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Analysis of antibiotic sensitivity profile of biofilm-forming uropathogenic *Escherichia coli*

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Abstract

Introduction: Biofilms are group of microorganisms which are embedded within a self-produced matrix of extracellular polymeric substance which adhere to each other. They are found to be involved in a wide range of infections in the body like urinary tract infections (UTIs). Biofilms are considered to be highly resistant to antimicrobial agents. *Escherichia coli* (*E. coli*) is the most common organism causing both community as well as hospital acquired UTI leading to serious health issues. **Objectives:** This study was conducted to analyse the antibiotic sensitivity profile of biofilm forming *Escherichia coli* (*E. coli*) isolated from patients with suspected UTI attending a Teaching hospital of North Karnataka. **Materials And Methods:** 388 *E. coli* isolates recovered from1000 suspected cases of UTI were tested for susceptibility to fourteen different antibiotics. In vitro biofilm formation was detected by Tube adherence method, Congo red agar method and Tissue culture plate method. **Results:** 277 isolates (71.39%) produced biofilm in-vitro by all the three methods. Biofilm forming *E. coli* developed significantly higher degree of resistance towards antimicrobial drugs Ampicillin (87.36%), Cefuroxime (81.58%), Amoxicillin clavulanic acid (77.61%), Ciprofloxacin (71.48%) and Ceftriaxone (71.48%). They were sensitive to higher antibiotics like Imipenem, Piperacillin-tazobactam, Nitrofurantoin, and Amikacin. **Conclusion:** Detection of biofilm in *E. coli* and its resistance to commonly prescribed antibiotics in the clinical practice is essential in improving the efficacy of empirical treatment. This study revealed the prevalence and antimicrobial susceptibility pattern of biofilm forming *E. coli* which helps clinicians to treat UTI effectively.

Keywords: Antibiotic resistance, biofilm, *Escherichia coli*, urinary tract infection

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Introduction

Urinary tract infections (UTIs) are the major and most important cause of serious health problems and morbidity. UTIs account for more than 7 million visits to physicians per year [1],[2],[3] affecting persons of all ages including children, women, and elderly but most predominant in women, especially in developing countries such as India.[4],[5]

Approximately 40% of women have had a UTI in their lifetime and over 20% of young sexually active women who had previous UTIs have recurrent UTIs.[6]

Escherichia coli is the most common organism causing both community and hospital-acquired UTIs, leading to serious secondary health issues worldwide.[7],[8] Currently, recurrent UTI is a serious health problem for many women despite our broad array of very successful antimicrobial agents. Recurrent and relapse UTIs may be due to bacterial virulence factors exhibited by uropathogenic *E. coli* (UPEC) which enable colonization of the bacteria and help the organism overcome host defenses and invade the urinary tract.[9]

Biofilm formation is one of the most important virulence factors exhibited by *E. coli* among other virulence factors. Microbial biofilms are community of bacteria and other microorganisms that are irreversibly attached to self-produced extracellular polymeric substances and adhere to a surface or each other. Biofilms are ubiquitous and can be found in a variety of niches or sites or devices. They play an important role in medicine and have been proven to cause a wide range of microbial infections in the human body such as UTIs, catheter-associated infections, or dental plaques.[10]

Biofilms decrease the susceptibility of organism to antimicrobial agents by enclosing them in an extracellular matrix.[11] A high content of polysaccharides in biofilm prevents the access of antimicrobial agents. Limited penetration of antimicrobial agents into the biofilm and slow rate of cell multiplication of organisms in the biofilm may contribute to the development of chronic infections. Biofilm-forming bacteria exhibit higher resistance to antimicrobial drugs used for the treatment of UTIs, which also lead to recurrent infections.[12]

Our study aimed to unveil the association of biofilm-forming *E. coli* and their antimicrobial susceptibility pattern. This study would help the clinicians in choosing suitable antibiotics for effective treatment of UTI.

