Original Article

DOI: 10.7860/NJLM/2019/41600:2358

Microbiology Section

# Biofilm Formation and Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Patients with Urinary Tract Infection

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## ABSTRACT

**Introduction:** Biofilm producing bacteria, which colonize the urinary tract and inherent catheters, indicate higher resistance to standard antibiotics used for the treatment of Urinary Tract Infections (UTI). Frequent studies of this type are required to formulate the impirical treatment strategy for UTI in a particular region.

**Aim:** To compare biofilm formation and antibiotic resistance pattern of *Escherichia coli* isolated from patients with UTI.

**Materials and Methods:** *E. coli* was isolated from 220 patients including hospitalized and OPD patients of a tertiary care hospital with symptoms of UTI was included in the study. The isolates were tested for biofilm production by microtiter plate method. The antibiotic susceptibility pattern of the isolates was determined by Kirby–Bauer disc diffusion method as per CLSI guidelines. Extended Spectrum β Lactamase (ESBL) production was detected by double disk approximation test using Ceftazidime 30µg and Ceftazidime/Clavulanic acid (30µg/10µg). Statistical analysis was done using Chi-Square test.

**Results:** Of the 220 *E. coli* isolates, 109 (49.54%) were ESBL producers and 154 (70%) were biofilm producers. Of the 109 ESBL producing *E. coli* 108 were sensitive to fosfomycin (99%), 101 ertapenem (92.67%), 99 amikacin (90.8%), 84 imipenem (77%), 83 meropenem (76.14%), 77 netillin (70.64%), 73 tigecycline (66.97%), 71 cefoperazone/sulbactam (65.14%), 67 piperacillin/tazobactam (61.47%) and resistant to rest of the antibiotics under study. Among the biofilm producers 134 (87%) were moderate biofilm producers and 20 (13%) were strong biofilm producers. More numbers of the biofilm producers were resistant to tigecycline than the non biofilm producers (p=0.005).

**Conclusion:** More numbers of ESBL producing *E. coli* were sensitive to fosfomycin, ertapenem, amikacin, imipenem, meropenem, netillin, tigecycline, cefoperazone/sulbactam, piperacillin/tazobactam and more numbers of the biofilm producers were resistant to tigecycline than the non biofilm producers.

**Keywords:** Anti-bacterial agents, Adherent bacterial aggregates, Beta-lactamases, Drug resistance, Uropathogenic *Escherichia coli*

## INTRODUCTION

*Escherichia coli* is a common causative agent causing UTI [1-3]. The treatment of *E. coli* infection has become difficult because of ESBL producing *E. coli* which are frequently resistant to many of the antimicrobial agents [1-3]. Control of infections is largely based on the awareness of drug resistance pattern in particular region which in turn decides the antibiotic policy of the hospitals [2,3].

The urinary tract and inherent catheters are colonized by biofilm-producing bacteria, implies that higher resistance to standard antibiotics used for the treatment of UTI and further it causes repeated episodes of UTI in the affected population [4]. This study was conducted to study biofilm formation and antibiotic resistance pattern of *E. coli* isolated from patients with UTI and frequent such studies of this type are required to formulate the impirical treatment strategy for UTI in a particular region.

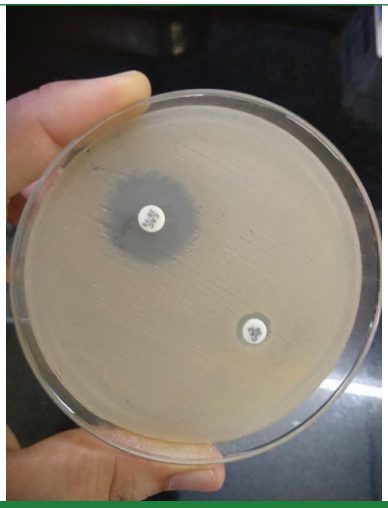
## MATERIALS AND METHODS

It was a cross-sectional study, wherein urine samples received from patients with symptoms of UTI at a microbiology laboratory attached to a tertiary care hospital from October 2016 – March 2017 and which yielded *E. coli* $>10^5$ colony forming units/ml were included. Samples other than urine samples and which did not yield *E. coli* or with colony count less than $10^5$ were excluded from the study. With 95% confidence level and 80% power, 20% relative precision with reference to the previous studies [5,6] the sample size came out to be 110 in each group. The urine samples were classified into two groups, those which were collected from hospitalised patients (Category A) and rest collected from patients visiting OPD with UTI (Category B). The present study was approved by the institutional ethics committee (IEC KMC MLR 11-16/306).

