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Study of biofilm production in *Escherichia coli* causing urinary tract infection and its correlation with antimicrobial resistance

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Abstract:

INTRODUCTION: Urinary tract infections (UTIs) are among the most common bacterial infections. *Escherichia coli* remains the most frequent cause of UTIs. More important is the increase in resistance to some antimicrobial agents. Furthermore, bacterial species are capable of living in a biofilm. There is increasing evidence for the role of bacterial biofilm in causing recurrent UTIs.

AIM: The present study aimed to evaluate the ability of *E. coli*, isolated from UTIs to form a biofilm, and its association with catheterisation and to correlate the role of biofilms with their antimicrobial resistance.

MATERIALS AND METHODS: A total of 403 urine samples were processed. All the isolated *E. coli* strains (226) were grown in Luria broth and were incubated overnight in high-glucose Dulbecco's modified Eagle's medium using a microtitre plate. The plate was stained with crystal violet, and the biofilm was quantified using an enzyme-linked immunosorbent assay plate reader at 570 nm. An optical density value more than that of the mean negative control plus three standard deviations is taken as positive for biofilm production. The antibiogram was done using the Kirby–Bauer disk diffusion method.

RESULTS AND DISCUSSION: Of 226 strains, 54.4% were found to produce biofilms. Of them, 81.3% of patients were catheterised. Most of them were found to be resistant to commonly used antibiotics such as Cephalosporins, Quinolones and Aminoglycosides. Imipenem and Nitrofurantoin are the most effective antibacterial agents, showing 77.3% and 73.2% sensitivity, respectively.

CONCLUSION: The biofilm assay using a microtitre plate is convenient and useful in screening the biofilm producers. Catheterisation is a risk factor for biofilm production, and catheter care is of paramount importance to prevent catheter-associated UTI.

Keywords:

Antimicrobial resistance, biofilm, *Escherichia coli*

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Introduction

One of the most common infections encountered in clinical practice is urinary tract infection (UTI). *Escherichia coli* is the most common causative agent for UTI.[1] In recent time, a great concern expressed regarding symptomatic UTI due to increased antimicrobial resistance to commonly used antimicrobials and its chronicity. This chronicity and increase in antimicrobial resistance is attributed to biofilm formation.[2]

The three essential components that constitute a biofilm are adherence of microorganism, a change in gene expression resulting in a different phenotype from the planktonic state and an extracellular matrix composed of host components and secreted bacterial products.[3,4]

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Thus, the objective of this study is to detect the biofilm production by *E. coli* isolated from urine samples and also to study the antimicrobial susceptibility pattern of these biofilm-producing *E. coli*.

Materials and Methods

This prospective study was conducted in the Department of Microbiology, S.C.B. Medical College and Hospital, Cuttack, for three months.

Inclusion criteria

Admitted patients of all age groups and both sexes who had symptoms of UTI (fever, urgency, frequency, dysuria or suprapubic tenderness in case of non-catheterised patients and new onset or worsening of fever, altered mental status, flank pain, costovertebral angle tenderness, rigors, pelvic discomfort and new or worsening malaise or lethargy in catheterised patients) were included in the study.

Sample collection and processing

The samples were collected under complete aseptic conditions in sterile containers. Ten microlitres of urine samples was inoculated onto cystine lactose electrolyte-deficient medium (HiMedia Laboratories, Mumbai) and incubated at 37°C overnight. The identification of isolates was done on the basis of the colony morphology, Gram-staining and the standard biochemical tests.

Detection of biofilm production

The method was adopted as described by Wakimoto *et al*.[5] Briefly, the isolates were inoculated into Luria broth (LB) (HiMedia Laboratories, Mumbai) and incubated at 37°C. In a flat bottom microtitre plate, 200 µl of Dulbecco's modified Eagle's medium (HiMedia Laboratories, Mumbai) was taken and 5 µl of inoculated LB was added and again incubated overnight at 37°C. Then, it was discarded, washed, heat fixed and stained at 0.5% crystal violet for 5 min. The biofilm was quantified in duplicate, after adding 200 µL of 95% ethanol, by an enzyme-linked immunosorbent assay plate reader (Bio-Rad Laboratories, USA) at 570 nm. The procedure was repeated three times, and the mean optical density (OD) value was calculated. *E. coli* ATCC 25922 procured from Microbiologics, USA, was taken as positive control while uninoculated LB was taken as negative control. If the OD of the medium exceeded 0.125, then it was classified the strain as biofilm producers. The number 0.125 was chosen as cut-off because it was three standard deviations (0.013) above the mean OD (0.086) of a clean microtitre plate stained by the method cited above.[6]

Antibiotic susceptibility test

Antibiotic susceptibility test was done using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines using Ampicillin, Amoxicillin/Clavulanate, Cefazolin, Amikacin, Ceftriaxone, Co-trimoxazole, Gentamicin, Norfloxacin, Nitrofurantoin, Piperacillin/Tazobactam and Imipenem which were also procured from HiMedia laboratories, Mumbai, India.[7]

Statistical analysis was done using IBM Statistical Package for the Social Sciences for Windows, version 21 (IBM Corp., Armonk, NY, USA). The data were presented as percentage and proportions. The Chi-square test was applied when two or more set of variables were compared. The critical value of P indicating the probability of significant difference was taken as <0.05.

