



## Study of biofilm formation and its correlation with highly drug resistant uropathogens in catheter-associated urinary tract infection

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### Abstract:

**Aims and objectives:** 1. To put culture and sensitivity of urine samples from catheterised patients with symptomatic and asymptomatic bacteriuria. 2. To detect the biofilm formation by tube adherence and tissue culture plate method. 3. To compare the antibiotic sensitivity between biofilm and non-biofilm forming organisms. **Materials and Methods:** The study was conducted in the Department of Microbiology of Yenepoya Medical College and Hospital, Mangalore, India. A total of 100 bacterial isolates obtained from urine samples of catheterised patients were included in the study. The processing of the samples (culture and sensitivity) were done according to standard microbiological techniques. The biofilm formation was done by tube adherence and tissue culture plate method. **Results:** Out of 100 uropathogens isolated, 96(96%) were Gram negative bacilli. Four isolates were Gram positive cocci. Amongst the Gram negative bacilli, *E.coli* were 69%, *Klebsiella pneumoniae* 19%, *Acinetobacter* species 5% and *Pseudomonas aeruginosa* 3%. All gram positive cocci were Enterococci (4%). In the tube adherence method, the number of isolates showing biofilm formation was 51% and non-biofilm producers was 49%. By tissue culture plate method, the number of isolates showing biofilm formation was 63% and non-biofilm producers was 37%. The predominant organism showing biofilm formation were *K.pneumoniae*(89%) followed by *Acinetobacter* spp. (40%). There was significant correlation between biofilm formation and multi-drug resistance. **Conclusion:** Significant correlation between biofilm production and multi-drug resistance was observed in our study. The study will help the clinician to take a call on non-responding uropathogens and decide on better therapeutic options amongst those available in CAUTIs.

**Key words:** CAUTI, tube adherence method, tissue culture plate method, HDRU

### Introduction:

Urinary tract infection is one of the most common nosocomial infection encountered in clinical practice. Catheterization is a potential predisposing factor of CAUTI (catheter-associated urinary tract infection).<sup>1</sup> The free floating micro-organisms attach to the polymeric surfaces resulting in the formation of biofilms. Biofilms are the population of multi-layered microbial cells enclosed in a matrix of polysaccharide materials.<sup>2</sup> Biofilms play a major role to the biofilm forming bacteria such as decreasing the susceptibility to the anti-microbial agents, helps in exchanging of nutrients and metabolites, exchange of genetic materials like plasmid from one bacteria to another. The plasmid exchange enhances the spread of anti-microbial resistance.<sup>3</sup> Hydrophobicity can also affect the ability of bacteria to form biofilms. Bacteria with

increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacteria. The bacteria within the biofilm communicate with each other by production of chemotactic particles. Some factors like surface adhesins and presence of surfactants influence the biofilm formation.<sup>4</sup> High anti-microbial concentrations are required to inactivate organisms within the biofilm, as the antibiotic resistance can increase by 1000 folds.<sup>5</sup> Highly drug-resistant uropathogens are the urinary gram negative rods(GNRs) that are resistant to third generation Cephalosporins, Ciprofloxacin and Gentamicin/Amikacin.<sup>6</sup> The therapeutic options become very limited when we correlate the biofilm production and Highly Drug Resistant Uropathogens(HDRU).

There is a large impact on newer empirical therapy and more potent anti-microbials. It is a matter of great concern to treat the urinary

tract infections caused by uropathogens with emerging resistance to the anti-microbial agents due to biofilm formation.

### Materials and Methods:

The study was conducted in a period of 3 months (March-May 2018) duration in the department of Microbiology, Yenepoya Medical College & Hospital, Mangalore, India.

**Selection of Isolates:** A total of 100 bacterial isolates obtained from all the urine samples were included in the study. Urine samples were collected from all age groups of catheterised patients, symptomatic and asymptomatic bacteriuria. Samples were collected under all aseptic precautions and transported immediately to the laboratory. All isolates were identified by standard microbiological procedures. Polymicrobial samples and other organisms except bacteria were excluded from the study.

**Biofilm detection:** The detection of the biofilms was done by the tube adherence method(TA) and Tissue culture plate method(TCP).

**Tube adherence method:** Described by Christensen et al,<sup>7</sup> this is a qualitative method for biofilm detection. A loopful of test organisms were inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes are incubated at 37°C for 24 hours. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. Only strong and high biofilms are considered as positive where as weak/none was taken as negative.

