

Study of catheter associated urinary tract infection and biofilm production by *Escherichia coli*

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Abstract

Introduction: Catheter associated urinary tract infection (CAUTI) is most common nosocomial infection accounting for 40% of all institutionally acquired infections and is significantly associated with increased morbidity and health care costs. The study was undertaken to identify the incidence of CAUTI, the causative organisms and their antibiotic sensitivity and biofilm production by *Escherichia coli* in patients with urinary catheter for more than 24 hours.

Materials and Methods: The study included 250 hospitalized patients who catheterized for more than 24 hours. The urine sample aspirated from the port of catheter and subjected to wet mount and Gram staining and inoculated on blood agar, MacConkey agar and CLED media by standard loop method. The isolates with significant counts ($\geq 10^2$ CFU/ml) were identified and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods as per CLSI guidelines. *Escherichia coli* isolates were tested for biofilm production qualitatively by Tube method and Congo Red Agar method.

Results: The present study showed CAUTI in 27.2% of the catheterized patients. *Escherichia coli* was the most common organism isolated (33.8%). The most effective antibiotics against Gram negative isolates were Imipenem (95.23%) and against Gram positive isolates were Vancomycin and Linezolid (100% each). 91.6% *Escherichia coli* isolates were biofilm producers.

Conclusion: The Study showed that the duration of catheterization (≥ 6 days) is the most important predisposing factor for developing CAUTI. *Escherichia coli* is the most common organism causing CAUTI. The high incidence of resistance to conventional antibiotic such as Ampicillin, Co-trimoxazole and Ciprofloxacin among the pathogens causing CAUTI.

Keywords: CAUTI, *Escherichia coli*, Biofilm.

Introduction

Urinary tract infection poses a serious health threat with respect to antibiotic resistance and high recurrence rate.¹ Urinary tract Infection (UTI) represents one of the most common diseases encountered in medical practice today whether hospital acquired or community acquired, occur in all age groups in both gender.²

Indwelling urinary catheters are essential components of modern medical care and are used in acute care setting, extended care facilities, in a person with injured spinal cord, intractable urinary incontinence and urinary retention. Unfortunately indwelling devices significantly increase the risk of iatrogenic Catheter associated urinary tract infection (CAUTI) particularly in an already fragile patient population with potentially pathogenic multidrug resistant organisms which are easily transmitted between patients.³ Incidence of UTIs in patients with indwelling catheter is directly related to the duration of catheterization and constitutes the largest institutional reservoir of nosocomial antibiotic resistant organisms.⁴

Catheter associated urinary tract infection (CAUTI) is defined as the new appearance of bacteriuria of predominate bacterial species or funguria of $\geq 10^2$ CFU/ml in a patient with indwelling catheter of more than 24 hours duration.⁵ CAUTI is most common nosocomial infection accounting for 40% of all institutionally acquired infections with an incidence of

more than 1 million per year. Nosocomial bacteriuria or candiduria develop in up to 25% of patients requiring a urinary catheter for ≥ 7 days with daily risk of 5%.⁶ CAUTI is second most common cause of nosocomial blood stream infection secondary to bacteraemia accounting for 1-4%³ and is most common source of Gram Negative bacteraemia in hospitalized patients.⁴

Biofilm formation on the surfaces of indwelling urinary catheter has important implications for pathogenesis, treatment and prevention of urinary tract infection of CAUTI.³ Biofilm consists of multi layered cell clusters embedded in a matrix of extracellular polysaccharide (slime) which facilitates the adherence of microorganisms to biomedical surfaces and protect from host immune system and antimicrobial therapy.⁷ The longer the urinary catheter remains in place, the greater the tendency of these organisms to develop biofilms and results in UTI.⁸

