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RESEARCH ARTICLE

A STUDY OF BACTERIAL PROFILE AND BIOFILM PRODUCTION IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Hospital acquired infections are a burden to patients as well as health care providers due to enhanced costs. Urinary tract infections are frequently noted in health care settings and are often associated with indwelling catheters. Biofilm producing pathogens are isolated frequently from patients with catheter associated urinary tract infections (CAUTI) and are more difficult to treat due their higher antimicrobial resistance and persistence. The present study was carried out over a period of two months in the Department of Microbiology, BMCRI, Bengaluru to determine the profile of uropathogens among patients with CAUTI and biofilm producing ability of these pathogens. Biofilm producing ability was confirmed by Tissue Culture Plate method using microtitre plates and the biofilm producers were categorized as Weak, Moderate and Strong. Escherichia coli was noted to be the predominant pathogen, followed by Klebsiella oxytoca in cases of CAUTI and a majority of these isolates were noted to be biofilm producers. The biofilm producing strains were noted to have increased antimicrobial resistance compared to non-biofilm producers in the study. Nitrofurantoin was the most effective antimicrobial in vitro among the isolates encountered in the study. Studies related to biofilm production in indwelling medical devices aid us in understanding the mechanisms associated with nosocomial infections and help in devising strategies to prevent infections due to colonization.

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INTRODUCTION

Biofilm producing pathogens are estimated to be associated with 65% of health care associated infections. These infections are more difficult to treatowing to their higher antimicrobial resistance and account for enhanced healthcare systemcosts. Urinary tract infections (UTI) cause significant morbidity in hospitalized patients. Among bacterial pathogens, *Escherichia coli* (*E.coli*) is reported as the predominant organism responsible for UTI and up to 92% of these strains have been reported to have ability to produce biofilm. Biofilms are frequently encountered in indwelling urinary catheters and are associated with increased antimicrobial resistance. (Abdallah *et al*., 2011; Suman *et al*., 2007) Abiofilmis an aggregate of microorganisms in which cells adhere to each other on a surface. These are embedded within a self-produced matrix of extracellular polymeric substance (EPS). The formation of biofilm is central to the pathogenesis of Catheter Associated Urinary Tract Infection (CAUTI) and affects both therapeutic and prevention strategies. (Longo 18th edition) Uropathogenic *E.coli* is known to produce intracellular communities in the bladder epithelium with biofilm-like properties. A dormant reservoir of pathogen is established in the bladder due to overcoming of the immune response by these pathogens, resulting in a potential source for recurrent infections. (Anderson *et al*., 2004; Anderson *et al*., 2003) A significant correlation has been reported between biofilm production and resistance to multiple antimicrobials such as Ampicillin (A), Co-trimoxazole (Co), Nalidixic acid (Na) and Norfloxacin (Nx). (Suman *et al*., 2007) The present study was carried out over a period of two months from June to July 2013 in the Department of Microbiology, Victoria Hospital, a tertiary care hospital attached to Bangalore Medical College and Research Institute, Bengaluru with the following objectives:

- To study the bacterial profile among inpatients with Catheter Associated Urinary Tract Infections (CAUTI)
- To demonstrate biofilm producing ability among these isolated uropathogens and to correlate the antimicrobial resistance.

MATERIALS AND METHODS

This prospective study carried out over a period of two months from June to July 2013 in the Department of Microbiology,

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Bangalore Medical College and Research Institute, Bengaluru involved processing of catheterized urine samples obtained from inpatients with suspected catheter associated urinary tract infections admitted in the tertiary care hospitals attached to BMCRI. Ethical committee clearance was obtained before the commencement of the study. After obtaining aninformed consent, detailed clinical history was elicited from the patients included in the study & reasons for urinary catheterization were noted. Catheterised but asymptomatic patients were excluded from the study. Collection of the clinical samples was done by appropriate methods under aseptic precautions. The urine samples were collected in a sterile wide mouth container after 30 minutes of clamping the catheter, through a syringe and needle inserted proximal to the site of clamping under aseptic conditions and labelled. The samples were transported immediately to the laboratory for processing. The clinical specimens were processed immediately in the microbiology laboratory. The specimens were inoculated by adopting the standard loop technique on 5% sheep blood agar and MacConkey agar. The inoculated plates were subjected to aerobic incubation at 37 ^{0}C for 24 hours. In positive cultures, the colony counts were counted using a hand lens. Significant bacteriuria was considered when the colony forming units were =/> 10^5. Phenotypic identification of bacterial pathogens were done following standard microbiological protocols. (Collee *et al*., 14th edition) A total of 50 consecutive non repetitive isolates obtained from suspected CAUTI cases were considered for the study. Antimicrobial susceptibility testing of these isolates were done by Kirby-Bauer disk diffusion technique as per recommendations of Clinical and Laboratory Standards Institute (CLSI).