**Tissue culture plate method:** This quantitative test described by Christensen et al<sup>8</sup> is considered the gold standard

method for biofilm detection.<sup>9</sup> Organisms isolated from fresh agar plates were inoculated in 10 ml of trypticase soy broth with 1% glucose. Broths were incubated at 37°C for 24 hours. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture treated plates were filled with 200 µL of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plates. Negative control wells contained inoculated sterile broth. The plates were incubated at 37°C for 24 hours. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilms formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilms was obtained by using micro ELISA autoreader at wavelength 570 nm.

**Antibiotic susceptibility testing:** All isolates were subjected to antibiotic susceptibility testing using Kirby-Bauer disc diffusion method, done on Mueller-Hinton Agar (MHA) plate as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

American Type Culture Collection (ATCC) strain *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as control strains.<sup>10</sup> The antibiotic sensitivity was compared between biofilm forming and non-biofilm forming organisms. Anti-microbial susceptibility was done using the following antibiotic discs-Amikacin(30µg), Ciprofloxacin(5µg), Ceftazidime(30µg) and Gentamicin(10µg).

## Results:

A total of 100 uropathogens were isolated from the catheterised urine samples. Among the 100 uropathogens, gram negative bacilli showed predominance. Amongst them, *E. coli* (69%) were the most frequently isolated pathogens followed by *Klebsiella pneumoniae* (19%), *Acinetobacter* species (5%), and *Pseudomonas aeruginosa* (3%). Among the gram positive pathogens, only *Enterococcus* spp. (4%) was isolated (Table I).

In this study, we evaluated 100 isolates by two screening methods for their ability to form biofilms, i.e. Tissue culture plate method (TCPM) and Tube adherence method (TAM). In the TCP method, the number of isolates showing biofilm formation was (63%), and non-biofilm producers were (37%). Tube method detected 51% isolates as biofilm producers and 49% as non-biofilm producers. From both the methods, 42% of isolates were showing biofilm formation (Table II).

The majority of the organisms associated with biofilm production were *K. pneumoniae* (89.4%) followed by *E.coli* (78.2%), *Acinetobacter* species(40%), *Pseudomonas aeruginosa*(33.3%) and *Enterococcus* species(25%) (Table III).

We also studied the antibiotic susceptibility pattern of all uropathogens and correlated that with biofilm production. Among the commonly used anti-microbials, Amikacin and Ciprofloxacin were the most sensitive to *E. coli* and *Klebsiella pneumoniae*. Sensitivity to other commonly used anti-microbials including Gentamicin and Ceftazidime were found to be lower than Amikacin and Ciprofloxacin.

Ceftazidime showed 91.6% resistance to *Escherichia coli* and 76.4% resistance to *Klebsiella pneumoniae*. Gentamicin showed 37.1% resistance to *E.coli* and 65.6% resistance to *Klebsiella pneumoniae* (Table IV).

The present study also showed significant correlation between biofilm production and multi-drug resistance, where 64% of strains producing biofilm were multi-drug resistant uropathogens. In this study, 48 (64%) isolates were HDRU while 27(36%) were non-HDRU. Similar to biofilm production, maximum HDRU were *Klebsiella pneumoniae* (70.5%), followed by *E. coli* (65.7%) . Out of 75 biofilm producing isolates 48(64%) were Highly Drug Resistance Uropathogens. (Table V)

**Table I:** Number and percentage of organisms isolated from urine samples

Bacteria isolated (n= 100)	Number (%)
<i>E. coli</i>	69(69%)
<i>Klebsiella pneumoniae</i>	19(19%)
<i>Acinetobacter</i> species	5(5%)
<i>Enterococcus</i> species	4(4%)
<i>Pseudomonas aeruginosa</i>	3(3%)

**Table II:** Distribution of biofilm detection by TAM and TCP method

Method	Number of biofilm producing organisms
Tube adherence method	51
Tissue culture plate method(TCPM)	63
Both methods	42

**Table III:** Distribution of biofilm forming uropathogens

Uropathogens	Biofilm producing	Non-biofilm producing
<i>E. coli</i> (69)	54(78.2%)	15(21.7%)
<i>Klebsiella pneumoniae</i> (19)	17(89.4%)	2(10.5%)
<i>Acinetobacter</i> species (5)	2(40%)	3(60%)
<i>Enterococcus</i> species (4)	1(25%)	3(75%)
<i>Pseudomonas aeruginosa</i> (3)	1(33.3%)	2(66.6%)
Total(n= 100)	75(75%)	25(25%)

**Table IV:** Antimicrobial resistance pattern of biofilm producing organisms

Organism	Ceftazidime (30µg)	Gentamicin (10µg)	Ciprofloxacin (5µg)	Amikacin (30µg)
<i>E.coli</i> (54)	49(91.6%)	20(37.1%)	19(35.1%)	1(1.85%)
<i>Klebsiella pneumoniae</i> ( 17)	13(76.4%)	11(65.6%)	10(59.7%)	6(35.2%)
<i>Acinetobacter species</i> (2)	1(50%)	2(100%)	-	1(50%)
<i>Enterococcus species</i> (1)	-	1(100%)	1(100%)	-
<i>Pseudomonas aeruginosa</i> (1)	1(100%)	1(100%)	-	-

