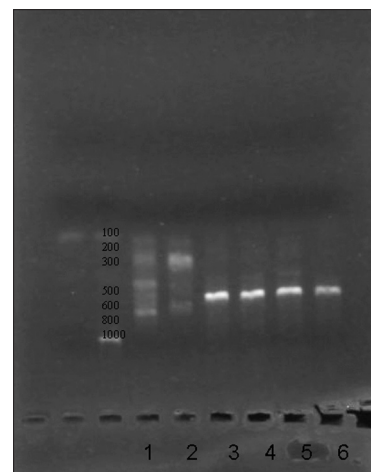
except three isolates (*Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*) causing CRBSI, showed moderate biofilm producing ability by the tube method.

Antimicrobial susceptibility testing was done against 13 drugs for 16 gram-negative bacilli and eight drugs for 5 gram-positive cocci [Table 2]. Eighteen of the twenty-one (85.7%, 95% confidence interval 64.5 to 95.8%) biofilm producing bacterial isolates, causing infection, were multidrug-resistant.

Fifteen out of sixteen (93.8%, 95% confidence interval 69.7 to 99.9%) biofilm producing gram-negative bacilli and three out of five (60%, 95% confidence interval 22.9 to 88.4%) Staphylococcal biofilms, causing DRI, were multidrug-resistant. Thirteen of sixteen (81.3%, 95% confidence interval 56.2 to 94.2%) gram-negative bacterial biofilm isolates were confirmed ES $\beta$ L positive by



**Figure 1:** SEM micrograph of urinary catheter showing mixed bacterial biofilm



**Figure 2:** Lane 1: MM lane Lane 2: negative, Lane 3 – gram negative bacilli (*bla*-<sub>CTX-M</sub> 593 bp)

**Table 1: Infection producing biofilm isolates obtained from patients with IMDs**

Type of infection/organism	Number of patients with device-associated infections	Type of device*		
		IVC	ETT	UC
<i>Monomicrobial</i>				
<i>Staphylococcus aureus</i>	2	2	-	-
<i>Staphylococcus epidermidis</i>	1	1	-	-
<i>Klebsiella pneumoniae</i>	9	6	2	1
<i>Escherichia coli</i>	1	-	-	1
<i>Pseudomonas aeruginosa</i>	1	1	-	-
<i>Acinetobacter baumannii</i>	1	1	-	-
<i>Bimicrobial</i>				
<i>Klebsiella pneumoniae</i> + <i>Staphylococcus epidermidis</i>	2	-	1	1
<i>Pseudomonas aeruginosa</i> + <i>Acinetobacter baumannii</i>	1	-	-	1
Total	18	11	3	4

IVC: Intravascular catheter, ET: Endotracheal tube, UC: Urinary catheter

**Table 2: Antibiotic resistance pattern of biofilm-based device-related infections**

Resistogram pattern*	Isolates	Medical device*
12 drugs G Ak Ci Of Pc Gf Cfs Cfx Nt Cs Cpm Pt	7 K.P	IVC(4), ETT (2), UC (1)
G Ak Ci Of Pc Gf Cfs Cfx Nt Cs Cpm Ca	1 P.A	IVC
11 drugs G Ak Ci Of Pc Gf Cfs Cfx Nt Cs Cpm	1 K.P	IVC
G Ak Ci Of Pc Cfs Cfx Nt Cs Cpm Pt	1 P.A, 1 A.B	UC
G Ak Ci Of Pc Gf Cfx Nt Cs Cpm Pt	1 A.B	IVC
10 drugs G Ak Ci Of Pc Gf Cfx Nt Cs Cpm	1 K.P	UC
G Ak Ci Pc Gf Cfs Cfx Nt Cs Cpm	1 K.P	IVC
G Ci Pc Gf Cfs Cfx Nt Cs Cpm Pt	1 K.P	ETT
9 drugs G Ci Of Pc Gf Cfx Nt Cs Cpm	1 K.P	IVC
G Ci Of Gf Cfs Cfx Cs Cpm Pt	1 E.C	UC
8 drugs G Ak Ci Pc Cfx Nt Cs Cpm	1 K.P	IVC
7 drugs G Ak Gf E C Cs Of	1 S.A	IVC
6 drugs G Ak Gf C Cs Of	1 S.A	IVC
G Ak E C Cs Of	1 S.E	UC
4 drugs G Ci CfxCs	1 P.A	IVC
3 drugs Gf C Of	2 S.E	ETT, UC

\*Gram-negative bacteria were tested for Gentamicin (G, [10] µg), Amikacin (Ak, [30] µg), Ceftriaxone (Ci, [30] µg), Ofloxacin (Of, [5] µg), Piperacillin (Pc, [100] µg), Gatifloxacin (Gf, [5] µg), Cefixime (Cfx, [30] µg), Netilmicin (Nt, [30] µg), Cefoperazone (Cs, [30]µg), Cefepime (Cpm, [5] µg), Cefoperazone sulbactam (Cfs, [75]/[10] µg), Piperacillin tazobactam (Pt, [100]/[10] µg), Imipenem (I, [10]µg). Gram-Positive bacteria were tested for Gentamicin (G, [10] µg), Amikacin (Ak, [30] µg), Gatifloxacin (Gf, [5] µg), Erythromycin (E, [15] µg), Chloramphenicol (C, [30] µg), Vancomycin (Va, [30] µg), Ofloxacin (Of, [5] µg), Cefoperazone (Cs, [30] µg). *Klebsiella pneumoniae* (KP), *Staphylococcus epidermidis* (S.E), *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Acinetobacter baumannii* (AB) <sup>§</sup>Intravascular catheter (IVC), Endotracheal tube (ETT), Urinary catheter (UC)

