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Multiple antibiotic resistance and biofilm formation of catheter associated urinary tract infection (CAUTI) causing microorganisms

Abstract

IUrinary Tract Infection (UTI) is the most common infection acquired in both hospitals and nursing homes and those UTI usually associated with catheterization are referred to as catheter associated urinary tract infection (CAUTI). Catheters provide entry along external and internal surface of catheters and organism contained within form biofilm. Biofilms are community based growth of infectious organisms encompassed in an exopolymeric matrix that protects the inhabitants from the immune response and antibiotics. In this study, 14 isolates were obtained from the catheter bag of UTI patients and their microbiological and biochemical characterization performed. The isolates isolated are *E. coli* (5), *Klebsiella* (4), *Pseudomonas* (2), Proteus (2) and *Enterococcus fecalis* (01). The isolates were differentiated based on Hi Chrome media and biochemical tests. Isolates were tested for antibiotic resistance using Kirby Bauer disk diffusion assay as per CLSI norms. 13 isolates are MDR (multiple drug resistant) and 3 NDR (non drug resistant). Based on Congo Red agar assay for biofilm formation all 13 MDR were found to be biofilm formers. Biofilm formation was confirmed using latex and silicone catheter tubes as adherent substrates. All the CAUTI isolates except for *E. coli* were found to be inhibited by Silicone catheters in comparison to those made of latex. *E. coli* isolates showed significantly lower biofilm formation in comparison to the other isolates. However, successful control strategies for UTI patients may be customized by including anti-biofilm forming measures along with alternatives to antibiotic treatments.

Keywords: UTI, catheter, biofilm, multiple drug resistance, MDR

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Dipti Singh, Pramila Devi Umrao, Shilpa Deshpande Kaistha
Department of Microbiology, Chhatrapati Sahuji Maharaj University, India

Correspondence: Shilpa Deshpande Kaistha, Department of Microbiology, Institute of Biosciences & Biotechnology, CSJM University, Kanpur 208016, Tel +98 3976 5491, Email shilpakaistha@gmail.com

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Introduction

Catheter associated urinary tract infections (CAUTI) account for 40% of nosocomial infections and up to 20% hospitalized patients are typically catheterize.[1] CAUTI is one of the most common care associated infections worldwide as they form an entry route for pathogenic bacteria.[2] Furthermore, abiotic surfaces such as the catheter form excellent substrates for micro-organisms to adhere and establish biofilm growth.[3] Most bacteria associated with urine produce the enzyme urease, the by product of which are ammonium ions which causes precipitation of Mg and Ca phosphate crystals. These crystals as well as the exopolymeric matrix produced by the biofilm on the catheter surface protects it from antimicrobial compounds as well as the immune response.[4] Once a mature biofilm has formed, it is highly recalcitrant to treatments of antibitoics as well as immune response components.

CAUTI usually occurs in catherized patients when the infectious organisms colonize the peri urethral skin and migrate to the bladder.[1] Bacterial biofilms adhere to the uroepithelium and can invade the renal tissue causing complications such as pyelonephritis and prostatisis. A number of studies have shown positive correlation between the duration of catherization and biofilm formation. A variety of microbial species have been implicated in colonization of the catheter. The prominent amongst them, which are causative agents of UTI, include *E. coli*, *Proteus*, Enterococcus, Pseudomonas, Enterobacter, Serratia, and Candida spp.[5–8]

The material of prosthetic devices such as Foley's catheter plays a major role in adsorption and subsequent biofilm formation of pathogenic organisms and extent of infection.[9] Originally, latex was used for the manufacturing of catheters due to its properties of high tensile strength, ease of processing and designing and due to the fact that it was highly economical. Its disadvantageous however are low biocompatibility and susceptibility to bacterial infections and encrustations. Coating of several types have since been added onto the latex surface such as silicone, polytetrafluoroethylene (PTFE) and poly hydroxylethyl methacrylate (PHMA).[9] However, mixed results about the role of the encapsulation on biofilm formation are reported. Herein, we have isolated CAUTI isolates and tested the latex and silicone catheters for their ability to serve as abiotic surfaces for biofilm formation by pathogenic clinical isolates.

