

Original Research Article

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A Study on Biofilm Phenotype and Antimicrobial Resistance Pattern of Clinical Isolates from Catheter Associated Urinary Tract Infection

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ABSTRACT

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Biofilms associated with indwelling medical devices are a major public health concern now-a-days as they are difficult to treat with the routine antibiotics. So the present study was taken up to study the biofilm producing phenotype and antimicrobial resistance pattern of pathogens in patients with catheter associate urinary tract infection (CAUTI). Method: this prospective study with 50 urine samples from catheterized patients (indwelling urinary catheter of more than 48 hrs) was carried out over a period of three months. Isolation and identification of the organism was followed by detection of biofilm phenotype by the tissue culture plate method. The results were analysed using SSPS 22.0 software. The most common organism isolated was *E. coli* (54%), followed by *Klebsiella pneumoniae* (16%). Among the total isolates, 31 (62%) were positive for the biofilm phenotype. Biofilm producing strains showed relatively higher antibiotic resistance than non-producers. 71% of the biofilm producers were multidrug resistant. Our study showed a significant association between biofilm production and multidrug resistant.

Introduction

Biofilm is a sessile microbial community which is characterized by the cells having capacity to irreversibly attach to the substratum (either animate or inanimate) or to each other and being embedded in a matrix of self-produced extracellular polymeric substances (Donlan *et al.*, 2002). Biofilms enhance the virulence of an organism, thereby rendering a less virulent organism or an avirulent microbe with huge power of being virulent². They are currently estimated to be responsible for over 65% of nosocomial infections and 80% of all microbial infections (Romling *et al.*, 2012).

Catheter associated urinary tract infection (CAUTI) is a common health care associated infection throughout the world, occurring due to one common reason of magnanimous usage of indwelling urinary catheters teamed with inappropriate antibiotic use (Majumder *et al.*, 2014). The mechanism of such infections is mostly due to the inert nature of the catheter material which is susceptible to microbial colonization and formation of biofilm, thus enhancing the pathogenic effects (Trautner and Darouiche, 2004). Medically, biofilms decrease the antimicrobial susceptibility and render treatment difficult.

CAUTIs are associated with recurrences and complication, thus posing a major health concern; eventually leading to urosepsis and death if not treated⁷. Also, due to extreme closeness of microbial cells in a biofilm, the spread of antimicrobial resistance hastens up due to plasmid exchange.

The present study was conducted to detect biofilm production and their antimicrobial susceptibility pattern in pathogens isolated from cases of CAUTI.

The main aim and objectives of this study includes the microbial profile of catheter associated urinary tract infections. To study the biofilm production in the isolates from catheter associated urinary tract infections. And also to study the antimicrobial resistance pattern of the isolates from catheter associated urinary tract infections.

Materials and Methods

Study design: Prospective

Ethical clearance: The study was approved by the institutional ethical committee

Place of study: Tertiary care hospital, Mangalore, Karnataka

Duration of the study: 3 months (June 2016 – August 2016)

Sample size: 50 non repetitive samples.

Inclusion criteria

Urine samples received with appropriately filled test requisition form (TRF's).

Samples which yielded monomicrobial growth

Exclusion criteria

Samples with polymicrobial growth.
Repeat sample from the same patient.

Statistical analysis

Descriptive data with frequency in numbers was collected and was statistically evaluated using the software SPSS 22.0. A chi square test was done to establish an association between biofilm production and Multidrug resistance.

Isolating the pathogen

The samples were inoculated on Blood agar, MacConkey's agar and Cysteine lactose electrolyte deficient medium using calibrated loops of 4mm.

Antibiotic sensitivity was performed on Muller Hinton agar plates by the Kirby Bauer disc diffusion method. The isolates were stored in nutrient agar in preservation vials at 2-8°C until further processing and detection of biofilms.

Biofilm production

Biofilm producing capacity of the isolate was detected by the tissue culture plate method as described by Mathur *et al.*, (2006).

