A Cross-Sectional Study on Association between Antibiotic Resistance Pattern and Biofilm Production of *E. coli* in Non-Catheterised UTI Patients at a Tertiary Care Hospital in Kolkata

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ABSTRACT

BACKGROUND

Urinary tract infections are some of the most common community-acquired as well as nosocomial infections with *E. coli* being the most common pathogen. There is increased antimicrobial resistance among bacteria worldwide. One of the important mechanisms of resistance and virulence of bacteria is biofilm formation. This study was conducted to find out the association between antibiotic resistance pattern and biofilm formation in *E. coli* in non-catheterised patients of UTI in a tertiary care hospital. We further wanted to determine the association between the ability of *E. coli* to form biofilm and their ability to produce extended-spectrum beta-lactamases (ESBLs) and carbapenemase in non-catheterised patients.

METHODS

Urine samples collected from 300 non-catheterised patients who had symptoms of UTI were inoculated into MacConkey's agar and blood agar media. Then identification and antibiotic susceptibility tests were done. Phenotypic detection of ESBL production was done by double disc diffusion test and carbapenemase production was done by mCIM (modified carbapenem inactivation method) and eCIM (EDTA carbapenem inactivation method) tests according to Clinical and Laboratory Standards Institute (CLSI) 2019 guideline. Biofilm detection was done by Congo red agar (CRA) method.

RESULTS

Out of 78 isolates *E. coli* were the commonest (61.5 %) isolate. Out of 48 *E. coli* isolates from non-catheterised UTI patients, 26 (54.1%) were biofilm producers. Antibiotic sensitivity pattern among the *E. coli* isolates showed the highest susceptibility of the strains to amikacin, whereas the least susceptibility was for amoxicillin. Out of 48 *E. coli,* 20 (41.6 %) were ESBL producers, 16 (33.33 %) *E. coli* were carbapenemase producers. Significant association was found between ESBL and biofilm production. However, no statistical significance was found between the association of carbapenemase production and biofilm formation.

CONCLUSIONS

Uropathogenic *E. coli* is not an uncommon pathogen for biofilm formation even in non-catheterised patients. The antibiotic-resistance rate was higher among biofilm producing *E. coli* isolates. The biofilm forming ability was found to be significantly higher among ESBL producing strains but was not statistically significant for carbapenemase producing strains of *E. coli*.

KEYWORDS

Biofilm, Uropathogen, Congo Red Agar (CRA) Method, UTI, ESBL, Carbapenemase

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BACKGROUND

Urinary tract infection (UTI) means an infection affecting any part of urinary tract, namely, the kidneys, the ureters, the urinary bladder or the urethra in singularity or in generalised pattern. UTI can present with fever, dysuria, urgency to urinate, frequent urination, incontinence, abdominal pain and suprapubic tenderness. It is the most common cause of both community-acquired and nosocomial infection, affecting all age group.[1,2] Every year there are almost 150 million reported cases that occur worldwide.[3] Common uropathogenic organisms include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* proteus species and some other gram-negative and gram-positive bacteria.[4,5] Among them *E. coli* ranks as the most common pathogen. *Escherichia coli is* responsible for 90 % of community acquired and 50 % of hospital acquired UTI.[6]

The non-judicious use of antibiotics has resulted in the global emergence and propagation of antibiotic resistance among bacteria.[7] According to WHO, antibiotic resistance is a global public health threat which humanity is facing now. The worldwide emergence of extended spectrum beta-lactamase (ESBL) and carbapenemase producing bacteria pose treatment problems resulting in high morbidity and mortality with increased health care costs.[8]

An intrinsic mechanism of bacterial resistance is biofilm formation which gives rise to chronic and recurrent infections.[9] Biofilm formation and its regulation is mediated by quorum sensing. Biofilms are essentially an assembly of microbial cells formed by certain bacterial species that on being associated with a surface can get enclosed in a matrix of polysaccharide and protein material.[10] Biofilm gives a number of advantages to the bacteria such as protection from antimicrobial agents, exchange of nutrients and exchange of genetic material.[11] Biofilm producing organisms are more resistant to antibiotic therapy.[12] In many studies it has been irrefutably shown that biofilm producing organisms are responsible for relapse and reinfection.[13,14] This ultimately increases the hospital burden, health care costs as well as higher morbidity and mortality of patients. A lot of studies have been done on biofilm formation by *E. coli* in catheterised patients but only a few works are there on biofilm formation by *E. coli* in non-catheterised patients. So, in this study we tried to focus on biofilm formation in non-catheterised patients.