Urine samples were cultured by semi-quantitative technique on 5% sheep blood agar and Cytine Lactose Electrolyte Deficient (CLED) agar plates [7]. *E. coli* isolates were identified by colony morphology, standard biochemical reactions and by VITEK2 Compact (C) system (bioMerieux, North Carolina, USA) using the Gram-negative Identification (GN-ID) 21341 card. On Mac Conkey's agar, *E. coli* colonies were pink colored, flat, transluscent with irregular margins [Table/Fig-1]. The isolates fermented glucose and mannitol with

**[Table/Fig-1]:** Colonies of *E.coli* grown on Mac Conkey's agar.

acid and gas, indole positive, reduce nitrate, Voges–Proskauer, citrate, $H_2S$ and urease negative, decarboxylate lysine. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method using Muller Hinton agar medium following standard CLSI guidelines [8]. ESBL production was detected which was done by double disk approximation test using Ceftazidime 30 µg and Ceftazidime/ Clavulanic acid (30 µg/10 µg). If the difference of diameter of zone of inhibition was more than 5 mm then the isolate was considered an ESBL producer [Table/Fig-2].



**[Table/Fig-2]:** Double disc approximation test for detection of Extended Spectrum β lactamase (ESBL).

Biofilm formation was detected as described previously [9]. Biofilm formation was graded as OD <0.5 as weak or non-biofilm producers; OD 0.5-2 as moderate and >2 as strong biofilm producers. *Pseudomonas aeruginosa* ATCC 27853 was used as positive control.

## STATISTICAL ANALYSIS

The data was collected, tabulated and analysed using chi-square test using SPSS version 16. In present study, antibiotic sensitivity pattern of *E. coli* isolates was compared with the two categories of UTI patients, category A (hospitalised patients) and category B (OPD patients). Antibiotic resistance pattern of *E. coli* isolates from patients with symptoms of UTI was also compared with the ability of the isolates to produce moderate or strong biofilm. The p <0.005 was considered to be statistically significant.

## RESULTS

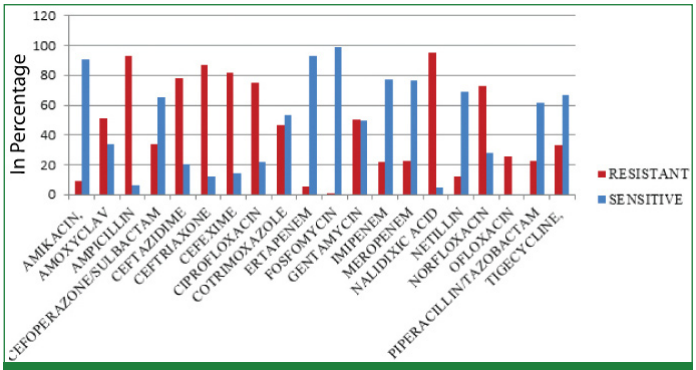
Out of the 220 patients under study, 148 (67.27%) were female and 72 (32.72%) were male; 133 (60.45%) belonged to age group > 45 years; 56 (25.45%) belonged to age group 20-45 years and 31(14%) belonged to age group 1-19. Of the 220 *E.coli* isolates, 109 (49.54%) were ESBL producers and154 (70%) were biofilm producers. Out of the 148 female patients under study, 62 (41.8%) yielded ESBL producing *E. coli* whereas out of 72 male patients 47 (65.27%) yielded ESBL producing *E. coli*. Male patients yielded more of ESBL producing E.coli in comparison with female patients (p = 0.001). Out of the 133 patients belonging to age group >45 years, 78 (58.64%) yielded ESBL producing *E.coli*; out of 56 patients belonging to age group 20-45 years 17 (30.35%) yielded ESBL producing *E. coli* and out of 31 patients belonging to age group 1-19 years 14 (45.16%) yielded ESBL producing *E. coli*. Maximum numbers of patients belonging to age group >45 years yielded ESBL producing *E. coli* (p=0.002).

