

## Bacteriology and Antibiotic Sensitivity Patterns of Urine and Biofilm in Patients with Indwelling Urinary Catheter in a Tertiary Hospital in Bangladesh

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### Abstract

Catheter-Associated Urinary Tract Infection (CAUTI) is a common health care associated infection worldwide and is result of wide spread use of urinary catheter and inappropriate antibiotics use. Cause of CAUTI is formation of pathogenic biofilm in the inner surface of indwelling urinary catheters and its early detection prevents various hazards as well as economic impact.

This observational prospective study was done to see relationship between the pattern of microorganism in urine and biofilm and their antibiotic sensitivity patterns in 100 patients in Comilla medical college hospital, Comilla, Bangladesh. Selected patients had undergone catheterization for urinary retention or incontinence. The urine collected by suprapubic puncture and biofilm from indwelling catheter for culture and sensitivity.

90% of urine samples and 100% biofilm showed growth of uropathogens. *E.coli* was the most frequently isolated pathogen (60%), followed by *Klebsiella* spp (14%). Multibacterial isolates was found from biofilm in 15 samples with long term catheterization. Biofilm strains displayed relatively high resistance against tested antibiotics. Highest sensitivity pattern found for *E.coli* in urine and biofilm for imipenem (95% vs. 92%), lowest for ciprofloxacin (20% vs. 16%). Catheter biofilm resistant to all tested drugs were found for *E.coli* in 6.95% and *klebsiella* in 5.55%. Urine samples resistant to all tested antibiotics only in *E.coli* 3.33%.

*E.coli* was the most frequent isolate which showed the higher sensitivity to carbapenems, and lowest to the quinolones. Correlation was observed between biofilm production and multidrug resistance. A large-scale prospective studies suggested to make a guideline to manage UTI specially CAUTI.

**Keywords:** *E.coli*; Catheter-associated urinary tract infection; Patients

### Introduction

Indwelling urinary catheters are standard medical devices utilized to relieve urinary retention and urinary incontinence. Due to its frequent and unnecessary use many patients are placed at risk of its complications like nosocomial Urinary Tract Infections (UTIs) also known as Catheter-Associated UTI (CAUTI) which has got considerable economic impact [1,2]. The underlying cause of CAUTI is formation of a pathogenic biofilm on the surface of the indwelling urinary catheter. The biofilm mode of growth is a basic survival strategy deployed by bacteria in a wide range of environmental, industrial and clinical aquatic settings [3]. The cells in these biofilm communities are protected from environmental stresses. Microorganisms those are apparently fully sensitive to antibiotics and may become fully resistant in the biofilm mode. The biofilm bacteria thrive in their matrix gel and the gentle flow of warm nutritious urine. Biofilms containing  $5 \times 10^9$  viable cells per centimeter can be found on long-term indwelling catheters removed from patients [4]. A variety of bacterial species colonize catheters, and many of these biofilms can induce serious complications [5-7]. Microscopic observations show that catheter biofilm-associated bacteria form polymicrobial

microcolonies that are embedded within an amorphous, protective extracellular matrix [8-10]. Isolated cases of single-species biofilms were observed, but most biofilms contained mixed bacterial communities. The risk of urinary tract infection is related to the length of time the catheter is in place. Long-term catheters colonized by extensive biofilms, which can have profound effects on the health of the patient.

Urinary tract infections in catheterized patients can occur by colonization of organism in periurethral skin can migrate into the bladder through the mucoid film that forms between the epithelial surface of the urethra and the catheter. In addition, contamination of the urine in the drainage bag can allow organisms to access the bladder through the drainage tube and the catheter lumen [11,12]. A clear understanding of the distinction between urinary tract infection (UTI) and asymptomatic bacteriuria is essential for prevention of CAUTI. However, more than 90% of cases of nosocomial catheter-associated bacteriuria are asymptomatic, or sub clinical infections [13]. Nosocomial UTI associated with catheterization occur in more than 1 million U.S. patients each year [14,15]. Although catheter-associated urinary tract infections (CAUTI) may not result in excess mortality but they significantly increases burden in the health care system by increasing both morbidity and treatment costs [16,17].