This study was conducted to find out the association between antibiotic resistance pattern and biofilm formation in *E. coli* in non-catheterised patient of UTI in a tertiary care hospital and also to determine the association between the ability of *E. coli* to form biofilm and their capacity to produce extended-spectrum beta-lactamases (ESBLs) and carbapenemase in non-catheterised patients.

METHODS

The study conducted was cross-sectional by design and took place in the Microbiology Department of a tertiary care hospital, Kolkata for a period of two months from 1st February to 31st March 2019. As advised by institute ethics committee, ethical clearance was not required for this study, though consent was taken from all subjects included in this study. On the basis of an earlier study by Dash D et al. in the year 2018 who found the prevalence of biofilm formation in *E. coli* in non-catheterised patient to be 30 %,[15] we calculated the sample size by using the formula:

$$Z_\alpha{}^2\, pq/\, L^2$$

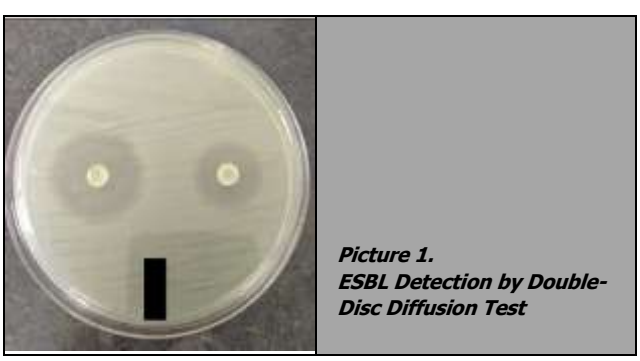
Where Z_α = 1.96 at 95 % confidence interval, p = 0.3, q = (1-p) = 0.7, L = allowable error i.e., 20 % of p. We found the sample size to be 224 by calculating with this formula. Assuming 20 % nonresponse rate final sample size was 268 (approximately). This was rounded to 300.

Inclusion Criteria

Non-catheterised patients from outpatient department (OPD) and inpatient department (IPD) of all age groups and both sexes who had symptoms suggestive of UTI were included in the study. A total of 300 patients were included as per these criteria. We excluded all catheterised patients from the study.

Sample Collection and Processing

The urine sample collected from the 300 patients was 'midstream clean catch' in nature and sterile containers were used for the purpose. Urinary tract infection (UTI) was considered to be present if Gram's staining of 'uncentrifuged' urine sample showed the presence of a single bacterium per oil immersion field or a 'centrifuged' urine sample showed the presence of > 5 white blood cells (WBCs) per high power field. Symptoms of UTI such as fever, dysuria, urgency, frequency, incontinence, abdominal pain and suprapubic tenderness if present was also noted. Urine samples were inoculated into both blood agar media as well as MacConkey's agar media and then subjected to overnight aerobic incubation at 37° C. Colony count was done semi-quantitatively followed by identification of the isolates on the basis of their colony morphology, Gram's staining characteristic and results of standard biochemical tests.



Picture 1.
ESBL Detection by Double-Disc Diffusion Test

Antibiotic susceptibility testing was done according to the CLSI 2019 guidelines by using the Kirby–Bauer disk-diffusion method. The following antibiotic discs were used in our study – amoxicillin (20 µg), ceftazidime (30 µg), ceftazidime-clavulanic acid (30 / 10 µg), ceftriaxone (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), norfloxacin (5

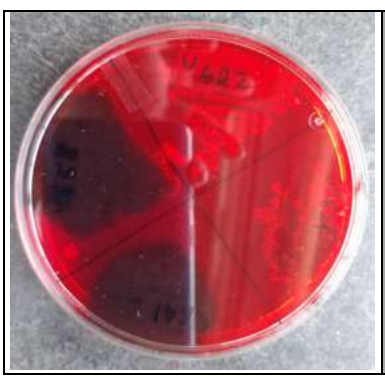
µg), amikacin (30 µg), gentamicin (10 µg), ertapenem (10 µg) and imipenem (10 µg). *E. coli* ATCC 25922 was used as control strains for antibiotic sensitivity testing.

