Isolation and Characterization of Biofilm Forming Multiple Drug Resistance Bacterial Pathogens from the Urinary Catheters

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ABSTRACT

Lengthy urinary tract catheterization leading to the bacterial colonization of the urinary tract is a significant determinant of colonization through adherence to the catheter surface. The collection of urine and urinary catheter bacterial isolates of 55 patients was shown to be multiple catheter colonisation, while the *Escherichia coli* isolates were usually contained in high number in the urine. Kirby Bauer disc diffusion system was used to conduct antimicrobial susceptible patterns of the isolated species. Based on the morphological characterization and some specific biochemical reactions of the most resistant, intermediate and sensitive bacteria, the isolates were identified as, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Enterococcus* Sps., *Staphylococcus epidermidis*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. Nearly 67 % the isolates were high biofilm producers with strong adherence.

KEY WORDS: URINARY CATHETERS, BIOFILM PRODUCERS, ANTIMICROBIAL SUSCEPTIBLE PATTERNS.

INTRODUCTION

Urinary tract infections (UTIs) are severe health distressing complications worldwide. The most common bacterial pathogens involved UTIs in human beings *E. coli*, *E. faecalis*, *K. pneumoniae*, *S. marcescens*, *P. aeruginosa*, *S. saprophyticus*, *S. aureus* and *Proteus mirabilis*. Around 85% of the population acquired UTIs and 50% of the hospital acquired UTIs are mainly caused by *E.coli*. Different factors like age, gender, immunosuppression and urological instruments may affect prevalence of UTIs.

Catheter-associated UTIs are one of the most dangerous health risks contributing 34% of all health care associated infections. The use of antibiotics and spread of antibiotic resistance in clinical settings is a well-recognized problem, but antibiotics and antibiotic resistance as environmental problems and pollutants have largely been overlooked. As a result, the increasing incidence of resistance to a wide range of antibiotic agents by a variety of organisms is a major concern facing modern medicine.

Detection of UTI causing pathogens and resistance of these pathogens to commonly prescribed antibiotics in clinical set ups is essential and helpful in improving the efficacy of empirical treatment. Objective of the present study was to highlight the bacterial etiology of UTIs and determination of resistance pattern of *E. coli* isolates.

Biofilm formation is an important mechanism by which bacteria survive and persist despite antibiotics and host immune responses. Bacterial biofilms are responsible for the failure of many medical devices and are associated

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with many infectious and non-infectious complications¹. Catheter associated urinary tract infections (CAUTI) are among the most commonly reported hospital acquired infections. Bacteria frequently associated with CAUTI include uropathogenic *Escherichia coli* (UPEC), *Enterococcus spp.*, *Klebsiella pneumoniae*, *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa*, etc. Intraluminal and extraluminal biofilms, caused by the attachment of bacteria to the catheter, lead to the entry and subsequent persistence of uropathogens in the bladder. Objective of the present study was to highlight the bacterial etiology of UTIs and determination of resistance pattern of biofilm forming pathogenic isolates.

MATERIAL AND METHODS

Sample collection and Isolation of Bacteria: Urinary catheter samples (n=55) were collected from patients in different wards. The catheter samples were sonicated and centrifuged with sterile phosphate buffered saline. Resultant residues were cultured mainly on blood agar and macConkey's agar by spread plate technique. Bacterial colonies with dissimilar morphology were designated, purified and known by their biochemical profiles.

Multiple drug resistance: Antibiotic sensitivity pattern of all the isolates were evaluated on Muller Hinton agar plates by Kirby-Bauer disc diffusion assay method. Isolates were stated as sensitive or resistant based on the zone of inhibition results after the measures of Clinical Laboratory standards Institute (CLSI).

Biofilm formation on abiotic surfaces

Glass surfaces: Biofilm formation was also examined in glass test tubes. The biofilms were formed by adding 0.1 mL of the culture to 5 mL Brain heart infusion broth (0.5 ×) distributed in glass test tubes. The trial was did in duplicates and the cultures were incubated at 37 °C for 72 h in immobile situation. After incubation, the medium

was detached, the tubes were eroded with sterile distilled water, air dried and biofilms were analyzed using the crystal violet staining method. Strain of *Pseudomonas aeruginosa* PA01 were used as control for the biofilm experiments.

Statistical Analysis Assessments of occurrence data were tested using Chi square (χ^2) test and Fisher's exact test with GraphPad prism (version 6) statistical software.

