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## Original Research Article

# Detection of biofilm formation, extended spectrum beta lactamase production and their correlation with antibiotic resistance among uropathogenic *Escherichia coli*

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## ABSTRACT

**Background:** Urinary tract infections (UTI) are most common bacterial infections encountered in clinical practice and *E.coli* is predominant organism causing UTI.

**Objectives of the study:** 1. To study antibiotic resistance pattern among uropathogenic *E.coli* (UPEC). 2. To study extended spectrum beta lactamase (ESBL) production and their correlation with antibiotic resistance among uropathogenic *E.coli* (UPEC). 3. To study biofilm formation and its correlation with antibiotic resistance among uropathogenic *E.coli* (UPEC)

**Materials and Methods:** A prospective study was conducted on first 100 *Escherichia coli* isolated from urine specimens of patients suspected to be having urinary tract infection between January 2016 and December 2016 received at Department of Microbiology, SIMS Shimoga. Fresh midstream urine samples were aseptically collected in sterile containers and plated on Blood agar & MacConkey agar plates using a standard loop technique & the growth was processed by standard bacteriological technique. Biofilm detection was done by tube and microtitre plate method. ESBL detection was done according to CLSI criteria. Antimicrobial sensitivity testing was done using Kirby-Bauer methods on Mueller-Hinton agar. Results were interpreted as per the CLSI guidelines.

**Result:** Antibiotic sensitivity of *E.coli* was nitrofurantoin (100%) fosfomycin (100%) imipenem (77%) cotrimoxazole (61%) amikacin (47%) aztreonam (53%) piperacillintazobactam (41%). cefotaxim (25%) ceftazidime (32%) norfloxacin (20%) ampicillin-sulbactam (12%) ciprofloxacin (15%), levofloxacin (12%), amoxiclavulanic acid (15%). ESBL positive were 83% (screening), 80% (confirmatory). Biofilm positive were 63% (tube method) 79% (Microtiterplate method).

**Conclusion:** This study showed Fosfomycin and Nitrofurantoin were the most sensitive drug. Study shows antibiotic resistance was seen more in ESBL and Biofilm producers compared to non ESBL and non biofilm producers.

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## 1. Introduction

Urinary tract infections (UTI) is one of most important causes of morbidity and mortality.<sup>1</sup> *Escherichia coli* is most common organism causing UTI. Subsets of fecal *E.coli* that can enter colonize urinary tract and cause infection are known as uropathogenic *Escherichia coli* (UPEC).<sup>1</sup>

According to Kass concept growth of >10<sup>5</sup> organisms per millilitre from properly collected clean catch midstream urine sample indicates significant bacteriuria.<sup>2</sup> Biofilm is a group of microorganisms encased in an exopolymer coat.<sup>3</sup> It is considered pathogenic determinant which allows strains to persist a long time in genitourinary tract and interfere with bacterial eradication.<sup>4,5</sup> It causes antibiotic resistance by limitation of antibiotic diffusion through matrix,

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transmission of resistance genes, expression of efflux pumps, inactivation by change in concentration of metallic ions.<sup>6,7</sup> Extended spectrum beta lactamase constitute growing class of plasmid mediated beta lactamases. These enzymes hydrolyse oxyamino beta lactams. In gram negative pathogens beta lactamase production remains most important contributing factor for beta lactam resistance.<sup>8</sup>

Hence the current prospective analysis of UPEC was undertaken to know their antimicrobial susceptibility pattern, detection of biofilm and ESBL production and their correlation with antibiotic resistance for the period of 1 year with UTI in a tertiary care hospitals has been undertaken.

## 2. Materials and Methods

The study was carried out in the Department of Microbiology, attached to McGann hospital, Shimoga Institute of Medical Sciences, Shimoga. The study was conducted during the period from January 2016 to December 2016 from clean catch midstream urine samples of both in-patient and out-patient departments and first 100 *Escherichia coli* isolates which were isolated from samples were considered for study.