The protocol followed was as follows: Mueller-Hinton Agar plates (Hi-Media, Mumbai) were used. Inoculum was prepared in sterile saline solution and turbidity was adjusted to 0.5 McFarland standard. Mueller Hinton Agar plate was inoculated by lawn culture. Antimicrobial discs (Hi-Media, Mumbai) were evenly placed on the inoculated surface using a sterile forceps in such a way that any two discs are not closer than 24 mm from centre to centre. The reporting of antimicrobial susceptibility of these isolates was done after 18 hours of incubation at 35^{0}C +/- 2^{0}C by measuring the zones of inhibition of the isolate for these disks and comparing the zone size with the interpretative zones as per CLSI 2012 recommendations. (Clinical and Laboratory Standards Institute, 2012) Bacterial isolates from CAUTI cases exhibiting significant bacteriuria were tested for production of biofilms qualitatively by Tube method and Congo red method. In Tube method, a loop of isolate was inoculated in 10 mL of Trypticase Soy Broth with 1% glucose and incubated at 37°C for 24 hours. The tube was then decanted and washed with phosphate buffer saline (pH 7.3) and dried. The dried tube was stained with crystal violet (0.1). Excess stain was removed and the tube was washed with water, followed by drying in inverted position and observed for biofilm formation.

Biofilm formation was considered positive when visible film lined the wall and bottom of the tube. In Congo red agar (CRA) method, the isolates were inoculated on CRA and incubated aerobically at 37°C for 24 – 48 hours. Positive results were indicated by black colonies with dry crystalline consistency. Biofilm producing ability was confirmed by Tissue Culture Plate method described by Stepanovic *et al*. as detailed in the research article by Abdallah *et al*. For interpretation of the results, the strains were categorized as Non biofilm producer, Weak biofilm producer, Moderate biofilm producer and Strong biofilm producer. (Abdallah *et al*., 2011; Mathur *et al*., 2006) Data collected during the study was analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) software for statistical significance.

RESULTS

Of the 50 patients included under the present study, post surgical catheterization was the most frequently noted reason for catheterization (n=12) (Table 1). *E.coli* was noted to be the predominant uropathogens (n=30), followed by *Klebsiella oxytoca* (n=9) and *Pseudomonas aeruginosa* (n=4) (Table 2). 27 of the 50 isolates (54%) included in the present study were detected to be biofilm producers (Figure 1), a majority of them being *E.coli* (n=13; 48.14%), followed by *K.oxytoca*. Both isolates of *P.vulgaris* encountered in the study were found to be biofilm producers (Table 3). The biofilm producing isolatesen countered in the present study were noted to be most susceptible to Nitrofurantoin (n=10; 37.03%), followed by Piperacillin-Tazobactam (n=6; 22.22%), Carbapenems (n=6; 22.22%), Cotrimoxazole (n=5; 18.51%) and Aminoglycosides (n=5; 18.51%). Nonbiofilm producing isolates were most sensitive to Nitrofurantoin (n=15; 65.23%), followedby Carbapenems (n=13; 56.52%), Fluoroquinolones (n=11; 47.82%) fourth generation cephalosporin (n=11; 47.82%), Cotrimoxazole (n=10; 43.47%) andPiperacillin-Tazobactam (n=10; 43.47%).