RESULTS

From the 55 catheter samples handled, 45 (81%) swabs produced at least five bacterial isolate and the remaining 10 (19 %) swabs did not produce any bacterial progression. Direct gram stain recovered 102 organisms from the catheters in the form of bacilli, cocci or a mixture of the two. On unique media the bacterial isolates were cultivated and the most frequent were observed and serial code numbers were given. Due to the morphological characterization of the most resistant, intermediate and vulnerable bacteria and unique biochemical reactions, isolates have been identified as, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Enterococcus Sps.*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Klebsiella pneumoniae*. Out of 102 bacterial isolates from patients, rate of *E. coli* (33.5 %) isolation was highest followed by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus species*, *Klebsiella pneumonia* (Table 1).

Multiple drug resistance: Of the 102 isolates 63 isolates were multidrug resistant. The consequences of this study designated that some of the isolated bacterial strains (Table 2) are vulnerable to most the verified antibiotics. From the results it was observed that *Pseudomonas aeruginosa* (the most resistant isolate) was resistance to Ceftriaxone (30 µg), Cefotaxime (30 µg); Gentamicin (300 µg); while, *Staphylococcus aureus* and *Klebsiella pneumoniae* (intermediate isolates) showed resistance toward, Cefotaxime (30 µg) and Ceftriaxone (30 µg), Netilmicin (300 µg), respectively.

Table 1. Microorganisms associated with catheter mediated infection

Microorganisms	Catheter associated with colonization (%)	Catheter associated with urinary tract infection (%)
<i>Pseudomonas aeruginosa</i>	24.7	42.5
<i>Staphylococcus aureus</i>	12.3	22.5
<i>Escherichia coli</i>	33.6	Nil
<i>Candida albicans</i>	7.2	Nil
<i>Enterococcus Sps.</i>	6.5	5.17
<i>Staphylococcus epidermidis</i>	6	13.17
<i>Proteus mirabilis</i>	5.5	20
<i>Klebsiella pneumoniae</i>	4.7	7

Biofilm Formation of Isolates: All the isolates have been investigated with ideal biofilm medium and Polystyrene as base, assessed by adhesiveness, pellicle, and clumping.

Though the degree of biofilm diverse inside a species and among dissimilar species, 95% of the isolates presented at least one type of biofilm formation. *P. aeruginosa*

isolates showed the maximum capability to form biofilm and adherence of all tested species. Although almost all *E. coli* isolates exhibit biofilm formation, the median

amount of biofilm in this study was lower than for most isolated species (Figure 1).

Table 2. Antibiotic susceptibility profile (sensitivity test) of clinical bacterial isolates against different antibiotics drugs.

Antibiotics	Percentage of isolates showing antibiotic resistance			
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterococcus Sps</i>
Amoxiclav	32	32	43	80
Ceftriaxone	94	93	77	82
Imipenem	55	NS	95	97
Gentamicin	61	NS	65	63
Ceftazidim	45	67	81	83
Oxacillin	NS	65	NS	NS
Netilmicin	87	88	21	NS
Clindamycin	NS	76	NS	NS
Vancomycin	NS	21	NS	NS
Cotrimoxazol	45	NS	32	42
Ciprofloxacin	56	83	81	47
Amikacin	57	62	43	47
Piperacillin	32	27	N	N
Piperacillin/tazobactam	13	14	43	41
Cefotaxim	55.7	67	52	NS

Figure 1: Representative images for the biofilm formation by the pathogens on glass surfaces when incubated at 37°C with stationary conditions. A – *Enterococcus* spp. B– *Staphylococcus aureus*, C– *Pseudomonas aeruginosa*, D– *E. coli*, E– *Pseudomonas aeruginosa* PA01

DISCUSSION

This research explores the prevalence of urinary catheter colonization and infection associated with a bacterial catheter, along with antibiotic resistance trends of isolated bacteria in tertiary care hospital in Indian countries with the quantitative and semi-quantitative techniques. The present study for isolation and identification of organism present in the catheter signifies that in the health care system now the organism which was earlier considered as nonpathogenic are now very much prevalent in a pathogenic form. The elimination of these pathogens is the highest need, as they will lead to an aggravation of already occurring diseases. In certain cases the capacity of certain pathogens to infect immune-skilled humans has been found. Their ability to build resistance scares

humans, and with those species it takes an hour to resolve. The findings of our bacterial isolates' antibiotic susceptibility showed that these species are well known to the antimicrobials tested and that they are evolving mechanisms that inhibit these antibiotics, which are completely compatible with Abdallah et al. A significant field of antibiotic science is currently the multiple resistant bacteria's search for new successful antibiotics against multi-drug pathogenic bacteria.

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Conflict of Interest: Authors does not have any conflict of interest

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