### 2.1. Isolation and Identification of *Escherichia coli*

On day 1-Urine sample was examined by wet mount preparation for the presence of pus cells, red blood cells, urinary casts, epithelial cells. These samples were processed by Standard loop technique (A semiquantitative method). A loopful (0.001 ml) of uncentrifuged urine sample was inoculated onto the surface of blood agar and MacConkey agar. Plates were then incubated at 37 degree celcius aerobically for 24hrs. On day 2 Positive urine culture was determined by significant bacteriuria. (count > 10<sup>5</sup> /ml in a carefully taken and promptly examined sample). The colonies were Lactose fermenting colonies on MacConkey media, Lack of cytochrome oxidase activity, Positive catalase test, Gram negative bacilli on gram stain, Motile bacilli, IMViC + + - - (Indole test, Methyl red test, Voges-Proskauer, Citrate test) Glucose, lactose, mannitol, maltose (but not sucrose) are fermented with production of acid and gas The organisms isolated were identified as *Escherichia coli* based on their colony morphology, Gram stain and relevant standard biochemical methods.<sup>9,10</sup>

### 2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out by Kirby Bauer Disk Diffusion method. Fosfomycin (200µg), Gentamicin (10µg), Nitrofurantoin (300µg), Cotrimoxazole (1.25/23.75µg) Norfloxacin (10µg), Aztreonam (30µg), Ceftazidime (30µg), Cefotaxim (30µg), Amikacin (30µg), Amoxiclavulanic acid (30µg), Ciprofloxacin (5µg) Levofloxacin (5 µg), Imipenem (10 µg), Piperacillin-Tazobactam (100:10 µg).<sup>11</sup>

### 2.3. Detection of extended spectrum beta lactamase production

1. Phenotypic screening test for detection of ESBL production -*E.coli* isolates were screened for resistance to 2 Cephalosporins: Ceftazidime (30µg) and Cefotaxime (30µg) by Kirby Bauer disk diffusion test. Isolates that displayed resistance to one or both of these antibacterials were considered positive for screening test 2. Phenotypic confirmatory test for detection of ESBL production -*E.coli* isolates positive on screening test was further confirmed by using both Ceftazidime (30µg)/ Ceftazidime-Clavulanic acid (30µg/10µg) and Cefotaxime (30µg)/ Cefotaxime-Clavulanic acid (30µg/10µg) disks. An increase in zone diameter by ≥5mm around the disk with Cephalosporin and Clavulanic acid versus the zone around disks with Cephalosporin alone was interpreted as POSITIVE as per CLSI guidelines. On Day 3: The final identification of *E.coli* and their antibiotic susceptibility pattern was noted and on Day 4: Confirmatory test result for ESBL production by the *E.coli* isolates was noted.<sup>11</sup>

### 2.4. Detection of biofilm formation by tube method

Biofilm production was estimated qualitatively for *E.coli* isolates by tube method as described by Christensen et al. Procedure: Glass test tube containing Brain Heart Infusion broth was inoculated with a loopful of pure culture of *E.coli* isolate and was incubated aerobically at a temperature of 35° C for a period of 2 days After incubation for 48hrs, the supernatant was discarded and the glass tube was stained by 1% safranin solution for 7 minute. The glass tube was then washed with distilled water for 3 times and dried. Result: A positive result was defined as the presence of a layer of stained material adhered to the inner walls of the tube.<sup>12,13</sup>

### 2.5. Detection of biofilm formation by Microtitre-plate method

Isolates were grown overnight in Brain Heart Infusion broth at 37° C. 200µl of this culture suspension was used to inoculate in the wells of a sterile 96-well flat bottomed polystyrene microtitre plate. Negative control well contained broth only. The plate was covered and incubated aerobically for 24 hours at 37°C. After 24hr of incubation, the content of each well was washed three times with 250 µl of sterile physiological saline. Then the plate was dried in inverted position. Plate was then stained for 5 min with 1% safranin. Excess stain was rinsed off by placing the plate under running tap water. The OD of each well was measured at 578 nm using ELISA reader. The cut-off optical density (ODc) for the microtitre-plate is defined as three standard deviations above the mean OD of the negative controls. For the purpose of comparative analysis of test results, the adherence capabilities of the test strains were classified into the following four categories: non-adherent (0), weakly (+),

moderately (++) , or strongly (+++) adherent, based upon the ODs of bacterial films. All the tests were carried three times and the results were averaged. Strains were classified as follows OD ≤ ODc -non-adherent, ODc < OD ≤ 2 x ODc- weakly adherent, 2 x ODc < OD ≤ 4 x ODc- moderately adherent, 4 x ODc < OD –strongly adherent.<sup>14,15</sup>

### 3. Result

The present study was conducted from January 2016 to December 2016 in Department of microbiology, attached to McGann hospital SIMS, Shimoga. First 100 *E.coli* which were isolated were included in the study. Out of 100 isolates 68(68%) isolates were from female patients and 32(32%) were from male patient.