## Materials and Methods

This was an observational prospective study done in the department of medicine and urology, Comilla Medical College Hospital, Comilla, Bangladesh during the period of 1<sup>st</sup> July, 2012 to 31<sup>st</sup> December, 2012. The patient's undergone indwelling catheterization for urinary retention or incontinence developed biofilm in inner surface of catheter lumen seen on naked eye was included in this study for culture of urine and biofilm. Those patients are on antibiotic advised to stop drug for 48 hours and then included in this study. Patient's clinical presentation, co morbid illness, duration of catheterization was noted in data sheet. Urine samples were collected in sterile container through suprapubic puncture. A cross section of indwelling catheter containing biofilm seen on naked eye was also collected. A modified semi-quantitative technique using a standard calibrated bacteriological loop of urine was performed to transfer the 0.001 ml of sample on Blood agar and MacConkey agar media. After allowing the urine to be absorbed into the agar, the plates were then inverted and incubated aerobically at 37°C for 24 hours. The colony count was done using semi quantitative method. Number of colonies obtained is multiplied by 1000 to obtain the colony forming units (cfu)/ml [18]. Identification of any bacteria from suprapubic puncture and 105 cfu/ml on more bacteria in biofilm considered as significant. The characteristic bacteria on the culture media were aseptically isolated. Antimicrobial sensitivity tests were carried out by disc diffusion technique using Muller Hinton Agar. The Antibiotics used for susceptibility testing were done with amoxyclav, cefotaxime, ceftriaxone, nitrofurantoin, ceftazidime, cefuroxime, ciprofloxacin, cotrimoxazole, amikacin, imipenem nalidixic acid.

## Observation and Results

Table 1 Shows the mean age of the patients were  $56.20 \pm 20.67$  years. Out of total 100 patients 39% were female and 61% were male. Total 92% patients were asymptomatic, 06% had lower abdominal pain, 03% had fever with chills and rigor, and 02% had loin pain (Table 2).

Variables	Categories	Frequency	Percentage
Age (M ± SD) in years	56.20 ± 20.67 years		
Sex	Male	61	61
	Female	39	39
Symptoms	Lower abdominal pain	06	06
	Loin pain	02	02
	Fever with Chills and rigor	03	03
	Asymptomatic	92	92

Table 1: Patients characteristics (n=100)

Figure 1 shows out of 100 of urine sample, no growth was found in 10 samples and *E.coli* was found in 60% samples followed by *Klebsiella* spp 14%, *Pseudomonas aeruginosa* 7%, *Acinetobacter* spp 4%, *Enterococci* 3%, *Proteus* 3%.

Duration	Number of patients (n=100)	Percentage (%)
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Short term catheter (1-7 days)	15	15
Medium term catheter (7-28 days)	71	71
Long term catheter (over 28 days)	14	14

Table 2: Duration since catheterization

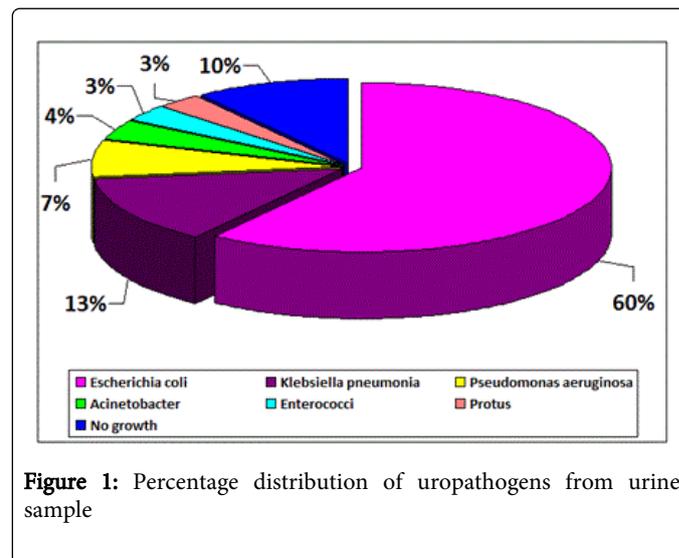


Figure 1: Percentage distribution of uropathogens from urine sample

Figure 2 shows distribution of organism isolates from catheter biofilm. There was profuse growth of microorganism and some samples showed growth of multiple organism. Total 115 microorganisms were isolated from 100 biofilms, among them, *E.coli* was found to be the most frequently isolated pathogen (72 samples) followed by *Klebsiella* spp (18 samples).