The organisms preserved in nutrient broth were inoculated in brain heart infusion broth (BHI) with 2% sucrose. The broth was incubated at 37°C for 18-24 hours. The broth culture was then diluted 1:100 with fresh brain heart infusion broth with 2% sucrose.

Individual wells of sterile 96 well flat bottom polystyrene tissue culture plates were filled with 200µl of diluted cultures. The tissue culture plates were then incubated at 37°C for 24 hours. After incubation content of each well was removed by gently tapping the plates on a surface. The wells were washed 4 times with 0.2ml of phosphate buffer saline (pH 7.2) to remove the free floating "planktonic" bacteria. Biofilms formed by adherent sessile

organisms in plate were fixed with 2% sodium acetate and stained with 0.1% w/v of crystal violet. Excess stain was removed by thoroughly washing the tissue culture plate with deionized water three times and then the plates were kept for drying. Optical densities (OD) of stained adherent bacteria were determined with an ELISA reader at wavelength of 570nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilm.

Results and Discussion

Out of 50 patients 39(78%) were male and 11 (22%) were female.

Most common age group was 25 to 40 years which made up 60% (30 out of 50) of total study population. Diabetes mellitus was found to be a co-morbid condition associated with 48% (n=24) of the patients. *E. coli* was the commonest organism seen in 54% (n=27) of samples, followed by *Klebsiella pneumoniae* (16%), *Enterococcus faecalis* (12%), *Pseudomonas aeruginosa* (10%), *Acinetobacter baumannii* and Coagulase Negative *Staphylococcus* (4% each) (Table 1).

62 % (n=31) of the strains were positive for *in vitro* biofilm production. With 70% of the isolates being positive for the biofilm phenotype, *E. coli* was a significant biofilm

producer in this study. It was followed by *Klebsiella* (63%), *Pseudomonas* (60%), *Acinetobacter* (50%) and *Enterococcus* (50%). All isolates of Coagulase Negative *Staphylococci* (CONS) were negative for *in vitro* biofilm formation (Table 2).

The antimicrobial resistant pattern of biofilm producers and non-producers was compared. In the present study, 80% of the total isolates were resistant to cotrimoxazole, of which 25 were biofilm producers and 15 were non biofilm producers. Similarly 80% isolates were resistant to Nalidixic acid, of which 27 were biofilm producers and 13 were non biofilm producers.

Among the biofilm producers maximum resistance was observed for Nalidixic acid (87%), followed by Norfloxacin (81%), Ampicillin (77%), Ceftotaxime (74%) (Table 3).

Multidrug resistance is an inherent feature of biofilm producing organisms, which further enhances their virulence. Out of the 31 biofilm producers, 22 (71%) of the isolates were multidrug resistant.

There was a strong correlation between biofilm production and multidrug resistance with p value <0.0001 (Table 4).

Table.1 Microbial profile of catheter associated urinary tract infections (n=50)

Organisms	No of isolates (%)
<i>E.coli</i>	27(54%)
<i>Klebsiella pneumoniae</i>	8(16%)
<i>Pseudomonas aeruginosa</i>	5(10%)
<i>Acinetobacter baumannii</i>	2 (4%)
<i>Enterococcus faecalis</i>	6(12%)
CONS	2(4%)

Table.2 Screening for biofilm production (n=50)

Bacterial organisms	Biofilm producers (%)		Total
	Positive	Negative	
<i>E. coli</i>	19(70%)	8(30%)	27
<i>Klebsiella pneumoniae</i>	5(63%)	3(37%)	8
<i>Pseudomonas aeruginosa</i>	3(60%)	2(40%)	5
<i>Acinetobacter</i>	1(50%)	1(50%)	2
<i>Enterococcus faecalis</i>	3(50%)	3(50%)	6
CONS	0(0%)	2(100%)	2
Total	31(62%)	19(38%)	50