Materials and Methods

Sample collection and processing

This study was conducted in the Department of Microbiology, Bidar Institute of Medical Sciences (BRIMS), Bidar, after getting approval from the Institutional Ethical Committee. One thousand patients of all age groups and both sexes complaining of burning micturition and other associated illness attending the outpatient department of BRIMS teaching hospital were included in this study. Informed consent was obtained from all the patients. Clean-catch midstream urine samples were collected in a sterile widemouthed container along with information about their age, sex, and brief clinical history. Samples were transported to the laboratory immediately and processed for culture and antimicrobial drug susceptibility testing as per the routine microbiological techniques and recommendations of Kass.[13] Further, the isolates were identified by standard biochemical tests, and a diagnosis of UTI was made when pathogens were grown at least 10^5 colony forming unit/ml of urine. Only *E. coli* isolates were included in this study.

Antibiotic sensitivity testing

Antibiotics (obtained from HiMedia Laboratories, Mumbai, Maharashtra, India) such as ampicillin (AMP 10 μg), amikacin (AK 30 μg), amoxicillin-clavulanic acid (AMC 30 μg), aztreonam (AT 30 μg), ceftriaxone (CTR 30 μg), cefuroxime (CXM 30 μg), cefepime (CPM 30 μg), ciprofloxacin (CIP 5 μg), chloramphenicol (C 30 μg), gentamicin (GEN 10 μg), imipenem (IPM 10 μg), nitrofurantoin (NIT 300 μg), norfloxacin (NX 10 μg), and piperacillin-tazobactam (PIT 100/10 μg) were tested according to Kirby–Bauer's disc diffusion method [14] as per the Clinical and Laboratory Standards Institute's (CLSI) guidelines.[15]

Quality control

The CLSI control strain of *E. coli* ATCC 25922 was used as a control for antimicrobial susceptibility testing.

In Vitro Biofilm Detection

In vitro detection of biofilm was done by three different methods as follows: tube adherence method, Congo red agar method (CRA), and tissue culture plate method (TCP).

Tube adherence method

This test described by Christensen *et al*. is a qualitative method for biofilm detection.[16] A loopful of test organisms was inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37°C for 24 h. After incubation, tubes were decanted and washed with phosphate-buffered saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water and dried. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.

Congo red agar method

Freeman *et al*.[17] have described a simple qualitative method to detect biofilm production using CRA medium. CRA medium was prepared with brain–heart infusion broth 37 g/L, sucrose 50 g/L, agar no. 1 10 g/L (HiMedia Laboratories, Mumbai, Maharashtra, India), and Congo red indicator 8 g/L (Nice chemicals, Cochin). Initially, Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121°C for 15 min) separately from the other medium constituents. It was then added to the autoclaved brain–heart infusion agar with sucrose at 55°C. CRA plates were inoculated with test organisms and incubated at 37°C for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production.

Tissue culture plate method

This quantitative test described by Christensen *et al*. is considered the gold standard method for biofilm detection.[18] Isolates were inoculated in 10 ml of trypticase soy broth with 1% glucose and incubated at 37°C for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile TCPs were filled with 200 μL of the diluted cultures including control strains. Plates were incubated at 37°C for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate-buffered saline (pH 7.2) four times. Biofilms formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were dried. Optical density of stained adherent biofilms was obtained using micro ELISA autoreader (model 680, Biorad, UK) at a wavelength of 570 nm. The interpretation of biofilm production was done according to the criteria of Stepanovic *et al*.[19]

Quality control

The biofilm producers such as *Staphylococcus epidermidis* ATCC 35984 (positive control) and the nonbiofilm producers such as *S. epidermidis* ATCC 12228 (negative control) were used as standard control strains.

Statistical analysis

Statistical software package SPSS version 22 (IBM SPSS Statistics for Windows, IBM Corp., Released 2013, Armonk, NY, USA) was used to analyze the data. Chi-square test was applied. $P < 0.05$ was considered statistically significant.

Results

Of 1000 urine specimens processed from patients of suspected UTI, 388 *E. coli* were isolated (38.8%). Infection was predominant in females with a rate of 80.92% between the age group of 20 and 29 years (39%). Among males, the infection rate was 19.07%.