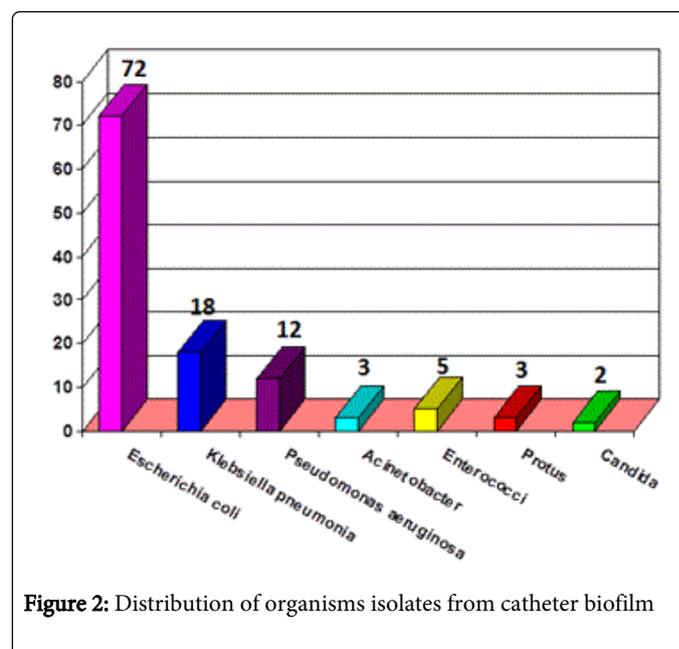


Figure 2: Distribution of organisms isolates from catheter biofilm

Antibiotic sensitivity pattern of organisms isolated in urine showed in Table 3, Sensitivity pattern of *E.coli* against ciprofloxacin, cotrimoxazole, ceftriaxone, cefuroxime, amikacin, nitrofurantoin, amoxycylav, amikacin and imipenem was 20%, 35%, 32%, 45%, 45%, 85%, 25%, 82% and 95% respectively.

Organism Isolated	Sensitive									Resistant to all
	Ciprofloxacin	Cotrimoxazole	Ceftriaxone	Cefuroxime	Amikacin	Nitrofurantoin	Imipenem	Amoxycylav	Ceftazidim	
Escherichia coli	12 20%	21 35%	19 31.66%	27 45%	49 81.66%	51 85%	57 95%	25 41.66%	27 45%	2 3.33%
Klebsiella pneumoniae	1 7.69%	2 15.38%	4 30.76%	3 23.07%	8 61.53%	9 69.23%	11 84.61%	2 15.38%	2 15.38%	0
Pseudomonas aeruginosa	2 28.57%	2 28.57%	1 14.28%	2 28.57%	4 57.14%	5 71.42%	6 85.71%	1 14.28%	1 14.28%	0
Acinetobacter	0	1 25%	1 25%	2 50%	2 50%	2 50%	4 100%	1 25%	1 25%	0
Enterococci	1 33.33%	1 33.33%	0	0	3 100%	2 66.67%	3 100%	0	1 33.33%	0
Proteus	0	1 33.33%	1 33.33%	0	2 66.67%	1 33.33%	3 100%	1 33.33%	0	0

**Table 3:** Antibiotic sensitivity pattern of organisms isolated in urine

The Table 4 shows antibiotic sensitivity pattern of organisms isolated in biofilm. Sensitivity pattern of *E.coli* against ciprofloxacin, cotrimoxazole, ceftriaxone, cefuroxime, ceftazidime, nitrofurantoin, amoxycylav, amikacin and imipenem was 16%, 22%, 31%, 36%, 40%, 70%, 26%, 74% and 92% respectively. The table also shows resistant to all tested drugs were found in case of *E.coli* in 5 samples (6.95%) and *klebsiella* in 1 sample (5.55%)

Organism Isolated	Sensitive										Resistant to all
	Ciprofloxacin	Cotrimoxazole	Ceftriaxone	Cefuroxime	Amikacin	Nitrofurantoin	Imipenem	Amoxycylav	Ceftazidim	Nalidixic acid	
Escherichia coli	11 15.27%	16 22.22%	22 30.55%	26 36.11%	53 73.61%	51 70.83%	66 91.66%	19 26.38%	29 40.27%	5 6.95%	5 6.95%
Klebsiella pneumoniae	2 11.11%	2 11.11%	6 33.33%	7 38.88%	12 66.66%	13 72.22%	16 88.88%	6 33.33%	10 55.55%	1 5.55%	1 5.55%
Pseudomonas aeruginosa	3 25%	1 8.33%	4 33.33%	4 33.33%	8 66.67%	9 75%	11 91.66%	3 25%	6 50%	1 8.33%	0
Acinetobacter	0	1 33.33%	1 33.33%	0	2 66.67%	1 33.33%	3 100%	0	1 33.33%	0	0
Enterococci	0	2 40%	2 40%	1 20%	3 60%	4 80%	5 100%	1 20%	2 40%	1 20%	0
Proteus	1 33.33%	0	1 33.33%	0	2 66.67%	2 66.66%	3 100%	1 33.33%	1 33.33%	0	0

**Table 4:** Antibiotic sensitivity pattern of organisms isolated in biofilm

Multiple drug resistant patterns of biofilm organisms shown in Table 5. There was a significant correlation between biofilm production and resistance to multiple antibiotics such as amoxycylav, ciprofloxacin, nalidixic acid, cotrimoxazole and ceftriaxone .Out of the

115 strains isolated, 79 (68.69%) strains were multidrug resistance phenotype.