Nosocomial CAUTI is most frequently caused by *Escherichia coli*, followed by *Klebsiella*, *Enterobacter*, *Pseudomonas aeruginosa*, *Enterococci*, *Staphylococcus aureus*, *CONS*, *Candida* species. The source of CAUTI may be endogenous that is colonic flora and flora around the perianal or periurethral region or exogenous that is hands of health care personnel, collecting bag or break in drainage system.⁴

Most patients with CAUTI are asymptomatic. Patients who develop nosocomial UTI have more

prolonged hospitalization than patients without such an infection.³ Seriously ill patients are at high risk for UTI related nosocomial bacteraemia and case fatality rate being approximately 13%.⁵ CAUTI is significantly associated with increased morbidity, mortality-rate being 5% and health care costs.³ Short term catheterization may be associated with fever and acute pyelonephritis whereas long term catheterization may be complicated by catheter obstruction, urinary tract stones and chronic renal failure.⁵

Hence this study is undertaken to identify the causative organisms and know their antibiotic sensitivity, to estimate production of biofilm qualitatively in *Escherichia coli*. The accurate and early diagnosis is essential to guide the management of catheter related infections.

Materials and Methods

The present prospective study was included 250 hospitalized patients who catheterized for more than 24 hours duration.

In this study, hospitalized patients who catheterized for more than 24 hours duration were included whereas pregnant women, patients with UTI before catheterization, patients in whom the catheter removed before one day and patients with intermittent catheterization were excluded from the study.

3ml of urine sample aspirated with a sterile syringe from the port of the catheter after disinfecting the port with 10% povidine-Iodine and transported to laboratory without delay.⁹

The urine sample after noting the macroscopic appearance, subjected to wet mount and Gram staining and inoculated on 5% sheep blood agar, MacConkey agar and CLED media by standard loop method and incubated at 37°C for 24-48 hours aerobically. Specimen was also inoculated into sabouraud agar slope and incubated at room temperature for 7 days and observed for growth.⁹

In positive cultures, a count of $\geq 10^2$ CFU/ml was taken as significant bacteriuria.⁵ Positive cultures with colony count $<10^2$ CFU/ml were considered as insignificant and if no growth was observed after 48 hours of incubation, culture were reported as no growth. The isolates with significant colony count were identified by standard laboratory procedures and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods as per CLSI guidelines.¹⁰

Escherichia coli isolates were tested for biofilm production qualitatively by Tube method and Congo Red Agar method.⁷

In Tube method- a loop of isolates were inoculated in 10 ml Trypticase Soy Broth with 1% glucose (TSB glu) and incubated at 37°C for 24 hours, the tubes decanted and washed with phosphate buffer saline (PH 7.3) and dried and dried tubes stained with crystal violet (0.1). Excess stain is removed and tubes were washed with water and then dried in inverted position and

observed for biofilm formation.⁷ Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. In Congo Red Agar method (CRA) – isolates were inoculated on CRA and incubated aerobically for 24-48 hours at 37°C. Positive results were indicated by black colonies with dry crystalline consistency.⁷

Results

In this study of 250 cases, 184(73.6%) were male and 66 (26.4%) were female. The mean age for males and females was 58.24 years and 56.7 years respectively. The most common age group affected was 51-60 years (20.4%) followed by 61-70 years (17.6%) and 41-50 years (17.2%).

Out of 250 cases, 68(27.2%) cases had developed significant bacteriuria whereas no growth observed in 182(72.8%) cases with total percentage of culture positivity being 27.2%.

Out of 68 positive cultures, 65 (95.58%) were monomicrobial and 3 (4.41%) were polymicrobial and the organisms found in polymicrobial infections were *Escherichia coli* and *Klebsiella pneumoniae*.