[Table/Fig-3] shows the antibiotic sensitivity pattern of the *E. coli* isolates from hospitalized patients and OPD patients. *E. coli* isolated from more numbers of OPD patients were resistant to ampicillin (p=0.015), cefexime (p=0.059), ceftriaxone (p=0.021), ciprofloxacin (p=0.027), nalidixic acid (p=0.044), norfloxacin (p=0.00), ofloxacin (p=0.014) in comparison to isolates from inpatients. Of the 109 ESBL producing *E. coli* 108 were sensitive to fosfomycin (99%), 101 ertapenem (92.67%), 99 amikacin (90.8%), 84 imipenem (77%), 83 meropenem (76.14%), 77 netillin (70.64%), 73 tigecycline (66.97%), 71 cefoperazone/sulbactam (65.14%), 67 piperacillin/tazobactam (61.47%) and resistant to rest of the antibiotics under study [Table/Fig-4].

| Antibiotic | Category | Sensitive n (%) | Resistant n (%) | Intermediate n (%) | p-value |
|---|---|---|---|---|---|
| Amikacin | A | 103 (93.6) | 7 (6.36) | 0 | 0.531 |
| | B | 101(91.1) | 9 (8.2) | 0 | |
| Ampicillin | A | 42 (38.1) | 68 (61.8) | 0 | 0.015* |
| | B | 24 (21.8) | 85 (77.2) | 1 (0.9) | |
| Amoxyclav | A | 60 (54.5) | 39 (35.4) | 11(10.0) | 0.510 |
| | B | 52 (47.2) | 46 (41.4) | 12 (10.9) | |
| Cefexime | A | 50 (45.5) | 58 (52.7) | 2 (1.8) | 0.059* |
| | B | 34 (30.9) | 72 (65.4) | 4 (3.6) | |
| Cefoperazone/ sulbactam | A | 87 (79.0) | 23 (20.9) | 0 | 0.095 |
| | B | 74 (67.2) | 35 (31.8) | 1 (0.9) | |
| Ceftazidime | A | 59 (53.6) | 49 (44.5) | 2 (1.8) | 0.087 |
| | B | 48 (43.6) | 62 (56.3) | 0 | |
| Ceftriaxone | A | 56 (50.9) | 53 (48.1) | 1 (0.9) | 0.021* |
| | B | 38 (34.5) | 72 (65.4) | 0 | |
| Ciprofloxacin | A | 52 (47.2) | 55 (50.0) | 3 (2.7) | 0.027* |
| | B | 34 (30.9) | 74 (67.2) | 2 (1.8) | |
| Cotrimoxazole | A | 68 (61.8) | 42 (38.1) | 0 | 0.249 |
| | B | 60 (54.5) | 50 (45.5) | 0 | |
| Ertapenem | A | 100 (90.9) | 6 (5.45) | 4 (3.63) | 0.403 |
| | B | 103 (93.6) | 6 (5.45) | 1 (0.9) | |
| Fosfomycin | A | 109 (99.7) | 1 (0.9) | 0 | 0.995 |
| | B | 109 (99.7) | 1 (0.9) | 0 | |
| Gentamycin | A | 73 (66.3) | 35 (31.4) | 2 (1.8) | 0.342 |
| | B | 72 (64.8) | 38 (34. 5) | 0 | |
| Imipenem | A | 89 (80.9) | 21 (19.0) | 0 | 0.191 |
| | B | 79 (71.8) | 30 (27.2) | 1 (0.9) | |
| Meropenem | A | 90 (81.8) | 20 (18.1) | 0 | 0.197 |
| | B | 82 (74.5) | 26 (23.6) | 2 (1.8) | |
| Nalidixic acid | A | 21 (19) | 89 (80) | 0 | 0.044* |
| | B | 11 (10) | 97 (88.2) | 2 (1.8) | |
| Netillin | A | 90 (81.8) | 9 (8.1) | 11(10) | 0.586 |
| | B | 84 (74) | 12(10) | 14(12) | |
| Norfloxacin | A | 61 (55.4) | 49 (44.5) | 0 | 0.00* |
| | B | 36 (32.7) | 74 (67.2) | 0 | |
| Ofloxacin | A | 56 (50.9) | 53 (48.1) | 1(0.9) | 0.014* |
| | B | 37 (33.6) | 73 (66.4) | 0 | |
| Piperacillin/ Tazobactam | A | 86 (78.1) | 19 (17.2) | 5 (4.54) | 0.132 |
| | B | 78 (70.9) | 19 (17.2) | 13 (11.8) | |
| Tigecycline | A | 65 (59.0) | 45 (40.9) | 0 | 0.522 |
| | B | 70 (63.6) | 40 (36.3) | 0 | |