Material and methods

Catheter sampling and growth media

Catheters bags of patients suffering from urinary tract infections were used for microbiological sampling using Hi Chrome UTI media (Hi Media, Mumbai) from hospitals around Kanpur. Samples were streaked on the media and incubated for 37C for 24h. Pure culture isolation was performed and isolates further characterized based on microbiological and biochemical characterization as described previously.[10] Standard strain *P. aeruginosa* P15 obtained

from Department of Microbiology, CSJM University, Kanpur was maintained on Tryptone Soya Agar (TSA) or Tryptone Soya Broth (TSB) medium (Hi Media, India) at 37°C for further study. Local Kanpur city market associated with catering to the needs of government and private hospitals was surveyed for catheters of different materials available. Catheters made of latex and pure silicone were included in this study.

Antibiotic susceptibility

Antibiotic susceptibility test was performed by Kirby Bauer disk diffusion method and their resistance was determined as per CLSI nomenclature.[11] For the analysis of susceptibility of strains, 24h log strains were spread on the MHA (Mueller Hinton Agar) plates, followed by placement of antibiotic discs, and incubated at 37°C for 18h. After incubation, inhibition zones were measured. Multiple antibiotic resistance (MAR) index was calculated as -:

MAR Index = Number of Resistance to antibiotics tested ÷ Total number of antibiotics tested

Congo red agar assay

Congo red agar was prepared by supplementing tryptone soyapeptone with congo red stain (0.8mg/L) was used for biofilm formation assay.[12] Congo red was prepared as a concentrated aqueous solution and autoclaved separately at 121°C for 15 minutes from other medium constituents. Plates were inoculated and incubated aerobically for 24 to 48 hours at 37°C. Black colonies with a dry crystalline consistency were considered as positive. Pink colonies and colonies with occasional darkening at the centers of colonies were considered as weak slime producers.

Crystal violet biofilm assay

Static biofilm formation assay was performed on microtiter plates as per O'Toole et al.[13] In brief, log phase isolates were inoculated on the microtiter plate containing 200µl TSB for 24h at 37°C. Microtiter well's surface was washed with saline and stained with 0.3% crystal violet for 20 min After incubation wells biofilms were washed, stained adhered bacteria were detached by using 200µl of dimethyl sulfoxide and solubilized biofilm was measured by using ELISA microreader (Thermo Fisher Scientific, USA) at A630. For biofilm assays on catheters, half inch catheter tubes were incubated in log phage pure cultures of 16 isolates for 72h at 37°C.

Biofilm formation on catheter tubes

Commonly used catheter samples were acquired from local Kanpur region which included latex and silicone catheters**.** Sterile Catheter tubes were divided into equal 1/4th inch sizes and immersed in 2ml TSB broth and incubated at 37°C for 3 days**.** Tubes were washed with 1X PBS and stained for biofilm formation with crystal violet stain as described previously**.**[13]

Statistical analysis

Student's t test was used wherever applicable.

Results and discussion

Sampling

A total of 16 catheter bags were sampled for bacterial growth using HiChrome UTI media which allows differentiation of the isolates based on enzymatic transformation of substrate which translates into different colored colonies. Brown colored colonies of *Proteus vulgaris*, blue colored colonies of *Klebsiella pneumoniae*, greening yellow of *Pseudomonas aeruginosa*, purple color for *Escherichia coli* and green for *Enterococcus feacalis* were isolates either as unispecies or multi species infections from urine sample. The most predominant infectious organisms were Gram negative *E. coli* and *K. pneumoniae*. Pure cultures of the isolates were transferred to respective selective media such as Eosin Methylene Blue Agar (*E. coli*), MacConkey's Agar (*P. vulgaris* and *K. pneumoniae*) and *Pseudomonas agar* (*P. aeruginosa*). Further microbiological and biochemical characterization was used to confirm the isolates to be Proteus **(**DS1A, DS1B**)**, *Klebsiella pneumoniae* **(**DS2, DS3A, DS3B, DS3C**)**, *Escherichia coli* **(**DS4, DS5, DS6, DS7, DS 9**)**, *Enterococcus fecalis* **(**DS10**)** and *Ps. aeruginosa* **(**DS11, DS12**)** (Table 1).