Among 388 *E. coli* strains subjected to *in vitro* biofilm production, 277 isolates (71.39%) produced biofilm by all the three methods.*In vitro* biofilm formation by different methods was as follows: 40 (10.3%) strains showed highly positive, 35 strains (9%) showed moderately positive, and 91 strains (23.5%) showed weakly positive by tube method [Figure 1]. Similarly, in CRA method, 254 strains (65.5%) showed highly positive [Figure 2], whereas in TCP method, 284 (73.2%) strains showed strongly positive, 23 strains (5.9%) showed moderately positive, and 81 strains (20.9%) showed weakly positive [Figure 3] and [Table 1].

Figure 1: Strong biofilm formation of *Escherichia coli* by tube adherence method

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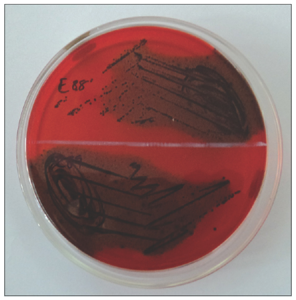

Figure 2: Biofilm formation of *Escherichia coli* on Congo red agar

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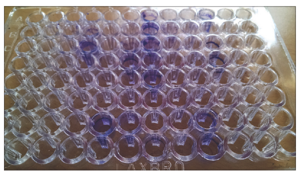

Figure 3: Positive biofilm formation of *Escherichia coli* by tissue culture plate method

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	Tube adherence method (%)	CRA method (%)	TCP method (%)
Strong	40 (10.3)	254 (65.5)	284 (73.2)
Moderate	35 (9)		23 (5.9)
Weak	91 (21.5)		81 (20.9)
Negative	222 (57.2)	134 (34.5)	
Total	388	388	388

CRA: Congo Red Agar, TCP: Tissue culture plate

Table 1: Screening of the *Escherichia coli* isolates for biofilm formation by tube adherence method, Congo Red Agar method, and tissue culture plate method

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Biofilm-producing isolates showed the highest resistance to the antibiotics compared to nonbiofilm-producing isolates. Biofilm producers demonstrated resistance to AMP (87.36%) followed by CXM (81.58%), AMC (77.61%), CIP (71.48%), CTR (54.6%), and CPM (64.98%) [Table 2]. Significant association was observed between biofilm formation and multidrug resistance which was proved to be statistically significant regarding antibiotics such as AK, AMC, AT, CTR, CXM, CPM, CIP, and C [Table 3]. Isolates were sensitive to antibiotics such as PIT (97.83%), IPM (97.14%), and NIT (92.41%).

Antibiotic	Biofilm producers (*n*=277)		Nonbiofilm producers (*n*=111)	
	Number of isolates showing resistance (%)	Number of isolates showing sensitive (%)	Number of isolates showing resistance (%)	Number of isolates showing sensitive (%)
AMP	242 (87.36)	35 (12.2)	88 (79.3)	23 (20.7)
AK	32 (11.55)	245 (88.4)	10 (9.1)	101 (90.9)
AMC	215 (77.61)	62 (22.3)	62 (55.9)	49 (44.1)
AT	139 (50.18)	138 (49.8)	37 (44.4)	74 (66.6)
CTR	198 (71.48)	79 (28.5)	57 (51.4)	54 (48.6)
CXM	226 (81.58)	51 (18.4)	61 (55)	50 (55)
CPM	180 (64.98)	97 (35)	47 (42.4)	64 (57.6)
CIP	198 (71.48)	79 (28.5)	56 (50.5)	55 (49.5)
C	33 (11.91)	244 (88)	19 (13.2)	92 (82.8)
GEN	128 (46.2)	149 (53.7)	34 (30.7)	77 (69.3)
IPM	8 (2.88)	269 (97.1)	5 (4.6)	106 (95.4)
NIT	21 (7.58)	256 (96)	6 (5.4)	105 (94.5)
NX	134 (48.37)	143 (51.6)	42 (37.9)	69 (62.1)
PIT	6 (2.16)	271 (97.8)	18 (12.3)	93 (83.7)

AMP: Ampicillin, AK: Amikacin, AMC: Amoxicillin-clavulanic acid, AT: Aztreonam, CTR: Ceftriaxone, CXM: Cefuroxime, CPM: Cefepime, CIP: Ciprofloxacin, C: Chloramphenicol, GEN: Gentamicin, IPM: Imipenem, NIT: Nitrofurantoin, NX: Norfloxacin, PIT: Piperacillin-tazobactam

