Microorganism and Antibiotic Sensitivity Pattern of Urine and Biofilm in Patient with Indwelling Urinary Catheter Developing Biofilm

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Catheter-associated urinary tract infection (CAUTI) is a common problem with considerable economic impact. The underlying cause of CAUTI is formation of a pathogenic biofilm on the surface of the indwelling urinary catheter. Understanding the pathogenesis of CAUTI is essential for designing and evaluating preventive strategies. This cross sectional study included 100 urine and biofilm samples from catheterized patients who have developed biofilm, seen on naked eye with or without symptoms of UTI admitted in medicine and urology department of Comilla medical college & hospital over a period of six months from 1st july 2012- 31st december, 2012. Urine was collected by suprapubic puncture with all aseptic precaution. The mean age of the participants was 56.20 ±20.67 years. Of them 61% were male and 31% were female.This study Showed that,out of these 100 samples, *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%. In catheter biofilm, there was profuse growth of multiple organisms. Out of 115 strains of catheter biofilm, *E.coli* was the most frequent isolated pathogen (62.60%).At the same time; we investigated antibiotic resistance patterns of biofilm and urine of a catheterized patient. The investigated biofilm strains displayed relatively high resistance against tested antibiotics. E. coli which is the most frequent pathogen in biofilm and urine was resistant to ciprofloxacin (84.73% vs. 80%), cotrimoxazole (77.78% vs. 65%), ceftriaxone (69.45% vs. 68.34%), cefuroxime (63.89% vs. 55%), ceftazidime (59.73 % vs. 55%), nitrofurantoin (29.17% vs. 15%), amoxyclav (73.62% vs. 58.34%), amikacin (26.39% vs. 18.34%), imipenem (8.34% vs. 5%).Here, 68.69% pathogen of catheter biofilm was multidrug resistant.Whereas, 6.95% E.coli and 5.55% of klebsiella of biofilm were found to be resistant to all tested drugs.So, from this study it has been showed that ciprofloxacin was the least active drug and the uropathogens showed the highest sensitivity to carbapenems, nitrofurantoin and aminoglycosides in urine samples. There was correlation between biofilm production and multidrug resistance was observed in our study. So, it is necessary to establish standard guidelines on the care of indwelling urinary catheter in admitted patients with a view to preventing nosocomial infections associated with this device in patients.

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Introduction

Indwelling urinary catheters are standard medical devices utilized in both hospital and nursing home settings to relieve urinary retention and urinary incontinence. The most common urinary catheter in use is the Foley's indwelling urethral catheter, a closed sterile system that is comprised of a tube inserted through the urethra and held in place by an inflatable balloon to allow urinary drainage of the bladder. Although these devices were originally designed for short-term use in patients, indwelling catheter use is now commonplace in the long-term setting. Due to the frequent and sometimes unnecessary use of indwelling catheters during hospitalization (21 to 50% of patients) many patients are placed at risk for complications associated with the use of these devices.[1] The most notable complication associated with indwelling urinary catheters is the development of nosocomial urinary tract infections (UTIs), known as catheter-associated UTIs (CAUTIs).[2] Catheter-associated urinary tract infection (CAUTI) is a common problem with 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 is a basic survival strategy deployed by bacteria in a wide range of environmental, industrial and clinical aquatic settings.[3] These cells have an array of adhesins in their cell walls that allow them to colonize many types of substrate, and, on contact with a surface, the cells secrete exopolysaccharides that secure their attachment. The cells in these biofilm communities are protected from environmental stresses, and this protection has particular advantages for the bacteria in biofilms that develop *in vivo.* Microorganisms that are apparently fully sensitive to antibiotics and antiseptics in conventional laboratory testing methods become fully resistant in the biofilm mode *in vivo*.The biofilm bacteria thrive in their matrix gel and the gentle flow of warm nutritious urine. Enormous populations develop, and become visible to the naked eye as thick coatings. Biofilms containing 5×10^9 viable cells per centimeter can be found on long-term indwelling catheters removed from patients.[4] The biofilm populations, therefore, often outnumber those in the urine.A variety of bacterial species colonize catheters, and many of these biofilms can induce serious complications.[5,6,7.] Microscopic observations show that catheter biofilm-associated bacteria form polymicrobial microcolonies that are embedded within an amorphous, protective extracellular matrix.[8,9,10] The most common species present in the mixed-population biofilms were *E. faecalis*, *P. aeruginosa*, *E. coli*, and *P. mirabilis*. In patients who develop bacteriuria during short-term catheterization, bacterial colonization of the catheter does occur.[11] The risk of urinary tract infection is related to the length of time the catheter is in place. Long-term catheters become colonized by extensive biofilms.UTI in catheterized patients can occur in several ways. Organisms that colonize the 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.[12,13] The presence of bacteria in the bladder, a normally sterile site, triggers an inflammatory response[14]. However, more than 90% of cases of nosocomial catheter-associated bacteriuria are asymptomatic, or subclinical infections.[15] Nosocomial UTI associated with catheterization occur in more than 1 million U.S. patients each year.[16,17] Although catheter-associated urinary tract infections (CAUTI) may not result in excess mortality,[18] they significantly burden the health care system by increasing both morbidity and treatment costs.[14]