Phenotypic detection of ESBL and carbapenemase: ESBL detection was done by double disc-diffusion test according to CLSI 2019 guideline. ESBL production was considered to be positive if there was an increase in the zone diameter of ≥ 5 mm between ceftazidime (30 µg) and ceftazidime-clavulanate (30 / 10 µg) discs.

E. coli which showed resistance to one or more carbapenem group of drugs particularly ertapenem underwent mCIM (modified carbapenem inactivation method) and eCIM (EDTA carbapenem inactivation method) tests for confirmation of carbapenemase production according to CLSI 2019 guideline.

Biofilm detection: There are various methods of detection of biofilm like tube adherence method, Congo red agar method and tissue culture plate method. Among them the Congo red agar (CRA) method was used in our study for detection of biofilm production. It is a screening method which is simple to perform and gives proper qualitative data pertaining to detection of biofilm production. The chief ingredients used for preparation of the Congo red agar (CRA) were brain-heart infusion broth (BHI) (37 g/l) with supplementation of sucrose (50 g/l), agar No 1 (10g/l) and the Congo red dye (0.8 g/l). The CRA plates were then inoculated with the test organisms followed by their aerobic incubation at 37° C for the next 24 hours.[11] For interpretation, the following colour scale was used:

Colour	Interpretation
Black, dry and crystalline colonies	Biofilm producer
Red or pink or Bordeaux colonies	Biofilm non-producer
Dark colonies without the dry and crystalline morphology	Indeterminate



Picture 2. Detection of Biofilm Production by Congo Red Agar Method

Statistical analysis was done by Excel spread sheet and Open Epi platform. To find out antibiotic resistance pattern and biofilm production Fisher's exact test and chi-square test was used. A P value < 0.05 was considered to be statistically significant.

RESULTS

In our study, out of 78 isolated organisms from non-catheterised patients, 90 % were gram negative and only 10 % were gram positive. Out of 78 isolates, *E. coli* were the commonest (61.5%) followed by klebsiella (15.3%), proteus (5%), pseudomonas (3%), acinetobacter (3%), *S. aureus* (2.6%), *S. epidermidis* (2.6%) and enterococcus (4%).

A total of 48 *E. coli* isolates were obtained from non-catheterised UTI patients. Among them 26 (54.1%) were biofilm producer. Of the 48 *E. coli* isolates, highest susceptibility from among the strains was noted for amikacin followed by imipenem and nitrofurantoin. Similarly, lowest number of the strains were susceptible to amoxicillin, norfloxacin and ciprofloxacin (Figure: 1).

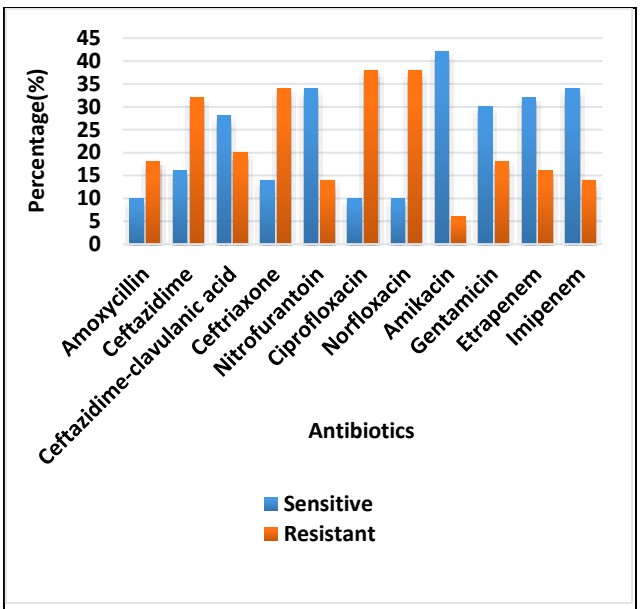


Figure 1. Susceptibility Pattern of E. coli Isolated from Non-Catheterised UTI Patients (N = 48)