**[Table/Fig-3]:** Antibiotic sensitivity pattern of *E.coli* isolates from patients with symptoms of UTI.
Category A: Hospitalised patients; Category B: OPD patients *Significant by Chi-Square test

Among the biofilm producers 134 (87%) were moderate biofilm producers and 20 (7.6%) were strong biofilm producers with average OD of 2.31±0.49. Out of the 20 strong biofilm producers 12 (60%) were from in patients, 13 (65%) were ESBL producers. ESBL production was equally distributed among the biofilm producers 50.7% and the non biofilm prodcuers 48.5%. More numbers of the biofilm producers were resistant to tigecycline than the non biofilm producers (p=0.005) [Table/Fig-5].




**[Table/Fig-4]:** Antibiotic sensitivity pattern of ESBL producing *E.coli* isolates from patients with symptoms of UTI (n=109).

| Antibiotics | Biofilm production | | | p-value |
|---|---|---|---|---|
| | Weak/no biofilm n=66 (%) | Moderate n=134 (%) | Strong n=20 (%) | |
| Amikacin | 5 (7.5) | 11 (8) | 1 (5) | 0.894 |
| Ampicillin | 44 (66.7) | 94 (70) | 16 (80) | 0492 |
| Amoxyclav | 28 (42.4) | 51(38) | 7 (35) | 0.929 |
| Cefexime | 39 (59) | 80 (59.7) | 12 (60) | 0.926 |
| Cefoperazone/ sulbactam | 17 (25.7) | 36 (26.9) | 6 (30) | 0.924 |
| Ceftazidime | 41 (62.1) | 60 ( 44.8) | 11 (55) | 0.204 |
| Ceftriaxone | 44 (66.7) | 71 ( 53) | 11 (55) | 0462 |
| Ciprofloxacin | 41 (62.1) | 74 (55.2) | 15 (75) | 0.351 |
| Cotrimoxazole | 25 (37.9) | 61(45.5) | 7 (35) | 0.412 |
| Ertapenem | 4 (6) | 7 (5.2) | 2 (10) | 0.774 |
| Fosfomycin | 1 (1.5) | 2 (1.5) | 0 | 0527 |
| Gentamycin | 21 (31.8) | 46 (34.3) | 7 (35) | 0.804 |
| Imipenem | 14 (21.2) | 31 (23.1) | 7 (35) | 0.633 |
| Meropenem | 11 (16.7) | 33 (24. 6) | 3 (15) | 0379 |
| Nalidixic acid | 58 (87.9) | 110 (82) | 19 (95) | 0.475 |
| Netillin | 11 (16.7) | 9 (6.7) | 2 (10) | 0.210 |
| Norfloxacin | 39 (59) | 72 (53.7) | 13 (65) | 0.578 |
| Ofloxacin | 41 (62.1) | 73 (54.4) | 13 (65) | 0.754 |
| Piperacillin/ Tazobactum | 16 (24.2) | 19 (14.2) | 4 (20) | 0.431 |
| Tigecycline | 18 (27.2) | 55 (41) | 13 (65) | 0.005* |
| In patients | 37 (56) | 62 (46.3) | 12 (60) | 0.317 |
| Out patients | 29 (43.9) | 72 ( 53.7) | 8 (40) | |
| ESBL | 32 (48.5) | 65 (48.5) | 13 (65) | 0.358 |
| Non ESBL | 34 (51.5) | 69 (51.5) | 7 (35) | |

**[Table/Fig-5]:** Antibiotic resistance pattern of *E. coli* isolates from patients with symptoms of UTI compared with the ability of the isolates to produce moderate or strong biofilm.
*Significant by Chi-Square test