Multiple drug combination	Number of isolates showing resistance	Percentage
Cft,Co,AC,Na,Cip	79	68.69%

**Table 5:** Multiple drug resistant pattern of biofilm organisms. Cft=Ceftriaxone, Co=Cotrimoxazole, Cip=Ciprofloxacin, AC=Amoxycylav, Na=Nalidixic acid

## Discussion

In this study, 91.11% urine culture positive patients were asymptomatic. Similarly Tambyah et al. found more than 90% CAUTI were asymptomatic [19]. CAUTI is the most common nosocomial infection in hospitals comprising >40% of all institutionally acquired infections [20]. The relevance of biofilm to CAUTI is that a foreign body, such as an indwelling urethral catheter, connecting a normally sterile, hydrated body site to the outside world which inevitably become colonized with microorganisms. This study showed 90% urine samples and 100% biofilm showed growth of uropathogens. *E.coli* was the most frequently isolated pathogen in both urine and biofilm (60% vs. 72%), followed by *Klebsiella spp* (13% vs 18%). Study by Hassin et al showing *E.coli* as the predominant organism (74%) followed by *Klebsiella spp* 17.7% [21]. Ronald et al. showed that *E.coli* remains the predominant uropathogen (80%) followed by *S. saprophyticus* (10-15%), *Klebsiella*, *Enterobacter* [22]. Different study shows *E.coli* is responsible for more than 80% of symptomatic UTI and asymptomatic bacteriuria by expression of its variety of virulence factors and toxins [23,24].

Sensitivity pattern of *E.coli* in urine sample against ciprofloxacin, cotrimoxazole, ceftriaxone, cefuroxime, ceftazidime, nitrofurantoin, amoxycylav, amikacin and imipenem was 20%, 35%, 32%, 45%, 45%, 85%, 25%, 82% and 95% respectively. Sensitivity pattern of *E.coli* against ciprofloxacin, cotrimoxazole, ceftriaxone, cefuroxime, ceftazidime, nitrofurantoin, amoxycylav, amikacin and imipenem was 16%, 22%, 31%, 36%, 40%, 70%, 26%, 74% and 92% respectively.

Study of biofilm shows there 15 samples yielding multibacterial isolates in biofilms and these patients were on catheter for a long time. Biofilms may be composed of a single species or multiple species of uropathogens, depending on the type of device and duration of its use. Biofilms may initially be composed of single species of uropathogens, but longer exposures inevitably lead to multispecies biofilms [25]. In our study 72% biofilm isolates of *E.coli* which was similar (67.5%) to other study [26].

Antibiotic sensitivity patterns of biofilm and urine shows biofilm strains displayed relatively high resistance against tested antibiotics. Highest sensitivity pattern was found for *E.coli* in urine and biofilm for imipenem (95% vs. 92%), lowest for ciprofloxacin (20% vs. 16%) Study shows bacterial biofilms are often associated with long term persistence of organisms in various environments and they display dramatically loss its sensitivity increased to antibiotics [27]. The present study also showed significant correlation between biofilm production and multidrug resistance, where 68.69% of strains producing biofilm were multidrug resistant phenotypes. In catheter biofilm resistant to all drugs were found in case of *E.coli* in 5 samples (6.95%) and in case of *klebsiella* in 1 sample (5.55%). Urine samples

resistant against all tested antibiotics were only in case of *E.coli* in 3.33% cases.

Therapy against UTI should be guided by antimicrobial susceptibilities as urinary isolates are developing resistance to commonly used antibiotics. Increasing antimicrobial resistance of uropathogens has led to reconsideration of traditional treatment of recommendations in many areas. Microbial biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy. This also helps in the spread of antibiotic resistance in nosocomial pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance. Antibiotic therapy against device associated biofilm organisms often fails without the removal of the infected implant although they are not resistant to antibiotics per se [28]. An elevated expression of the efflux pump and physiological heterogeneity play important role for the development of antibiotic resistance in biofilm bacteria by affecting the rate of growth, metabolism, interbacterial quorum signals, the accumulation of toxic products and change in the local micro environment. To lower our economic burden of the hospital admitted patient with CAUTI we have to broaden our knowledge regarding safe use of indwelling urinary catheter.

## Conclusion

*E.coli* was the most frequent isolate which showed the higher sensitivity to carbapenems, and lower to the quinolones. Correlation was observed between biofilm production and multidrug resistance. To establish a standard guideline for the indwelling urinary catheter management in our tertiary hospital it requires a large-scale prospective study.

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