Table 1 Microbiological and biochemical characterization of CAUTI isolates

Isolates	Indole	MR	VP	Citrate	Urease	Catalase	Oxidase	Gram staining	Identification
DS1A	+	+	-	+	+	+++	-	GNR*	*Proteus vulgaris* DS1A
DS1B	+	+	-	+	+	+++	-	GNR	*Proteus vulgaris* DS1B
DS2	-	-	+	+	+	+	-	GNR	*Klebsiella pneumoniae* DS2
DS3A	-	-	+	+	+	+	-	GNR	*Klebsiella pneumoniae* DS3A
DS3B	-	-	+	+	+	+	-	GNR	*Klebsiella pneumoniae* DS3B
DS4	+	+	-	-	-	++	-	GNR	*Klebsiella pneumoniae* DS4
DS5	+	+	-	-	-	++	-	GNR	*E. coli* DS5
DS6	+	+	-	-	-	++	-	GNR	*E. coli* DS6
DS7	+	+	-	-	-	++	-	GNR	*E. coli* DS7
DS8	-	-	+	-	-	-	-	GNR	*E. coli* DS8
DS9	+	+	-	-	-	+	-	GNR	E. coli DS9
DS10	-	-	+	+	+	+	+	GPC	*Enterococcus fecalis* DS10
DS11	-	-	-	+	+	+++	-	GNR	*Pseudomonas aeruginosa* DS11
DS12	-	-	-	+	+	+++	-	GNR	*Pseudomonas aeruginosa* DS12

* GNR, gram negative bacteria; +, positive test; -, negative test

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The congo red agar test was performed to check for production of exopolymerix substances typical in biofilm formation (Table 2). All isolates except for *Proteus vulgaris sp.* and *E. coli* DS9 showed black precipitate commonly observed for organisms with exopolysaccharides as predominant components in thier biofilms (Figure 1). All isolates except *E. coli* isolates were also found to urease positive, a factor which is known to help in biofilm formation on catheters.[4]

Table 2 Multiple antibiotic resistance and biofilm formation ability of CAUTI isolates

Sr. No	Isolate	Biofilm CRAssay	Antibiotic resistance profile1	MAR Index#
1.	*Proteus vulgaris* DS1A	-	AMP^{R},GEN^{S},CIP^{R},AMC^{R}, IMP^{S}	3/5
2.	*Proteus vulgaris* DS1B	-	AMP^{R},GEN^{S},CIP^{R},AMC^{R}, IMP^{S}	3/5
3.	*Klebsiella pneumoniae* DS2	+	IMP^{S},FOS,CTR^{R},GEN^{R},CFM^{R},CXM^{R}	4/6
4.	*Klebsiella pneumoniae* DS3A	+	IMP^{S},FO^{S},CTR^{R},GEN^{R},CFM^{R},CXM^{R}	4/6
5.	*Klebsiella pneumoniae* DS3B	+	IMP^{S},FO^{S},CTR^{S},GEN^{R},CFM^{R},CXM^{R}	3/6
6.	*Klebsiella pneumoniae* DS4	+	IMP^{S},FO^{S},CTR^{R},GEN^{R},CFM^{R},CXM^{R}	4/6
7.	*E. coli* DS5	+	AMP^{R},AMC^{R},CIP^{R},FO^{S},IMP^{S}	4/5
8.	*E. coli* DS6	-	AMP^{R},AMC^{R},CIP^{R},FO^{S},IMP^{S}	3/5
			AMP^{S},VA^{S},GEN^{S},S^{S},CTR^{S},IMP^{S}	3/5
9.	*E. coli* DS7	+	AMP^{R},GEN^{R},TOB^{S},AMC^{R},IMP^{S}	3/5
			AMP^{R},GEN^{R},TOB^{R},AMC^{R},IMP^{I}	3/5
10.	*E. coli* DS8	+	AMP^{R},GEN^{S},TOB^{S},AMC^{I},IMP^{S}	0/5
			AMP^{R},GEN^{S},CIP^{R},AMC^{R}, IMP^{S}	3/5
11.	*E. coli* DS9	-	AMP^{R},GEN^{S},CIP^{R},AMC^{R}, IMP^{S}	3/5
			IMP^{S},FO^{S},CTR^{R},GEN^{R},CFM^{R},CXM^{R}	1/5
12.	*Enterococcus fecalis* DS10	+	IMP^{S},FO^{S},CTR^{R},GEN^{R},CFM^{R},CXM^{R}	3/5
			IMP^{S},FO^{S},CTR^{S},GEN^{R},CFM^{R},CXM^{R}	3/5
13.	*Pseudomonas aeruginosa* DS11	+		4/6
14.	Pseudomonas aeruginosa DS12	+		4/6
15.	Pseudomonas aeruginosa ATCC 15542	+		3/6

