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In vitro biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern

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ABSTRACT

Objective: To detect *in vitro* biofilm formation of uropathogenic *Escherichia coli* (*E. coli*) (UPEC) strains isolated from urine specimens and also to determine their antimicrobial susceptibility pattern using 13 commonly used antibiotics. **Methods:** The present study comprised of 166 urine specimens collected from tertiary care hospitals in and around Coimbatore, South India. All the specimens were subjected to gram staining, bacterial culture and the *E. coli* strains were screened for biofilm formation using Tube Method (TM), Congo Red Agar (CRA) and Tissue Culture Plate method (TCP) respectively. Subsequently, the antimicrobial susceptibility test was performed by Kirby Bauer-disk diffusion method for the biofilm and non-biofilm producing *E. coli* strains. **Results:** Of the 100 (60.2 %) *E. coli* strains, 72 strains displayed a biofilm positive phenotype under the optimized conditions in the Tube Method and the strains were classified as highly positive (17, 23.6%), moderate positive (19, 26.3 %) and weakly positive (36, 50.0 %), similarly under the optimized conditions on Congo Red agar medium, biofilm positive phenotype strains were classified as highly positive (23, 23 %), moderate positive (37, 37 %) and weakly positive (40, 40%). While in TCP method, the biofilm positive phenotype strains were also classified as highly positive (6, 6 %), moderate positive (80, 80 %) and weakly positive (14, 14 %), it didn't not correlate well with the tube method for detecting biofilm formation in *E. coli*. The rates of antibiotic resistance of biofilm producing *E. coli* were found to be 100 % for chloramphenicol and amoxyclav (amoxicillin and clavulanic acid), 86% for gentamicin and cefotaxime, 84% for ceftazidime, 83% for cotrimoxazole and piperacillin/tazobactam, 75% for tetracycline and 70% for amikacin. **Conclusions:** This study reveals the prevalence and antimicrobial susceptibility pattern of biofilm and non-biofilm producing uropathogenic *E. coli* strains.

1. Introduction

Urinary Tract Infection's (UTI's) pose a serious health threat with respect to antibiotic resistance and high recurrence rates[1]. *Escherichia coli* (*E. coli*) are one of the most prevalent pathogens among gram-negative bacteria, capable of causing complicated and uncomplicated UTI's[2,3]. According to Foxman[4], uropathogenic *E. coli* (UPEC) are the primary cause of community-acquired urinary tract infections (UTI) (70%-95%) and a large portion of nosocomial UTI's (50%). Mulvey *et al*[5] and Bower *et al*[6] have reported that, UPEC strains act as a opportunistic intracellular pathogens which colonizes the bladder of the urinary tract, causing cystitis and also ascend through the ureters into the kidneys, causing pyelonephritis. Uropathogenic *E. coli* forms intracellular bacterial communities with many biofilm like properties within the bladder epithelium[7].

Biofilms have a role in up to 60% of human infections and they are difficult to eradicate with antimicrobial treatment. *In vitro* susceptibility tests have shown considerable increase in resistance of biofilm cells to killing[8]. Biofilms can be regarded as a universal strategy for bacterial survival which positions them to effectively use the available nutrients. They largely consist of polysaccharides, which prevents the access of antibacterial agents, antibodies and white blood cells. Planktonic cells are highly susceptible to antibiotics than the sessile bacterial cells in the biofilms which can withstand the host immune responses[9]. So the

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concentrations of antibiotics needed to kill bacteria in the sessile phase are often much higher than those required for bacteria in the planktonic phase[10]. Resistance can be due to production of inactivating enzymes and there is evidence that the relatively large amounts of antibiotic inactivating enzymes such as beta-lactamases which accumulate within the glycocalyx produce concentration gradients can protect underlying cells[11].

The *in vitro* detection of biofilms of uropathogenic *E. coli* and antimicrobial susceptibility pattern among UTI patients has been documented across the globe. This study would serve as a useful guidance for the health care providers especially the physicians' choice of antibiotics for the treatment of biofilm infections among UTI patients.

2. Materials and methods

A total of 100 consecutive non-repetitive *E. coli* strains were isolated from 166 urine specimens of UTI patients attending tertiary care hospitals in and around Coimbatore, South India over a period of 1 year and they were subjected for biofilm production. Identification of the strain was based on cultural characteristics and reactions in standard biochemical tests[12]. All *E. coli* strains were included in the study and were analyzed for the production of biofilm and antimicrobial susceptibility pattern.

2.1. Detection of biofilm formation and antibiotic susceptibility pattern

All the 100 *E. coli* strains were subjected to biofilm production and the numbers of tests are available to identify biofilm producing *E. coli* by methods including Tissue Culture Plate method[13], Tube method[14] and Congo Red Agar method[15]. The above strains were tested for antimicrobial susceptibility by disc diffusion technique according to Clinical and Laboratory Standards Institute guidelines[16] with commercially available discs (Hi-Media, Mumbai). The following antibiotic discs (drug concentration in μg) were used: ampicillin (10), amikacin (30), amoxicillin / clavulanic acid (20/10, 30), co-trimoxazole (25), ceftazidime (30), cephotaxime (30), chloramphenicol (30), gentamicin (30), imipenem (10), norfloxacin (10), piperacillin/tazobactam (100/10), tobramycin (10) and tetracycline (30).