## DISCUSSION

Indian studies in past have shown *E. coli* as the most prevalent isolate from cases of UTI [1-5]. Various studies have shown varying prevalence of ESBL producers among the *E. coli* causing UTI. Studies have shown 20%, 34%, 41.6%, 48%,65.43% of the uropathogenic *E. coli* were ESBL producers [1-6]. In a study by Mittal S et al.,13.5% isolates were biofilm producers which were 88% were ESBL producers [4]. In a study by Borah VV et al., ESBL producing *E. coli* isolates were resistant to all cephems and monobactams and ertapenem but susceptible to imipenem and doripenem [5]. A study by Poovendran P et al., showed ESBL producers were100% susceptible to imipenem. The study also emphasizes that ESBL producers have greater biofilm producing ability among uropathogenic *E. coli* thereby increasing the antibiotic resistance [6]. In the present study it was found that more numbers of ESBL producing *E. coli* were sensitive to fosfomycin (99%), ertapenem (92.67%), amikacin (90.8%), imipenem (77%), meropenem (76.14%), netillin (70.64%), tigecycline (66.97%), cefoperazone/sulbactam (65.14%), piperacillin/tazobactam (61.47%) and resistant to rest of the antibiotics under study.

In a surveillance study conducted in a hospital in Odisha, India, showed that ESBL strains were uniformly circulated in both community or hospital units and many of the isolates showed high drug resistsnce against (79 to 92%) gentamycin, (69 to 94%) to oxacillin, (58 to 85%) ceftriaxone, (47 to 69%) norflox in both nosocomial and community isolates equally [10]. In the present study *E. coli* isolated from more number of OPD patients were resistant to ampicillin (p= 0.015), cefexime (p=0.059), ceftriaxone (p=0.021), ciprofloxacin (p=0.027), nalidixic acid (p=0.044), norfloxacin (p=0.00), ofloxacin (p= 0.014) in comparison with hospitalised patients.

A study by Tayal RA et al., showed that among the uropathogens, *Enterococccus* spp showed maximum biofilm production followed by *Escherichia coli*. Most of the biofilm producers were multidrug resistant [11]. In a study by Murugan S et al, 84.37% of *E. coli* isolates showed biofilm production by tube method and it was associated with resistance to multiple antibiotics, signifying their correlation [12]. A study by Hancock V et al., showed that the *E. coli* causing asymptomatic bacteriuria were better biofilm formers than the *E. coli* which caused symptomatic bacteriuria [13].

A study conducted by Suman E et al., revealed that 92% of *E. coli* isolates show significant biofilm production and they also showed significant correlation between biofilm and multidrug resistance. Among the biofilm producers 54% were resistant to a combination of four drugs ampicillin, cotrimaxzole, nalidixic acid and norfloxacin [14]. A study done by Ponnusamy P et al., showed that both biofilm producers and non biofilm producers were equally resistant to amikacin, amoxyclav, cephalosporins, piperacillin tazobactam, gentamycin and sensitive to imipenem. But biofilm producers were more resistant to chloramphenicol, norfloxacin and co-trimaxazole [15].

An invitro study on the effect of antibiotics on biofilm production by uropathogenic *E. coli* isolated from children with UTI, except for ampicillin, the other antibiotics tested like cephalothin, ceftriaxone, ceftazidime, amikacin and ciprofloxacin, induced a significant reduction of biofilm biomass produced by uropathogenic *E.coli* [16]. A study analysed biofilm production, antibiotic resistance and fimbrial genes in various uropathogenic *E. coli* isolates. There was not much correlation [17]. In the present study ESBL production was equally distributed among the biofilm producers 50.9% and the non biofilm producers 49.1%. The biofilm producers were more resistant to tigecycline than the non biofilm producers (p=0.005).

## LIMITATION

In the present study gene detection for antibiotic resistance and biofilm formation has not been done. It can be considered for the future studies.

## CONCLUSION

In the present study more numbers of *E. coli* isolated from OPD patients were resistant to ampicillin, cefexime, ceftriaxone, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin in comparison with hospitalised patients. Maximum numbers of patients belonging to age group >45 years and most of the male patients yielded ESBL producing *E. coli*. More numbers of ESBL producing *E. coli* were sensitive to fosfomycin, ertapenem, amikacin, imipenem, meropenem, netillin, tigecycline, cefoperazone/sulbactam, piperacillin/tazobactam and more numbers of the biofilm producers were resistant to tigecycline than the non biofilm producers (p=0.005).

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**FINANCIAL OR OTHER COMPETING INTERESTS:** None.

Date of Submission: **Mar 23, 2019**
Date of Peer Review: **Apr 13, 2019**
Date of Acceptance: **May 25, 2019**
Date of Publishing: **Jul 01, 2019**