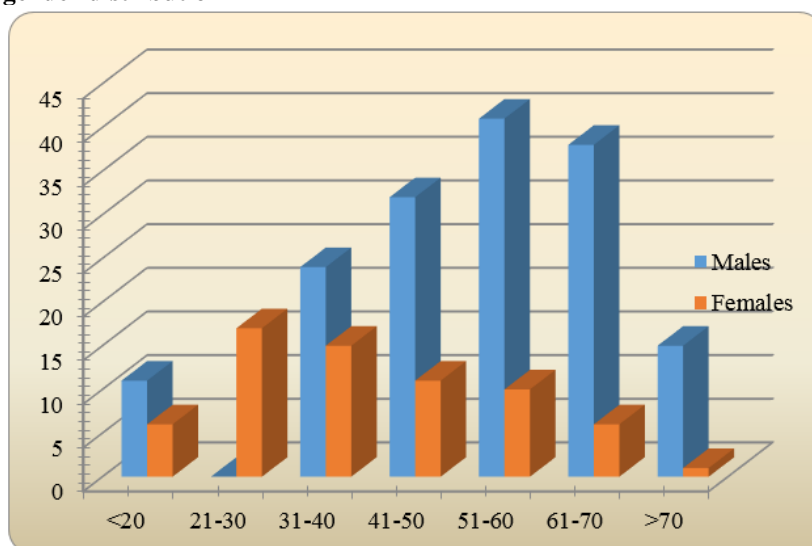
166 patients were with <6 days of catheterization, 9 (5.42%) of them had developed significant bacteriuria and 84 patients were with ≥ 6 days of catheterization, 59 (70.23%) of them had developed significant bacteriuria.

In this study, the most common organism isolated was *Escherichia coli* 24 (33.8%) followed by *Klebsiella* spp 15 (21.12%), *Pseudomonas aeruginosa* 12 (16.9%), *Enterobacter aerogenes* 6 (8.45%), *Proteus mirabilis* 5 (7.04%), *Staphylococcus aureus* 4 (5.63%), *Candida albicans* 3 (4.25%), *Enterococcus faecalis* and *Citrobacter freundii* (1.4% each).

The most effective antibiotic against *Escherichia coli* was Imipenem (100%), followed by Nitrofurantoin (70.83%), Amikacin, Netilmicin and Tobramycin (58.33% each). *Klebsiella* spp showed 93.33% sensitive to Imipenem, followed by 73.33% to Nitrofurantoin, 53% to Amikacin, Netilmicin and Tobramycin. *Pseudomonas aeruginosa* was 91.61% sensitive to Imipenem, followed by 66.66% to Piperacillin-Tazobactam, 58.33% to Amikacin, Netilmicin and Tobramycin.

The most effective antibiotic against *Staphylococcus aureus* was Vancomycin and Linezolid (100% each), followed by Gentamicin and Nitrofurantoin (75% each) and Norfloxacin (50%) and against *Enterococcus faecalis* was Vancomycin, Linezolid and Nitrofurantoin.

The total number of *Escherichia coli* isolates were 24 and 22 (91.6%) of them were biofilm producers.

Graph 1: Age and gender distribution**Table 1: Culture results**

Culture results	No. of cultures	Percentage
Significant bacteriuria ($\geq 10^2$ CFU/ml)	68	27.2%
No growth	182	72.8%
Total	250	100%

Table 2: Association between duration of catheterization and CAUTI

Duration of catheterization	No. of patients with catheterization	No. of patients with SBU	Percentage
<6 days	166	9	5.42%
≥ 6 days	84	59	70.23%
Total	250	68	27.2%

Table 3: Uropathogens isolated in the study group

Isolates	No. of isolates	Percentage
Escherichia coli	24	33.80%
Klebsiella spp	15	21.12%
Klebsiella pneumoniae (13)		
Klebsiella oxytoca (2)		
Pseudomonas aeruginosa	12	16.90%
Enterobacter aerogenes	6	8.45%
Proteus mirabilis	5	7.04%
Staphylococcus aureus	4	5.63%
Candida albicans	3	4.25%
Enterococcus faecalis	1	1.40%
Citrobacter freundii	1	1.40%
Total	71	100%