Antibiotic susceptibility pattern: In our study *E.coli* was highly sensitive to nitrofurantoin (100%), fosfomycin (100%), imipenem (77%). Moderately sensitive to cotrimoxazole (61%), amikacin (47%), aztreonam (53%), piperacillin tazobactam (41%). Least sensitive to cefotaxim (25%), ceftazidime (32%), norfloxacin (20%), ampicillin-sulbactam (12%), ciprofloxacin (15%), levofloxacin (12%), amoxyclavulanicacid (15%).

Detection of ESBL production: Screening method-Out of 100 *E.coli* isolates 83(83%) were ESBL positive by screening method. Confirmatory test- A total of 83 isolates were subjected to ESBL confirmatory test by combined disc diffusion method .Among 83 isolates 80 were ESBL positive by confirmatory method.

Detection of biofilm formation.: Out of 100 isolates 63(63%) were biofilm producers by tube method and 79(79%) were biofilm producers by microtiterplate method.

Total number of ESBL producers (%)N=100 80(80%),Number of biofilm producers showing ESBL Production (%),N=70 70(100%),Number of biofilm non-producers showing ESBL production (%),N=30 13(43.3%)

All the biofilm producers were ESBL producers and 43.3% biofilm non producers were ESBL producers.

Antibiotic resistance in ESBL producers and ESBL non producers:. ESBL producers resistance (%), Piperillin Tazobactam (77.5%), gentamycin (71.25%), amikacin (58.75%), cotrimoxazole (62.5%), norfloxacin (93.75%), amoxiclavulanic acid (86.25%), imipenam (75%), levofloxacin (93.75%), nitrofurantoin ( 0%), fosfomycin (0%), ciprofloxacin (93.75%), aztreonam (50%) ESBL non producers resistance (%) Piperillin Tazobactam (50%), gentamycin (55%), amikacin (45%), cotrimoxazole (35%), norfloxacin (80%), amoxiclavulanic acid (65%), imipenam (50%), levofloxacin (80%), nitrofurantoin (0%), fosfomycin (0%), ciprofloxacin (80%), aztreonam (50%).

Antibiotic resistance in biofilm producers and biofilm non producers: Biofilm producers resistance (%): Gentamycin (73.41%), cefotaxime (87.34%), ceftazidime (87.34%), amikacin (69.6%), cotrimoxazole (72.15%), norfloxacin (88.60 %), imipenem (78.48%), nitrofurantoin

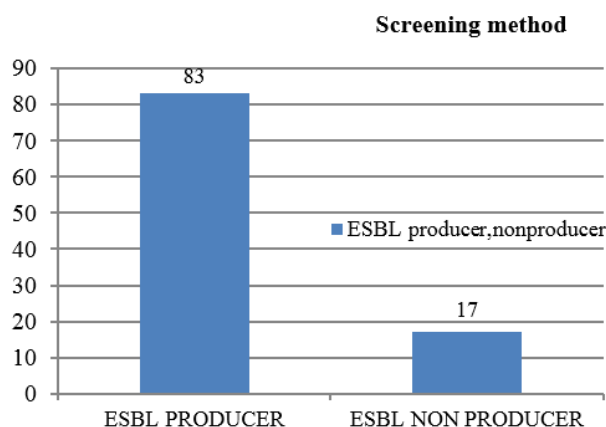
(0), fosfomycin (0), ciprofloxacin (75.94%), aztreonam (74.68% c). Biofilm non producers resistance (%): Gentamycin (52.38%), cefotaxime (76.19%), ceftazidime (76.19%), amikacin (57.14%), cotrimoxazole (66.66%), norfloxacin (85.71%), imipenem (71.42%), nitrofurantoin (0), fosfomycin (0), ciprofloxacin (57.14 %), aztreonam (66.66%).

**Table 1:** Sex distribution

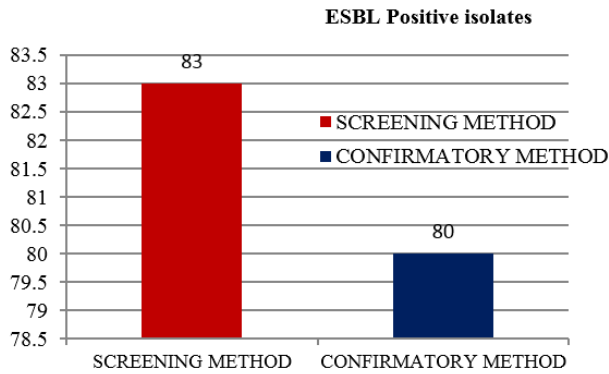
Sex	Number	Percentage
Male	32	32%
Female	68	68%
Total	100	100%