**Table 3: Phenotypic and genotypic ESBL detection in various gram-negative bacilli**

Organisms	Number of isolates	ESBL positivity	
		Phenotypic methods	Genotypic <i>bla</i> <sub>CTX-M</sub>
<i>Klebsiella pneumoniae</i>	11	9	8 (88.8%)
<i>Escherichia coli</i>	1	1	1 (100.0%)
<i>Pseudomonas aeruginosa</i>	2	1	1 (100%)
<i>Acinetobacter baumannii</i>	2	2	1 (50%)
Total	16	13 (81.25%)	11 (68.7%)

phenotypic methods [Table 3]. eleven of sixteen (68.7%, 95% confidence interval 44.2 to 86.1%) gram-negative bacterial biofilm isolates on genotyping showed presence of the *bla*<sub>CTX-M</sub> gene (593 base pairs) [Figure 2].

## DISCUSSION

The rates of device-specific infection caused by bacterial biofilms found in hospitalized pediatric patients of our institution were almost similar to those found in other studies;<sup>[14-16]</sup> however, the overall device-associated infection rates in any hospital can be determined only if an institutional surveillance study is designed, enrolling all hospitalized patients. DRI infections are known to be responsible for higher morbidity and therapeutic failure independent of the initial severity of illness.<sup>[17]</sup> Moreover, limited data is available on the profile and resistance rates of such infections in our country; therefore, active surveillance of hospital-associated infections from time to time is necessary.

Another noteworthy finding in our study was the biofilm producing ability of bacteria causing DRI. Biofilm formation could be demonstrated as surface adhesion on a bioprosthetic surface by the tube method and could be commonly used in clinical practice to demonstrate biofilms, where sophisticated microscopy techniques are not available. The tube method correlated well with other methods for biofilm demonstration of moderate-to-strong biofilm producing isolates, but the difficulty arose in discriminating between the weak and biofilm-negative isolates.<sup>[5,6]</sup> Detection of biofilms by the SEM and tube method was well correlated in this study, as all isolates were moderate-to-strong biofilm producers.

The most common bacterial biofilms causing DRI were *Klebsiella pneumoniae* (61.1%). *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus* spp. Prominent pathogens most often associated with biofilm-



based infections of IMDs were either normal commensal flora or nosocomial in origin.<sup>[2,18]</sup> The gram-positive *Staphylococcus aureus*, Coagulase Negative *Staphylococci* (CoNS), and the gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* might form biofilms and could cause DRI whenever the human immune response was compromised, as seen in hospitalized patients.<sup>[1]</sup> Nelson *et al.* (2003) also reported that *K. pneumoniae* was often involved in biofilm-related infections.<sup>[19]</sup> Prevalent gram-positive cocci, which often included *Staphylococcus* sp., were also found in our study and their ubiquity as skin flora and their adherence to IMD's surface was also documented by others.<sup>[1,5]</sup> Bimicrobial biofilms were found on urinary catheters and endotracheal tubes only. Biofilms on urinary catheters and endotracheal tubes might initially be composed of a single species, but longer exposures inevitably lead to multispecies biofilms.<sup>[20,21]</sup>

The drug resistance was alarming in the clinical isolates obtained from patients with DRI in our setup. Resistance was found to all the drugs checked, except for vancomycin, among gram-positive and imipenem among gram-negative strains. Gram-negative biofilm producers of 93.8%, causing DRI, were multidrug-resistant, 81.3% biofilm producing gram-negative bacilli were ES $\beta$ L producers, and 68.7% of the isolates particularly showed a presence of *bla*<sub>CTX-M</sub> alleles although other genotypes needed to be evaluated. The prevalence of *bla*<sub>CTX-M</sub> in gram-negative bacilli was high in our hospital strains and due to financial constraints we could check only their presence among the ES $\beta$ L genotypes.<sup>[22]</sup> Of late, few workers also discovered similar findings about biofilm-positive *Klebsiella pneumoniae* strains, which had the ability to produce ES $\beta$ Ls.<sup>[19,23]</sup> *Staphylococci* were important etiological agents of DRI, and a variety of drug-resistant mechanisms were investigated in biofilm isolates.<sup>[24]</sup> In our study Methicillin-resistant *Staphylococci* biofilms causing DRI were also found. A significant correlation was found between the exhibition of drug resistance and the biofilm producing ability, especially of gram-negative bacteria, in our hospital. High resistance and biofilm production among isolates of DRI, in our study, showed that bacteria in biofilms displayed altered character, and high resistance could be attributed to the biofilm producing ability of isolates.

The rates of DRI infection in our study are almost similar to those found in hospitals in developed parts of world, but the astonishing fact is the resistance associated with them is much higher when compared with the others.<sup>[17,25-27]</sup> The countries with preliminary studies carried out in hospitals in the developing parts of the world, where regulations for the implementation of infection control programs and

antibiotic prescribing policies are lacking, have had the same scenario as ours.<sup>[28,29]</sup> The wide difference in resistance rates associated with DRI may be attributed to lack of infection control programs plus the injudicious and inappropriate use of antibiotics in our country. The intricate nature of bacteria is changed due to such widespread misuse of antibiotics in our country, plus, the biofilm producing ability of the bacteria further complicates the resistance problem.

The tube method can be an effective alternative to sophisticated microscopy techniques for screening the biofilm producing ability of bacteria, in resource-constrained countries. The device-related infections and associated antibiotic resistance by biofilm producing isolates is now an emerging problem. The varying resistance pattern of organisms isolated in our setup emphasizes the importance of studying the pattern of infection in every setting and providing antibiotic guidelines in the management of such infections.

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