Table 1. Reasons for urinary catheterization

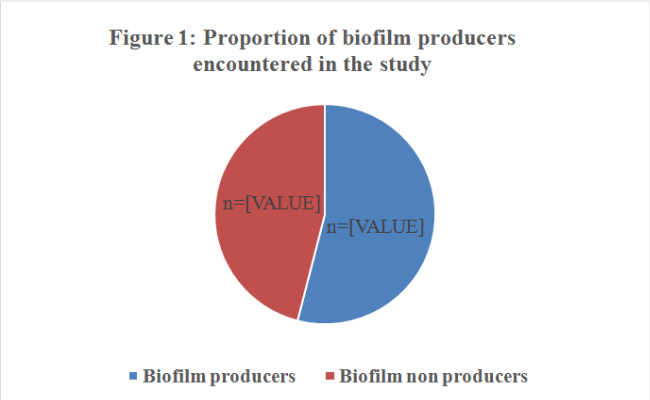
Reason	Numberofcases catheterized (n=50)	Percentage (%)
Postsurgical catheterization	12	24
ICU patients	11	22
Bladderpathology	09	18
Prostatepathology	06	12
Urethralpathology	06	12
Burns	06	12

Table 2. Bacterialisolatesfromcatheterisedurinespecimens

Pathogen	Numberofisolates (n=50)	Percentage (%)
Escherichiacoli	30	60
Klebsiellaoxytoca	09	18
Pseudomonas aeruginosa	04	08
Proteusmirabilis	03	06
Proteusvulgaris	02	04
Citrobacterkoseri	01	02
Enterococcus spp.	01	02

Table 3. Biofilmforminguropathogensincasesof CAUTI

Pathogen	Degree of biofilmformation			
	Non producers	Weak biofilm producer	Moderate biofilm producer	Strongbio film producer
Escherichia coli (n=30)	17	08	03	02
Klebsiellaoxytoca (n=9)	02	01	02	04
Pseudomonasaeruginosa (n=4)	02	02	00	00
Proteusmirabilis (n=3)	01	01	00	01
Proteus vulgaris (n=2)	00	02	00	00
Citrobacterkoseri (n=1)	01	00	00	00
Enterococcus spp. (n=1)	00	01	00	00



Biofilm producers (n=27); Non-producers (n=23).

DISCUSSION

Urinary tract infections are a set of commonly encountered, but misunderstood and challenging infectious diseases in clinical practice. Catheter Associated Urinary Tract Infections have a significant impact on the clinical outcomes in health care facilities and are associated with higher hospitalization costs.[1] The biofilm producing ability of bacterial pathogens have been evaluated using various methods, including Tube method, Congo red agar method and Tissue culture plate method by various studies. Tissue culture plate method using microtitre plate is a preferred method of determining the biofilm producing ability due to its ease of performance and low cost. This technique is used for direct detection of polysaccharide production because spectrophotometric measurements provide quantitative information on the ability of bacterial strains to rapidly grow while adhering to the substratum. Bellifa *et al*. have reported that Tissue culture plate technique can be considered as the most reliable to detect the ability to form biofilm in vitro compared to Tube method and Congo red agar method. (Samia Bellifa *et al*., 2016) Bacterial biofilms are linked to long-term persistence of organisms in various environments. Bacteria in biofilms have been reported to be associated with increased resistance to antibiotics. (Anderson *et al*., 2004) *E.coli* has been reported to be the commonest pathogen responsible for CAUTI in this study, which is similar to the findings of studies by Abdallah *et al* and Bagshaw *et al*. (Abdallah *et al*., 2011; Bagshaw and Laupland, 2006) Abdallah *et al* and Bellifa *et al* have reported significant biofilm producing ability among uropathogens. These biofilm producers are noted to have higher antimicrobial resistance as compared to their non-biofilm producing counterparts. The uropathogens with biofilm producing ability in the present study have been noted to have significant antimicrobial resistance when compared to non-biofilm producing pathogens. This corroborates with the findings reported by Suman E *et al* and Abdallah *et al*. (Abdallah *et al*., 2011; Suman *et al*., 2007; Samia Bellifa *et al*., 2016) Abdallah *et al* have demonstrated a statistically significant difference in antimicrobial susceptibility between planktonic and biofilm populations of the same organism in their study and recommend the use of Minimum Biofilm Eradication Concentration (MBEC) for antimicrobial susceptibility assay in biofilm producing isolates. (Abdallah *et al*., 2011)

Conclusion

From the present study, we conclude that *E.coli* is the predominanturo pathogenin patients with indwelling catheters, with a majority of them exhibiting the ability to form biofilm. Nitrofurantoin was the most effective antimicrobial in vitro among the isolates encountered in the study. Producing pathogens are difficult to treat as they are associated with increased antimicrobial resistance and increased hospitalization costs. Strategies to prevent colonization of urinary catheters would include utilization of appropriate biomaterials for producing catheters, strict infection control measures and judicious catheterization.

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