3. Results

Of the 166 urine specimens of urinary tract infection processed, 146 (87.9%) specimens showed culture positive and the rest 20 (12.0%) were negative. Among the strains, aerobic gram negative *E. coli* was 100 (68.5%) and other organisms were 46 (31.5%). Among 100 *E. coli* strains subjected to biofilm production, 17(17%) strains showed highly positive, 19 strains (19%) showed moderate positive, 36 strains (36%) showed weakly positive in tube method. Similarly, in Congo Red Agar method (CRA), 23 strains (23%) showed highly positive, 37 strains (37%) showed moderate positive and 40 strains (40%) were weakly positive, whereas in Tissue Culture Plate Method (TCP), 6(6%) strains showed highly positive, 80 strains (80%) showed moderate positive and 14 strains (14%) showed weakly positive.

3.1. Correlation of biofilm producing strains with multiple drug resistance strains

When analyzed among the strains exhibiting resistance to various commonly used antibiotics with the strains producing biofilm, it was found that the resistance pattern of the strains producing strong positive (17^{+++}), weakly positive (19^{++}) and moderate positive (36^{+}) were found to be 88.2%, 84.2% and 38.8 % respectively. There was also a significant correlation between biofilm production and resistance to multiple antibiotics such as ampicillin, amikacin, co-

Table 1
Antibiotic susceptibility result of the biofilm producing uropathogenic *E. coli* (%).

Antibiotics	Biofilm producer		Non-biofilm producer	
	Resistance	Sensitive	Resistance	Sensitive
Amikacin	70	30	66	34
Amoxyclav	100	–	88	12
Ampicillin	64	36	50	50
Co-trimoxazole	83	17	53	47
Ceftazidime	84	16	70	30
Cephotaxime	86	14	74	26
Chloramphenicol	100	–	3	97
Gentamicin	86	14	83	17
Imipenem	–	100	–	100
Norfloxacin	59	41	33	67
Piperacillin/Tazobactam	83	17	76	24
Tobramycin	66	34	55	45
Tetracycline	75	25	50	50

trimoxazole, norfloxacin and these strains were found to show increased biofilm production. In this study, resistance of 70.6% against ampicillin, amikacin and co-trimoxazole, 58.8% against ampicillin, amikacin and norfloxacin, 47.1% against ampicillin, amikacin, co-trimoxazole, norfloxacin, 41.2% against gentamicin and tetracycline and 35.3% against ampicillin, amikacin, co-trimoxazole, norfloxacin and piperacillin/tazobactam were observed. Thus the different combination of antibiotics resulted in varying degree of resistance among the biofilm producing uropathogenic *E. coli*.

3.2. Antibiotic susceptibility result of the biofilm producing uropathogenic E. coli

The multi-drug resistant pattern of the biofilm producing *E. coli* is shown in Table 1. All the biofilm forming strains showed maximum resistance to amoxyclav (100%), followed by chloramphenicol (100%), gentamicin and cephotaxime (86%), ceftazidime (84%), co-trimoxazole (83%), and amikacin (70%). Both biofilm producer and non- biofilm producer were highly resistant to amoxyclv, followed by gentamicin and piperacillin/tazobactam. However, resistance to other four antibiotics such as co-trimoxazole (83% *vs.* 53%), tetracycline (75% *vs.* 50%) and ampicillin (64% *vs.* 50%) was comparatively higher among biofilm producer than non-biofilm producer. Resistance among biofilm producer to norfloxacin was also higher (59% *vs.* 33%), when compared with non-biofilm producers. 100% sensitive was noticed for both biofilm and non-biofilm producer only against imipenem.

4. Discussion

In the community, bacterial infection of the urinary tract is one of the common causes for an individual to seek medical attention[17]. The pathogens causing UTI′s are almost always predictable, with *E. coli* being the primary etiological agent among the patients[18,19]. Easier methods for diagnosing and quantifying biofilm associated infection and ideal device surface would surely help in the fight against biofilm formation[20]. In the current study, 6% of strains were *in vitro* positive for biofilm production by TCP method. This is relatively lower than biofilm forming capabilities of uropathogenic *E. coli* reported in other studies[21].

Bacterial biofilm are often associated with long-term persistence of organism in various environments. Bacteria in biofilm display dramatically increased resistance to antibiotics[22]. The findings of the current investigations are in agreement with the reports of Reisner *et al*[23]; Ong *et al*[24]; Ulett *et al*[25] and Ulett *et al*[26] in which a greater variation was observed against the uropathogenic *E. coli* forming biofilms under different conditions. Another finding of this study is that strong biofilm producers were less susceptible to antimicrobial agents than the non-biofilm producer. This result may agree with the previous studies showing that the sessile bacterial cells seems to exhibit higher resistance than the planktonic cells[27-33], so the findings of the current investigation indicated that resistance mechanisms are associated with the formation of biofilm among uropathogenic *E. coli*.

Understanding the nature of intracellular bacterial communities in recurrent urinary tract infections will help in the development of new and more effective antimicrobial agents for treating infections due to biofilms[34]. It is commonly accepted that biofilms are more resistant to antibiotics than planktonic cells. Even in the present study, UPEC biofilm were highly resistant to antibiotics. The beta-lactam antibiotics cephotaxime and ceftazidime, and the aminoglycosides gentamicin and amikacin were hardly effective. There are several reasons why these antibacterial agents are not as effective on biofilm cells as they are on planktonic cells. Some antibiotics, such as beta-lactams, require rapid bacterial growth to kill cells[7]. The biofilm producing *E. coli* strains were resistant to at least 6 antimicrobial agents which calls for an urgent need to regulate the overuse of antibiotics. This would limit the spread of resistant microorganisms in the community as well as in hospital settings.

Biofilm formation is closely related with the resistance of *E. coli* towards the antimicrobial drugs and also it increases the chronicity of urinary tract infection. There is an association between biofilm production and antibiotic resistance. Therefore, the UTI caused by biofilm producing *E. coli*, may promote the colonization and increased the incidence rate of UTI′s.

Conflict of interest statement

We declare that we have no conflict of interest.

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