Methods

This was an observational cross sectional study done for six months period among the patients who were being catheterized with indwelling urinary catheter admitted into the medicine and urology ward of Comilla medical college hospital and those who developed biofilm within catheter tube seen on naked eye were randomly assigned for culture of urine and biofilm of catheter irrespective of duration and antibiotic treatment. The study physician saw the patient and examined thoroughly. After fulfilling the inclusion and exclusion criteria, patient was enrolled with unique ID Subjects and briefed about the objectives; risk, benefits and freedom for participating in the study. Informed consent was taken accordingly. Urine samples were collected with all aseptic precaution in sterile container through suprapubic puncture from urinary bladder to avoid contamination and at the same time, cross section of indwelling catheter containing biofilm seen on naked eye was also collected and immediately sent to Microbiology laboratory of comilla medical college and hospital for culture and sensitivity test which was done by disc diffusion method, modified kirb-bauer technique. The antibiotics to which sensitivity tested were Amoxiclav, Cefotaxime, Ceftriaxone, Nitrofurantoin, Ceftazidime, Cefuroxime, Ciprofloxacin, Cotrimoxazole, Amikacin, Imipenem.Concommitantly,10 ml of urine in a sterile glass test tube was collected and sent for routine microscopic examination.

Results

A total of 100 catheterized patients who got admitted in medicine and urology ward of Comilla medical college and hospital were recruited. Mean age of the participants was 56.2 years. Of them 39% were female and 61% were male.82 patients out of 90 urine culture positive patients were asymptomatic which is 91.11% and 06 participants presented with lower abdominal pain, 05 with fever , 02 with chills and rigor, 02 with loin pain.

Table I: Patients characteristics (n=100)

Variables	Categories	Frequency	Percentage
Age	20-30 years	16	16
	31-40 years	08	08
	41-50 years	12	12
	51-60 years	12	12
	61-70 years	28	28
	71-80 years	16	16
	81-90 years	04	04
	91-100 years	04	04
Sex	Male	61	61
	Female	39	39
Symtomps	Lower abdominal pain	06	06
	Loin pain	02	02
	Fever	05	05
	Chills and rigor	02	02
	Asymptomatic	92	92

Table II: Duration since catheterization

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

Table III: Percentage distribution of uropathogens from urine sample

Bacterial organisms	Frequency of isolation
Escherichia coli	60
Klebsiella Pneumonia	13
Pseudomonas Aeruginosa	07
Acinetobacter	04
Enterococci	03
Proteus	03
No growth	10
Total	100

Out of 100 strains of urine samle, no growth was found in 10 samples.Out of rest 90 samples, *E.coli* was found to be the most frequent pathogen which is 66.66% followed by *Klebsiella spp* 14.44%, *Pseudomonas aeruginosa* 7.77%, *Actinobacter spp* 4.44%, Enterococci 3.33%, *Proteus* 3.33%

Table IV: Percentage distribution of organisms from catheter biofilm

Bacterial organisms	Frequency of isolation
Escherichia coli	72
Klebsiella pneumoniae	18
Pseudomonas aeruginosa	12
Enterococci	05
Acinetobacter	03
Proteus	03
Candida	02
Total	115

In catheter biofilm, there were profuse growth of multiple organism.There were total 115 strains of organism. Out of 115 strains, *E.coli* was found to be the most frequent pathogen which was 62.60% followed by *Klebsiella spp* 15.65%, *pseudomonas aeruginos*a 10.43%, *Enterococci* 4.34%, *Actinobacter spp* 2.60% , *Proteus* 2.60%, *candida* 1.73%.