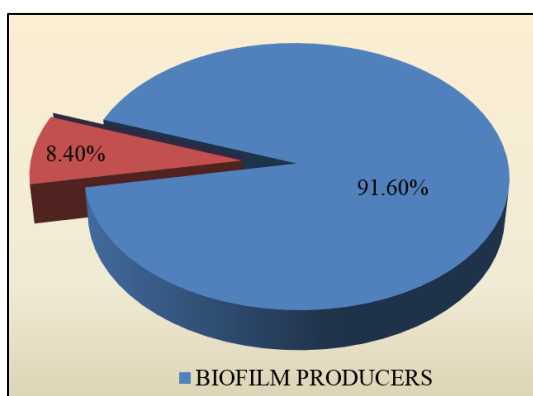
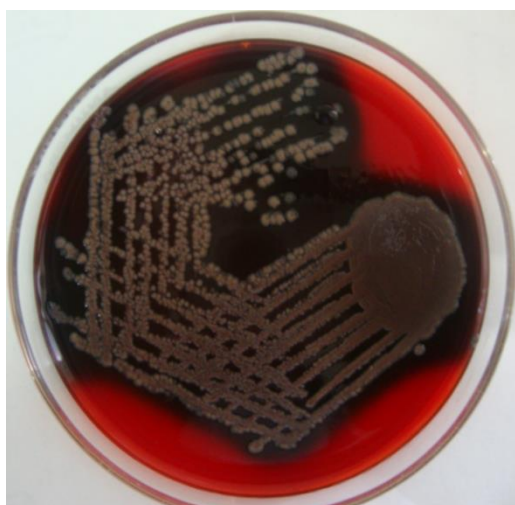
Table 4: Antibiotic susceptibility pattern of Gram negative isolates

Antibiotic	Susceptibility (in Percentage)
Imipenem	95.7%
Nitrofurantoin	63.4%
Amikacin, Netilmicin and Tobramycin	58.7%
Amoxicillin-clavulanic acid	36.5%
Cefotaxime	33.3%

Gentamicin	21.1%
Cefuroxime	19%
Ciprofloxacin and Norfloxacin	17.4%
Co-trimoxazole	7.9%
Ampicillin	1.5%

Table 5: Antibiotic susceptibility pattern of Gram positive isolates

Antibiotic	Susceptibility (in Percentage)
Vancomycin	100%
Linezolid	100%
Gentamicin	75%
Nitrofurantoin	75%
Norfloxacin	40%
Erythromycin, clindamycin, Co-trimoxazole and Tetracycline	20%
Penicillin	0%

**Fig. 1: E.coli isolates producing biofilm****Fig. 2: Biofilm producer forming black colonies with dry crystalline surface on Congo Red Agar****Fig. 3: Tube method; a. Biofilm producer forming thin layer coating the bottom and walls of the tube; b. Non biofilm producers forming no layer in the tube**

Discussion

Incidence of UTIs in patients with indwelling catheter is directly related to the duration of catheterization and constitutes the largest institutional reservoir of nosocomial antibiotic resistant organisms.⁴

In this study, incidence of CAUTI was 27.2%. This is in comparison with the studies conducted by Moataz M. Abdul Fallah et al.¹¹ (26.7%) and Umesh S et al.² (33.6%).

The study showed CAUTI was common in the age group of 51-60 years, the mean age being 58 years. This is in comparison with study conducted by Verhaz A et al.¹² (mean age 60) and Savas L et al.¹³ (mean age 61).

In the present study of 250 cases, females (28.78%) were more commonly affected than males (26.63%) & this correlates with studies of Garibaldi et al.¹⁴ (32.2%), Platt R et al.¹⁵ (30%), and Stamm WE et al.⁶ (32%) & this

difference is attributed to shorter length of female urethra and its proximity to anal canal.