[1]AMP, ampicillin; [2]CIP, ciprofloxacin; [3]GEN, gentamicin; [4]IPM, imipenem; [5]VA, vancomycin; [6]S, streptomycin; [7]CTR, ceftriaxone; [8]FO, Fosfomycin; [9]CFM, cefxime; [10]CXM, cefuroxime; [11]TOB, Tobramycin; [12]AMC, Amoxyclave; #MAR Index, Multiple Antibiotic Resistance Index

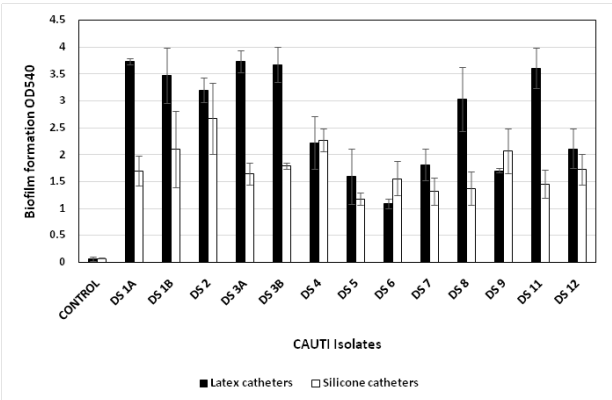

Figure 1 Comparison of biofilm formation on latex and silicone catheter tubes for the various CAUTI isolates. Control specifies negative control with catheter tubes suspended in media without inoculum

Antibiotic resistance and biofilm formation

Antibiotic resistance patterns were assayed using the Kirby Baeur disk diffusion assay as per the CLSI norms.[11] The different isolates were tested for antibiotic typically used in clinical settings. Both the Proteus vulgaris DS1A and *Proteus vulgaris* DS1B were found to be non-biofilm formers by the congo red agar test. The isolates were resistant to Ampicillin, Amoxycillin and Cirpofloxin but sensitive to extended spectrum βlactamase Imipenem and Gentamicin antibiotics. *Klebsiella pneumonia* isolates were multiple drug resistant biofilm formers with resistance to all drugs belonging to Cephalosporin class of antibiotics such as ciprofloxacin, ceftrizxone, cefxime and cefuroxime which are typically used in clinical settings in addition to Gentamycin but sensitive to fosfomycin. *E. coli* isolates were sensitive to fosfomycin and imipenem while resistant to Gentamicin and βlactam antibiotics.

Pseudomonas aeruginosa isolates were all biofilm formers and sensitive to imipenem amongst the antibiotics tested. *Enterococcus fecalis* was sensitive to all antibiotics tested and found to be a non biofilm former based on Congo Red Agar assay (Table 2). CAUTI is a common diseases and the data suggests that while maximum resistance of all the isolates is seen to ampicillin and amoxycillin (90%). All are found to be sensitive to imipenem class of antibiotics while *Klebsiella pneumoniae* and *E.coli* isolates were also sensitive to fosfomycin, and *Pseudomonas aeruginosa* to tobramycin (Table 3). A long term study (1996-2001) in the UK regarding antibiotic resistance pattern in CAUTI uropathogens showed a changing trend of pathogens from most frequented *E. coli* to *Enterococcus*. A changing trend of

least resistance to co-amoxiclav (22.5%), followed by ciprofloxacin (27.2%) and nitrofurantoin (28.8%) in 2001 in contrast to ciprofloxacin (8.0%) followed by co-amoxiclav (18.5%) and cephalexin (25.4%) in 1996.[5] Several other studies suggest that there is increasing resistance to fluoroquinolones, extended beta lactamase in *Enterobactericeae* and MDR *Pseudomonas aeruginosa*.[14]