Antimicrobials	Susceptibility pattern (N = 48)		Biofilm Producer (N = 26)	Non-Biofilm Producer (N = 22)	p value Significant at p < .05	
Amoxicillin	Sensitive	10	1	9	0.004152	Fisher's exact test
	Resistant	38	25	13		
Ceftazidime	Sensitive	16	2	14	0.000151	X^2 (Yates' corrected) =14.36
	Resistant	32	24	8		
Ceftazidime-clavulanic acid	Sensitive	28	10	18	0.006106	X^2 (Yates' corrected) = 7.519
	Resistant	20	16	4		
Ceftriaxone	Sensitive	14	2	12	0.001196	X^2 (Yates' corrected) = 10.5
	Resistant	34	24	10		
Nitrofurantoin	Sensitive	34	20	14	0.489921	X2 (Yates' corrected) = 0.4767
	Resistant	14	6	8		
Ciprofloxacin	Sensitive	10	2	8	0.037484	X2 (Yates' corrected) = 4.328
	Resistant	38	24	14		
Norfloxacin	Sensitive	10	2	8	0.037484	X2 (Yates' corrected) = 4.328
	Resistant	38	24	14		
Amikacin	Sensitive	42	21	21	0.2734	Fisher's exact test
	Resistant	6	5	1		
Gentamicin	Sensitive	30	12	18	0.024841	X2 (Yates' corrected) = 5.035
	Resistant	18	14	4		
Ertapenem	Sensitive	32	16	16	0.608587	X2 (Yates' corrected) = 0.2622
	Resistant	16	10	6		
Imipenem	Sensitive	34	16	18	0.221882	X2 (Yates' corrected) = 1.496
	Resistant	14	10	4		

Table 1. Antimicrobial-Resistance Patterns between Biofilm and Non-Biofilm Producer Bacterial Isolates from Non-Catheterised UTI Patients

The association between biofilm production and antibiotic resistance was found to be statistically significant ($p < 0.05$) for most of the antibiotics (amoxicillin, ceftazidime, ceftazidime-clavulanic acid, ceftriaxone,

ciprofloxacin, norfloxacin and gentamicin) but the same was not found to be significant in case of nitrofurantoin, amikacin, ertapenem and imipenem (Table: 1).

ESBL Status	Biofilm Producer	Biofilm Non-producer	Total
ESBL Producer	18 (90 %)	2 (10 %)	**20 (41.6 %)**
ESBL Non-producer	8 (28.5 %)	20 (71.4 %)	**28 (58.33 %)**
Total	**26 (54.1 %)**	**22 (45.8 %)**	**48**
Table 2. Association between ESBL Production and Biofilm Production			
Yates' corrected chi square =15.34; p value = 0.00008957			

Among 16 (33.33%) carbapenemase producer, 10 (62.5%) were biofilm producer and 6 (37.5%) were biofilm bon-producer whereas out of 32 (66.66%) carbapenemase non-producer 50% were biofilm producer and 50% were biofilm non-producer (Table 3).

Carbapenemase Status	Biofilm Producer	Biofilm Non-producer	Total
Carbapenemase Producer	10 (62.5 %)	6 (37.5 %)	**16**
Carbapenemase Non-producer	16 (50 %)	16 (50 %)	**32**
Total	**26 (54.1%)**	**22 (45.8%)**	**48**
Table 3. Association between Carbapenemase Production and Biofilm Production			
Yates corrected chi square=0.2622; p value =0.6086			

DISCUSSION

In our study, 78 organisms were isolated from non-catheterised patients. Among them 90 % were gram negative and only 10 % were gram positive. Out of 78 isolates *E. coli* were the commonest (61.5 %) isolates like other studies.[1,2]