The study showed 27.2% samples were culture positive with significant bacteriuria & this is in comparison with the study of Moataz M. Abdul Fallah et al.¹¹ (26.7%) and Umesh S et al.² (33.6%).

In this study, 95.5% showed monomicrobial growth and 4.41% showed polymicrobial growth, is in comparison with the study of Tambyh et al.¹⁶ (94% and 65%) and Maki DG et al.¹⁷ (93.58% and 4.41%). The organism isolated in polymicrobial growth were E.coli and Klebsiella pneumoniae, is in comparison with organism isolated in Tambyh et al study.¹⁶

The study showed 70.23% of patients with ≥ 6 days of catheterization and 3.5% patients with < 6 days of catheterization developed CAUTI, is in comparison with study of Garibaldi et al.¹⁴ (68.25% & 3.5%) and Shapiro et al.¹⁸ (72% & 2.65%).

In this study, Escherichia coli was the most predominant organism isolated (33.8%) and this finding correlated with Muresul E et al.¹⁹ (32%), Leblebicioglu H et al.²⁰ (32.4%), and Johansen TE et al.²¹ (31%). The next predominant organism isolated was Klebsiella spp (21.12%) and this finding correlated with and Custovic A et al.²² (22.6%). Pseudomonas aeruginosa was the third most common organism isolated (16.9%) and is in comparison with the study of Leblebicioglu H et al.²⁰ (16.8%). Enterobacter freundii was the fourth most common organism isolated (8.45%) and is in comparison with study of Johansen TE et al.²¹ (6.9%). Staphylococcus aureus was isolated in 5.63% of cases and is in comparison with study of Muresu E et al.¹⁹ (5%) and Moataz et al.¹¹ (4.7%). Candida albicans was isolated in 4.25% of cases and this finding correlated with Muresu E et al.¹⁹ (3%) and Moataz et al.¹¹ (5.8%). Enterococcus faecalis was isolated in 1.4% cases, is in comparison with study of Muresu E et al.¹⁹ (1%) and Moataz et al.¹¹ (1.2%).

In this study, predominant Gram negative organisms isolated were Escherichia coli, Klebsiella spp and Pseudomonas aeruginosa and predominant Gram positive organisms isolated was Staphylococcus aureus & this is in comparison with other studies.

In general, the most effective antibiotics against Gram negative isolates were Imipenem (95.23%), Nitrofurantoin (63.49%), Amikacin, Netilmicin and Tobramycin (58.73%). They were least sensitive to Amoxicillin-clavulanic acid (36.5%), Cefotaxime (33.33%), Gentamicin (21.12%), Cefuroxime (19.04%), Ciprofloxacin and Norfloxacin (17.46% each), Co-trimoxazole (7.93%) and Ampicillin (1.58%) and this is in comparison with other studies.

In general, most effective antibiotic against Gram positive isolates were Vancomycin and Linezolid (100% each), followed by Gentamicin and Nitrofurantoin (75% each) and Norfloxacin (40%). They were least sensitivity to Erythromycin, clindamycin, Co-trimoxazole and

Tetracycline (20% each) & this is in comparison with other studies.

In the present study, 91.6% of Escherichia coli isolates from CAUTI showed significant biofilm production and is in comparison with study of Suman E et al.¹ (92%). There is significant correlation between biofilm production and resistance to multiple antibiotics.

Conclusion

From the study, it can be concluded that the duration of catheterization is the most important predisposing factor for developing CAUTI. Escherichia coli is the most common organism isolated and high incidence of resistance to conventional antibiotic such as Ampicillin, Co-trimoxazole, Ciprofloxacin, Norfloxacin and gentamicin among the pathogens causing CAUTI. Biofilm production by pathogens is responsible for pathogenesis of UTI and resistance to multiple antibiotics in catheterized patients.

The justified urethral catheterization is the key measure for the prevention of CAUTI and decreasing in CAUTI related morbidity and mortality.

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