Table V: Antibiotic sensitivity pattern of organisms isolated in urine

Organism Isolated	Sensitive									Resistant to all
	Ciprofl-oxacin	Cotrim-oxazole	Ceftria-xone	Cefur-oxime	Amik-acin	Nitrofu-rantoin	Imipenam	Amoxyclav	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 VI: Antibiotic sensitivity pattern of organisms isolated in biofilm

Organism Isolated	Sensitive									Resistant to all
	Ciprofloxacin	Cotrim-oxazole	Ceftria-xone	Cefuroxime	Amikacin	Nitrofurantoin	Imipenam	Amoxyclav	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 VII: Multi-drug resistant organisms within Biofilm

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

Cft=Ceftriaxone, Co =Cotrimoxazole, Cip=Ciprofloxacin, AC=Amoxyclav, Na=Nalidixic acid

Out of the 115 strains isolated in Biofilm,79 (68.69%) strains were resistant to multiple antibiotics such as Ceftriaxone, Cotrimoxazole, Ciprofloxacin, Amoxyclav, Nalidixic acid .

Discussion

CAUTI is the most common nosocomial infection in hospitals and nursing homes, comprising > 40% of all institutionally acquired infections.[19] 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.[20] The present study Showed out of these 100 strains found in urine samples, *E.coli* was the most frequently 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% which was similar to the Findings of a study[21] showing *E.coli* (74%) as the predominant organism followed by *Klebsiella spp* 17.7% & *Pseudomonas spp* 2.5%. Another study found that *E. coli* remains the predominant uropathogen (80%) in community acquired infections followed by *S. saprophyticus* (10-15%), *Klebsiella, Enterobacter, Proteus spp*.[22] *Escherichia coli* is responsible for more than 80% of all UTIs and causes both symptomatic UTI and asymptomatic bacteriuria (ABU).[23,24] Biofilms may be composed of a single species or multiple species, depending on the device and its duration of use in the patient. Urinary catheter biofilms may initially be composed of single species, but longer exposures inevitably lead to multispecies biofilms.[25] In our study, there were also 15 specimens yielding more than one type of Bacteria in culture of biofilms which were mostly from long term catheterized samples .*E. coli* was found in 72 (62.60%) isolates of biofilm whereas one study have reported a similar finding in biofilm culture where *E. coli* was 67.5%.[26] We investigated antibiotic resistance patterns of biofilm and urine of a catheterized patient of different duration against drugs currently used in treatment of UTI. The investigated biofilm strains displayed relatively high resistance against tested antibiotics. Resistance pattern of mostly found organism E.coli in biofilm and urine were successively for Ciprofloxacin (84.73% vs. 80%), Cotrimoxazole (77.78% vs. 65%), Ceftriaxone (69.45% vs. 68.34%), Cefuroxime (63.89% vs. 55%), Ceftazidime (59.73 % vs. 55%), Nitrofurantoin (29.17% vs. 15%), Amoxyclav (73.62% vs. 58.34%), Amikacin (26.39% vs. 18.34%), Imipenam (8.34% vs. 5%). Bacterial biofilms are often associated with long term persistence of organisms in various environments and they display dramatically increased resistance to antibiotics.[26] 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%).In this study, 82 out of 90 (91.11%) urine cultures positive patients were asymptomatic and this is similar to one study where more then 90% CAUTI were asymptomatic. Therapy against

UTI should be guided by antimicrobial susceptibilities as increasing numbers of urinary isolates are developing resistance to commonly used antibiotics.

Conclusion

In conclusion, E.coli was the most frequent isolate both in urine (66.66%) and biofilm (62.60%) of catheterized patients. The antibiotic susceptibility pattern in the present study showed quinolones were the least active drug and the uropathogens showed the highest sensitivity to carbapenems, nitrofurantoin and aminoglycosides in urine samples. Significant correlation between biofilm production and multidrug resistance was observed in our study. So, it is necessary to establish standard guidelines on the care of catheter for all in the hospital to prevent nosocomial infections associated with indwelling catheter.

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