Table.3 Antimicrobial resistance pattern of biofilm and non-biofilm producers

Antibiotics	Resistance		
	Biofilm positive (n= 31)	Biofilm negative (n= 19)	Resistance of all isolates (n = 50)
Ampicillin (10µg)	24(77)%	9(47)%	33(66)%
Amikacin(30 µg)	9 (29)%	2(11)%	11(22)%
Cefotaxime(30 µg)	23(74)%	8(42)%	31(62)%
Cotrimoxazole	25(81)%	15(78)%	40(80)%
Norfloxacin(10 µg)	25(81)%	11(58)%	36(72)%
Gentamycin(10 µg)	14(45)%	8(42)%	22(44)%
Nalidixic acid(30 µg)	27(87)%	13(68.4)%	40(80)%
Nitrofurantion (300 µg)	18(58)%	6(32)%	24(48)%

Table.4 Co-relation between multidrug resistant phenotype and Biofilm production

Drug resistance	Biofilm producer	Non biofilm producer	p.value
MDR	22	3	
Non MDR	09	16	p < 0.0001

In the present study, *E. coli* was the predominant gram negative pathogen isolated, accounting for almost 54% of the total isolates (27 out of 50); followed by *Klebsiella pneumoniae* (16%), *Pseudomonas aeruginosa* (10%) and *Acinetobacter baumannii* (4%). Amongst the gram positive isolates *Enterococcus faecalis* was most common being 12% of the total isolates, followed by Coagulase negative *Staphylococcus* (4%).

Similar findings of *E. coli* being the commonly associated pathogen with CAUTI

have been reported by Deotale *et al.*,(2015) (51.4%) and Pramodhini *et al.*,(2012) (70%).

The present study reveals 62% (31 out of 50) of the strains were in vitro positive for biofilm production. Similar results were observed by Niveditha *et al.*, (2012), who reported 60% of their strains to be positive for in-vitro biofilm production. On the contrary Abdullah *et al.*,⁴ reported only 43.3% of the isolates to be biofilm producers. In our study, significant proportion of biofilm formation was seen in *E.coli* (70%), followed by *Klebsiella* (63%),

Pseudomonas (60%) and *Acinetobacter* and *Enterococcus* which were 50% each whereas coagulase negative staphylococci (CONS) did not display biofilm positive phenotype.

Similar findings have been reported by Niveditha *et al.*, (2012). Sharma *et al.*, (2012) reported a similar rate of biofilm production in *E. coli* (67.5%). Ponnuswamy *et al.*,⁶ reported 72% of *E. coli* strains having biofilm positive phenotype.

It is a well-known fact that antimicrobial resistance is an innate feature of bacterial biofilms that may further lead to treatment failure. Keeping this in mind, we studied the antibiotic resistance pattern of all uropathogens isolated and found the isolates positive for biofilm formation to have a higher drug resistance as compared to the non-biofilm producing isolates. In the present study, resistant to antibiotics like ampicillin, cefotaxime, norfloxacin and nalidixic acid was noted in 78%, 76%, 70% and 87% of the total biofilm producing isolates; which is much higher than non-biofilm producers.

About 71% of the biofilm producers were found to be multidrug resistant. Similar findings have been documented by Pramodhini *et al.*, (2012), who reported about 80% of their isolates to be multidrug resistant. In our study we found a very strong correlation between biofilm production and multidrug resistant pattern with p value < 0.0001.

E. coli was the most common isolate and 70% of them were found to be biofilm producers. Biofilm producing isolates displayed higher degree of antibiotic resistance as compared to the biofilm non producers. We also observed a significant correlation between biofilm production and multidrug resistance.

CAUTIs have a definite economical concern

as it increases the hospital stay as well as the cost of medication. Judicious use of antibiotics and good catheter care can go a long way in reducing the burden of Catheter associated urinary tract infections.

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