Table 2: Antibiotic sensitivity profile of biofilm-forming and nonbiofilm-producing *Escherichia coli* isolates

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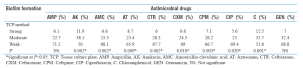
Table 3: Association between antimicrobial resistance and biofilm-forming



uropathogenic *Escherichia coli* isolates

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Discussion

E. coli is the most prominent causative agent of both symptomatic and asymptomatic UTIs, which accounts for more than 80% of the infections.[20],[21] In our study, we found that the frequency of UTI was higher in females compared to males, which concord with other studies conducted.[22],[23] This difference in frequency may be due to several clinical factors, including anatomic differences and hormonal effects. UTI is associated with an expression of different virulence factors including biofilm formation. Biofilm formation is closely related to the susceptibility pattern of *E. coli* toward the antimicrobial drugs which are commonly used to treat UTIs. The resistance pattern in UTI patients of this region is not known. Understanding the resistance pattern will be helpful for treatment. Resistance to antibiotics by biofilm-producing *E. coli* increases the chronicity and recurrence of UTI as bacteria are enclosed within the biofilm and do not allow the antibiotic access to the bacteria.

In this study, the incidence of *in vitro* biofilm formation by UPEC was 71.39%, which was similar to the studies conducted by Subramanian *et al*.,[24] Sharma *et al*.,[25] and Suman *et al*.[26] who reported biofilm formation at the rates of 63%, 67.5%, and 92.0%, respectively. In our study, we analyzed the *in vitro* biofilm formation by three different methods. Nearly 42.78% of isolates were found positive by tube adherence method, and we classified them as highly positive (10.3%), moderately positive (9%), and weakly positive (23.5%). In CRA method, 65.5% isolates were found positive. In TCP method, again it was classified as strongly positive (73.2%), moderately positive (5.9%), and weakly positive (20.9%). These findings were much closer to the study results reported by Tabasi *et al*.[27] In our study, we detected biofilm formation in all the 388 *E. coli* isolates (100%) by TCP method which was similar to the findings reported by Fattahi *et al*.[28] who outlined biofilm formation in 100% of isolates by TCP method.

We studied antibiotic susceptibility pattern for all UPEC isolates. We analyzed the antibiotic resistance pattern of biofilm- and nonbiofilm-forming *E. coli* isolates. Biofilm-forming isolates demonstrated increased resistance to the commonly used antibiotics to treat UTI compared to nonbiofilm producers. Our study results revealed significant correlation between biofilm formation and multidrug resistance. There was an increase in resistance pattern of the drugs such as AK, AMC, AT, CTR, CXM, CPM, CIP, and C which were routinely used to treat UTIs from a long time. This pattern of resistance coincides with the study findings reported by Mittal *et al*. and Ponnusamy *et al*.[29],[30]

Bacterial biofilms are associated with long-term persistence of the organisms in various environments. Biofilms make the organisms impermeable to antibiotics and bind the agents at the outer surface of the matrix layer which protects the bacteria from penetration of the antibiotics. This causes recurrent infection and results in the organism developing multidrug resistance. These strains respond poorly or not respond at all to conventional and routine antimicrobial

therapies.

In the present study, the drugs PIT, IPM, and NIT were effective against biofilm-producing UPEC isolates and these drugs can serve as useful reserved drugs for the treatment of UTI. Understanding biofilms in UTIs will help clinicians in decision-making toward effective treatment guidelines for recurrent UTI in this geographical region.

Conclusion

Conclusively, we have noticed significant association between biofilm production and multidrug resistance. We believe that the detection of biofilm formation might be worth in the management of UTI therapy. Therefore, the knowledge of biofilm formation by *E. coli* and their antibiotic susceptibility pattern will help in deciding on an appropriate antibiotic treatment for UTI. It also helps restrain the emergence of drug-resistant strains.

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Conflicts of interest

There are no conflicts of interest.

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Figures

[Figure 1], [Figure 2], [Figure 3]

Tables

[Table 1], [Table 2], [Table 3]

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