**Table 2:** Antibiotic susceptibility pattern of the UPEC isolates

Antibiotic	Sensitive (%)	Resistance (%)
Gentamicin	43(43)	57(57)
Ampicillin-Sulbactam	12(12)	88(88)
Nitrofurantoin	100(100)	0
Fosfomycin	100(100)	0
Aztreonam	53(53)	47(47)
Norfloxacin	20(20)	80(80)
Ciprofloxacin	15(15)	85(85)
Levofloxacin	11(11)	89(89)
PiperacillinTazobactam	41(41)	59(59)
Amikacin	47(47)	53(53)
Ceftazidime	32(32)	68(68)
Cefotaxim	25(25)	75(75)
Imipenem	77(77)	23(23)
Cotrimoxazole	61(61)	39(39)
Amoxiclavulanic acid	15(15)	85(85)



**Graph 1:** ESBL production by screening method and confirmatory method



Graph 2: ESBL Positive isolates

**Table 3:** Detection of biofilm formation of UPEC isolates by different methods

Test method	Biofilm producers
Tube method	63(63%)
Microtiter plate method	79(79%)

**Table 4:** ESBL production among biofilm producer and biofilm non-producer UPEC isolates

Total number of ESBL producers (%) N=100	Number of biofilm producers showing ESBL Production (%), N=70	Number of biofilm non-producers showing ESBL production (%), N=30
80(80%)	70(100%)	13(43.3%)

**Table 5:** Antibiotic resistance in ESBL producers and ESBL non producers

Antibiotics	ESBL producers resistance (%)	ESBL non producers resistance (%)
Piperacillin tazobactam	77.5	50
Gentamycin	71.25	55
Amikacin	58.75	45
Cotrimoxazole	62.5	35
Norfloxacin	93.75	80
Amoxyclavulanic acid	86.25	60
Imipenem	75	50
Levofloxacin	93.75	80
Nitrofurantoin	0	0
Fosfomycin	0	0
Ciprofloxacin	93.75	80
Aztreonam	50	50

**Table 6:** Antibiotic resistance in biofilm producers versus biofilm non producers

Antibiotics	Biofilm producers resistance (%)	Biofilm non producers resistance (%)
Ciprofloxacin	75.94	57.14
Norfloxacin	88.60	85.71
Gentamycin	73.41	52.38
Amikacin	69.6	57.14
Cotrimoxazole	72.15	66.66
Cefotaxim	87.34	76.19
Ceftazidime	87.34	76.19
Aztreonam	74.68	66.66
Imipenem	78.48	78.12
Fosfomycin	0	0
Nitrofurantoin	0	0

#### 4. Discussion

Urinary tract infection (UTI) is the most common and a serious health problem both in the community and hospital settings each year worldwide.<sup>5</sup> The ascending route infection accounts for more than 90% cases. Causative organisms are derived from fecal flora inhabiting the periurethral region. Urinary pathogens have shown a changed pattern of susceptibility to antibiotics because of biofilm formation and ESBL production resulting in an increase in resistance to commonly used antibiotics.<sup>6</sup>

In the present study out of 100 *E.coli* positive UTI cases 32(32%) were males and 68(68%) were females. According to study done by Chaterjee et al 50% isolates were from female patients and 50% isolates were from male patient, Rajani et al showed that 43.1% isolates were from male patient and 56.9% isolates were from female patient. Predominance in female is due to colonisation of urethra by colonic gram negative bacilli because of its proximity to anus, short length of urethra (about 4cm) & sexual intercourse (introduction of bacteria into bladder).<sup>16,17</sup>

Antibiotic resistance is a growing threat worldwide. Studies from various parts of India have shown occurrences of high rates of antimicrobial resistance among *E.coli*. In our study *E.coli* was highly sensitive to nitrofurantoin (100%), fosfomycin (100%), imipenem (77%). Moderately sensitive to cotrimoxazole (61%), amikacin (47%), aztreonam (53%), piperacillintazobactam (41%). Least sensitive to cefotaxim (25%), ceftazidime (32%), norfloxacin (20%), Ampicillinsulbactam (12%), ciprofloxacin (15%), amoxyclavulanic acid (15%).