Out of 48 *E. coli* isolates, 26 (54.1 %) were biofilm producer. Our finding is similar to findings by Verma S et al. and Nivedita et al.[1,2] but not similar to the finding by Ruchi T et al. who found only 27 % biofilm producing *E. coli*.[11] Our finding is also much higher than the finding of Dash D et al. who had found only 30 % biofilm production in case of non-catheterised patients.[15]

Out of total 48 *E. coli* isolates, the highest susceptibility was noted for amikacin followed by imipenem and nitrofurantoin. Similarly, lowest number of the strains were susceptible to amoxicillin, norfloxacin and ciprofloxacin (Fig: 1) which is very similar to the findings of Tadepalli S et al.[16] Another study by Dash D et al. showed that 77.3 % biofilm producing *E. coli* were susceptible to Imipenem and 100 % non-biofilm producing *E. coli* were susceptible to imipenem but in our study out of 22 non-biofilm producing *E. coli,* 4 (18%) isolates were imipenem resistant.[15]

The association between biofilm production and antibiotic resistance was found to be statistically significant ($p < 0.05$) for most of the antibiotics (amoxicillin, ceftazidime, ceftazidime-clavulanic acid, ceftriaxone, ciprofloxacin, norfloxacin and gentamicin), but the same wasn't found to be significant in case of nitrofurantoin, amikacin, ertapenem and imipenem (Table: 1). Our finding is very similar to the studies by Neupane S et al. except few antibiotics like amoxicillin, ciprofloxacin and norfloxacin.

Among 48 *E. coli* isolates, 20 (41.6 %) were ESBL producer (Table 2) comparable to the findings of Neupane S et al. and Dhamru R et al.[17,18] Whereas 16 (33.33 %) *E. coli* were carbapenemase producers (Table 3) which is quite higher than observations of Dhamru R et al.[18]

Among 20 ESBL producers, 18 (90 %) were biofilm producers and 2 (10 %) were biofilm non-producers whereas out of 28 ESBL non-producers, only 8 (28.5 %) were biofilm producers and 20 (71.4 %) were biofilm non-producers (Table 2). Significant association was found between ESBL production and biofilm formation (chi-square statistic was 15.3447; the P-value was 0.00008957, significant at $p < .05$). Significant association also was found by Neupane S et al. but not by Dhamru R et al.[17,18]

Conversely, the association between carbapenemase production and biofilm formation was not statistically significant (The chi-square statistic was 0.2622; the P-value was 0.608587, not significant at $p < .05$) which was not similar to the observations of Dhamru R et al.[18] Among 16 (33.33 %) carbapenemase producers, 62.5 % were biofilm producers and out of 32 (66.66 %) non-carbapenemase producers, 50 % were biofilm producer (Table 3).

CONCLUSIONS

From our study we can conclude that biofilm production by uropathogenic *E. coli* is not an uncommon phenomenon nowadays in non-catheterised patients. The antibiotic-resistance rate was higher among biofilm producing *E. coli* isolates and the spectrum of resistance covered almost all the antimicrobial agents except a few. The ability to form biofilm was significantly higher among ESBL producing strains of *E. coli* compared to other strains. The carbapenemase producing *E. coli* also showed greater propensity to produce biofilms figuratively but statistically the difference was not significant. This observation could be a consequence of the small number of carbapenemase producing *E. coli* that was part of the study.

This study emphasizes that biofilm production has enhancing effects on expression of many complex mechanisms of multidrug resistance. Biofilm producing bacteria can form the same not only within indwelling catheters, but also within the human urinary tract itself leading to failure of antimicrobial therapy, persistence and chronicity of the ongoing infection and further development of life-threatening sequelae and complications ultimately adding to the disease burden and even mortality in few cases harboring additional comorbidities. Emergence, survival and propagation of multi drug resistant superbugs within organized biofilm still remains a large-scale threat to the ignorant humanity. Proper antimicrobial supervision and strict antibiogram guided therapy must be followed.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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