Results

A total of 403 consecutive samples (254 from catheterised patients and 149 from non-catheterised patients) were processed for culture. A total of 293 (72%) samples were found to be culture positive, of which 226 (77.13%) were *E. coli* and others being *Pseudomonas aeruginosa* (7.5%), *Klebsiella pneumoniae* (4.1%), acinetobacter spp. (3.4%), enterococcus spp. (3.1%), enterobacter spp. (2.3%), candida spp. (1.7%) and *Staphylococcus aureus* (0.77%). All the isolated *E. coli* (226) were evaluated for biofilm production. A total of 123 (54.42%) samples were biofilm producers. Of 226 isolates, 100 (67.11%) were from catheterised patients while 23 (29.87%) were from non-catheterised patients (P = 0.03). The susceptibility pattern showed highest susceptibility to Imipenem (77.3%) followed by Nitrofurantoin (73.2%) among the biofilm producers, while the susceptibility of Imipenem was 100% in case of biofilm non-producing *E. coli* (P < 0.001 for Imipenem susceptibility).

Discussion

UTIs are gradually becoming a major threat to the society due to increasing antimicrobial resistance. The situation is more complicated in catheterised patients due to biofilm production. This results in limited treatment options, complicate medical management and prolonged hospital stays.[8]

In the current study, 226 (77.13%) were *E. coli* of the 293 culture-positive cases. In most of the other studies also, *E. coli* was found to be the most common isolated organism.[1,9-11]

Of 226 *E. coli*, 123 (54.42%) were biofilm producers. A similar study by Abdaagire *et al*. from India showed 44.85% of biofilm production by uropathogens.[9] It is estimated that more than 90% of microorganisms live in a structured community of cells, i.e. biofilms, since they

constitute a more efficient way of surviving in hostile environments.[12]

Among the isolated biofilm producing *E. coli*, 67.11% were from the catheterised patients. Studies done by Sayal *et al*. in Punjab, India,[13] Subramanian *et al*.[14] and Amuthamani *et al*. in Puducherry, India,[10] on catheterised patients had similar results of 71%, 60% and 71% biofilm production, respectively. Organisms got access to the urinary tract during catheterisation because of poor hygienic practices followed during the procedure. The daily risk of acquisition of bacteriuria varies from 3% to 7% when an indwelling urethral catheter remains *in situ*. Formation of biofilm along the catheter surface is a contributing factor for chronic, indolent infection in catheter-associated UTI (CAUTI).[15] Hence, the implementation of 'care bundle approaches' such as insertion of catheter by strict aseptic non-touch technique, catheter care by proper hand hygiene, mental care, perineal care and closed drainage system through the entire period of catheter stay has been advocated for prevention of CAUTI.

Biofilm-producing strains showed relatively high drug resistance against all antibiotics tested as compared to non-biofilm producing counterparts. This may be because bacterial biofilms are often associated with long-term persistence of organism in various environments, decreased bacterial growth rate in a biofilm, expression of resistance genes and restricted penetration of antibiotics into biofilm. Furthermore, proximity of cells within a biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance.[1,16]

The susceptibility pattern showed the highest susceptibility to Imipenem (77.3%) followed by Nitrofurantoin (73.2%) among the biofilm producers, while the susceptibility of Imipenem was 100% in case of biofilm non-producing *E. coli*. This finding was in close association with a study done by Abdaagire *et al*. on uropathogens who found that the most effective antibiotics against Gram-negative bacteria were Imipenem and Meropenem.[9] Furthermore, Rewatkar and Wadher in their study on various clinical isolates found that the most effective antibiotics against Gram-negative bacteria were Imipenem and Colistin.[16] From North India, Panda *et al*. in uropathogens found that Imipenem was the most effective drugs against both biofilm producer and non-biofilm producers.[2] Sharma *et al*. have shown that the susceptibility pattern of the biofilm-producing isolates of *E. coli* ranged from 16% to 57% while that of the non-film-producing isolates ranged from 38 to 76%.[17] Hence, it can be said that the role of biofilm is definite in case of increased antimicrobial resistance. Biofilm production is affected by the type of medical devices, duration of stay of medical devices, extracellular production of polysaccharide by bacteria and bacterial quorum sensing. Some of the effective ways of preventing biofilm formations are closed drainage system and the removal of catheter when not necessary, intermittent catheterisation and changing catheter material while antimicrobial prophylaxis, antimicrobial drainage bag solutions and antimicrobial bladder washes were found to be ineffective.[18]

This study is, however, has its limitations as the duration of the study is short. The isolates were not tested for Extended-spectrum β-lactamase, AmpC or Carbapenemase production. Furthermore, the blood cultures of the patients were not compared to know the extent of bacteraemia.

Conclusion

Biofilm production by uropathogens is not an uncommon phenomenon nowadays and more so in catheterised patients. In the present study, antibiotic resistance was significantly higher in biofilm-producing organism, highlighting the role of biofilm production in spreading drug resistance among the uropathogens.

Imipenem and Nitrofurantoin are the few antimicrobial agents that are effective against both biofilm-producing and non-biofilm-producing organisms. The biofilm mode of living is a highly advantageous response of the microorganisms to the environmental stresses of the urinary tract environment. Whether or not we human beings can overcome or subvert this ancient survival mechanism is an open question as few therapeutic options exist for treatment.

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Conflicts of interest
There are no conflicts of interest.

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