Table 3 Results of antibiotic resistivity as per disk diffusion assay

Antibiotics	Isolates	N(% resistant) [CLSI range in mm]*
Ampicillin (AMP)	*Proteus vulgaris*	2(100)[13-17]
	Klebsiella pneumoniae	4(100)[13-17]
	E. coli	5(100)[13-17]
	Enterococcus faecalis	1(0)[13-17]
	Pseudomonas aeruginosa	2(100)[13-17]
Amoxycillin (AMC)	*Proteus vulgaris*	2(100)[13-18]
	Klebsiella pneumoniae	4(100)[13-18]
	E. coli	5(100)[13-18]
	Pseudomonas aeruginosa	2(100)[13-18]
Ciprofloxacin (CIP)	*Proteus vulgaris*	2(100)[19-22]
	E. coli	5(100)[19-22]
Cefixime (CFM)	*Klebsiella pneumoniae*	4(100)[13-17]
Ceftriaxone (CTR)	*Klebsiella pneumoniae*	4(100)[13-17]
Cefuroxime(CXM)	*Klebsiella pneumoniae*	4(100)[13-17]
Gentamicin	*Proteus vulgaris*	2(0)[12-15]
	Klebsiella	4(100)[12-15]
	E.coli	5(50)[12-15]
	Enterococcus	1(0)[12-15]
	Pseudomonas aeruginosa	2(50)[12-15]
Fosfomycin (FO)	*Klebsiella pneumoniae*	4(0)[12-16]
	E. coli	5(0)[12-16]
Imipenem (IPM)	*Proteus vulgaris*	2(0)[19-23]
	Klebsiella pneumoniae	4(0)[19-23]
	E. coli	5(0)[19-23]
	Pseudomonas aeruginosa	2(0)[19-23]
Streptomycin (S)	*Enterococcus faecalis*	1(0)[11-15]
Tobramycin (TOB)	*Pseudomonas aeruginosa*	2(50)[12-15]
Vancomycin (VA)_	*Enterococcus faecalis*	1(0)[14-17]

*N, No of isolates; (), percentage resistance;[], CLSi norms for zone of inhibition interpretations

Biofilm formation on latex and silicone catheters

An analysis of the ability of the isolates except *Enterococcus faecalis* DS10 to form 72h biofilms on latex and silicone catheters was tested by the crystal violet biofilm formation assay (Figure 1). Figure 1 shows that all the isolates were capable of biofilm formation on catheters tubes. All the isolates except *E. coli* DS5,DS6 and DS7 showed higher biofilm formation on latex catheters in comparison to silicone catheters (p 0.05). However in our study, *E.coli* DS5, DS6 and DS7 showed comparable biofilm formation on the two catheter materials. Biofilm on latex surface was several fold lower in comparison to the other isolates (Figure 1). Other studies showing that showed that silicone was superior to latex in comparison.[15,16]

The surface of pure silicone is smoother in comparison and it has been previously reported that surface roughness has remarkably enhanced biofilm formation. The latex catheter had approximately 1.5 times the biofilm formation compared to the silicone-based urinary catheter which was attributed to rough surface with pores and uneven surface as evidences by scanning electron micrography images.[15]

Conclusion

Biofilm formation on urinary tract catheters is a common cause of recurrent and persistent bacterial infections. In this study, widely used catheters made of latex and silicone were tested for their abilities to be susceptible to biofilm formation by CAUTI isolates. All the isolates were found to be biofilm formers with higher propensity for biofilm formation on latex catheters in comparison to silicon. Latex catheters are still widely used due to their low cost and higher manoeuvrability and biocompatibility. Silicone catheters showed remarkable lower biofilm formation by the CAUTI isolates, a trait similar to that reported in literature. There are several antimicrobial coated catheters available which however are still not used in mainstream health care as well as due to poor availability in the markets in Kanpur, India due to their higher costs. Considering the high usage of catheterization in hospitalized patients, it is imperative that measures be made to make anti biofilm catheters economical and available to healthcare givers and patients in order to prevent the further development of antibiotic resistant biofilm forming UTI causing isolates.

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Conflict of interest

Authors declare that there is no conflict of interest.

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