**Table V:** Distribution of HDRU and Non-HDRU among biofilm producing isolates

Biofilm producing uropathogen	High drug resistant uropathogens (HDRU)	Non High drug resistant uropathogens (non HDRU)
<i>E.coli</i> (54)	35(65.7%)	19(35.2%)
<i>Klebsiella pneumoniae</i> ( 17)	12 (70.5%)	05 (29.4%)
<i>Acinetobacter species</i> (2)	01 (50%)	01 (50%)
<i>Enterococcus species</i> (1)	0	01 (100%)
<i>Pseudomonas aeruginosa</i> (1)	0	01 (100%)
<b>Total (75)</b>	48 (64%)	27 (36%)

CAUTI is the most common nosocomial infection in hospitals and nursing homes, comprising > 40% of all institutionally acquired infections.<sup>11</sup> A foreign body, such as an indwelling urethral catheter, connecting a normally sterile, hydrated body site to the outside world will inevitably become colonized with microorganisms.<sup>12</sup> The present study showed that out of 100 isolates found in urine samples, *E.coli* was the most frequently isolated pathogen 69%, followed by *Klebsiella* spp. 19%, *Acinetobacter* spp. 5%, *Enterococci* 4% and *Pseudomonas aeruginosa* 3% which was similar to the findings of a study<sup>13</sup> done by Das SC et al in Dinajpur Medical College, where *E.coli* was the most frequent isolated pathogen (66.66%), followed by *Klebsiella* spp. 14.44%, *Pseudomonas aeruginosa* 7.77%,

*Acinetobacter* spp. 4.44%, *Proteus* 3.33% and *Enterococci* 3.33%.

*E. coli* is the most common organism reported from CAUTI cases; the source of these strains of *E. coli* is frequently the patient's own intestinal flora<sup>14</sup>. Virulence factors of uropathogenic *E. coli* (UPEC) include the ability to adhere to uroepithelial cells and certain specific serotypes O and K antigens that are resistant to phagocytosis and bactericidal action of normal serum.<sup>15</sup>

From our study, the TCP is a quantitative and reliable method to detect biofilm forming microorganisms. When compared to Tube adherence method, TCP can be recommended as a general screening method for detection of biofilm producing bacteria in laboratories as TCP method detected 63 isolates and Tube adherence method detected 51 isolates.

Similar results were seen in the study done by Hassan A et al<sup>16</sup> in Army Medical College, Rawalpindi, Pakistan in which the TCP method was considered to be superior to TAM. From the total of 110 clinical isolates, TCP method detected 22.7% as high, 41% moderate and 36.3% as weak or non-biofilm producers.

In the current study, amongst the gram negative bacteria, the biofilm producing isolates showed high resistance to Ceftazidime and Gentamicin. Amikacin and Ciprofloxacin were the most effective drugs.

Ceftazidime showed 91.6% resistance to *Escherichia coli* and (76.4%) resistance to *Klebsiella pneumoniae*. Gentamicin showed 37.1% resistance to *E.coli* and (65.6%) resistance to *Klebsiella pneumoniae*. Anti-microbial resistance is a leading concern and efforts should be made to ensure an appropriate therapy for symptomatic UTI.<sup>17</sup>

In our study, antibiotic resistance was significantly higher in biofilm producing organisms, re-emphasising the role of biofilm production in spreading high drug resistance uropathogens (HDRU). Out of 75 biofilm producing isolates, 48(64%) were Highly Drug Resistance Uropathogens. The present study showed maximum HDRU were *Klebsiella pneumoniae* (70.5%) followed by *E. coli* (65.7%).

Similar study was done by Deotale VS et al<sup>18</sup> in Mahatma Gandhi Institute of Medical Sciences. Out of total 37 uropathogens isolated from catheterized urine samples, 30 (81.1%) were positive in vitro for biofilm production and 22 (59.5%) isolates were HDRU.

### Conclusions:

In conclusion, *E.coli* was the most frequent isolate (69%) in urine of catheterized patients. The antibiotic susceptibility pattern in the present study showed Ceftazidime and Gentamicin were

the least active drug and the uropathogens showed the highest sensitivity to Ciprofloxacin and Amikacin. Significant correlation between biofilm production and multi-drug resistance was observed in our study. So, the study will help the clinicians to take a call on non-responding uropathogens and decide on better therapeutic options amongst those available to prevent nosocomial infections associated with indwelling catheters.

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