According to study done by E.Sabharwal et al showed high sensitivity for nitrofurantoin (94.5%), imipenem (93.5%), fosfomycin (97.2%), cefotaxim (70.2%) moderately sensitive to gentamicin amikacin (56.7), ciprofloxacin (50.2%) least sensitive to norfloxacin (46.7%), cotrimoxazole (45.9%) and aztreonam (36.6%).<sup>18</sup>

According to study by Sultan et al 100% sensitivity was seen for fosfomycin, amikacin, imipenem and least sensitivity to norfloxacin (18%) and ceftriaxone (5.4%).<sup>18</sup> Study done by V Niranjana et al showed high sensitivity for nitrofurantoin (82%), imipenem (98.9%), amikacin (82.6%).<sup>4</sup> Study done by Mandira et al showed different results where sensitivity of nitrofurantoin (77.5%), cotrimoxazole (17.5%), gentamicin (62.5%), amikacin (72.5%) is different. Results of other studies are not in accordance with present study.<sup>19</sup>

Fosfomycin and nitrofurantoin have various advantages over other antibiotics which has led to their 100% sensitivity among UPEC isolates in our study.

Fosfomycin is broad-spectrum antibiotic and bactericidal that acts by inactivating the enzyme phosphoenolpyruvate synthetase, leading to disruption of bacterial cell-wall synthesis.<sup>18</sup> Common side effects are diarrhea, nausea, vomiting, skin rash, heartburn, vaginitis, headache, chills and asthenia. Advantageous feature of low molecular weight and long half life helps penetration of various tissues with ease, achieving the minimum inhibitory concentrations required to inhibit the growth of most pathogens.<sup>20</sup>

Nitrofurantoin used for the prophylaxis and treatment of uncomplicated cystitis. Increased emergence of antibiotic resistance has made nitrofurantoin a suitable for the treatment of UTI caused by multidrug-resistant pathogens. The mechanism of action requires enzymatic reduction within the bacterial cell and the reduced derivatives appear to be capable of binding to ribosomal proteins.<sup>21,22</sup> The fluoroquinolones susceptibility of urine pathogens, changing over the years, is influenced by factors such as the changing patient population and the extensive use and misuse of the antimicrobial agent as empirical treatment in UTI, which contribute to alterations in the microbial profile of urine isolates.<sup>23</sup>

Use of fosfomycin and nitrofurantoin as the first line therapy contributes to a reduction in overall fluoroquinolone use thereby helping to reduce selection pressure for increased resistance to fluoroquinolones.<sup>23</sup> In our study imipenem shows good sensitivity profile (77%). Carbapenems still remain as the antibiotic with highest sensitivity in ESBL *E.coli*. Doripenem is one of the newer carbapenem, which is advocated in complicated UTI. Aminoglycosides have least sensitivity according to our study. Parenteral administration and low safety profile especially among the elderly is the major drawback. This can be overcome by opting for single day dosing or by using in combination with other antimicrobial agents.<sup>21,23</sup>

In present study ESBL production among uropathogenic *E.coli* was detected in 80% of isolates. According to study done by Mandira et al 64.28% were ESBL producers.<sup>19</sup> Study done by Mahesh et al showed 56.2% were ESBL positive.<sup>24</sup> According to various other studies very few

UPEC isolates were ESBL producers and results are as follows: Rajani et al 39.66%, Shravani tadepalli et al 38%, Ponnuswamy et al 20.4% and Datta et al showed 21.4%.<sup>16,25–27</sup> These results are not in accordance with results of present study.

ESBL are mutation of various amino acid among which mutation at position 238 wherein glycine is replaced by serine, alanine or aspartate is the most common. Mechanism of action is by structural remodelling of active site of beta-lactamases leading to hydrolysis of extended spectrum cephalosporins, all penicillins and monobactams.<sup>16,27</sup>

In our study biofilm detection showed better results with microtitre plate method (79%) as compared to tube method (63%). Study done by Shravani tadepalli et al showed detection of biofilm by tube method 32% and microtitre plate method 35%.<sup>27</sup> Some studies used only single method for biofilm detection. Study done by Bajpai et al showed biofilm formation 62% by tube method, Suman et al showed 92% biofilm formation by microtitre plate method and study by Ponnuswamy et al showed 57.40% biofilm formation by microtitre plate method.<sup>27–29</sup> Results of above studies are not in accordance with present study.

Biofilm production is high in our study compared to other studies.<sup>28</sup> Biofilm are like intracellular pods that allow bacteria to outlast a strong host immune response to establish dormant reservoir of pathogens inside bladder cells. Re-emergence of bacteria from this reservoir causes recurrent infections. This also causes antibiotic resistance.<sup>27,29</sup>

The resistance pattern of the isolates to various antibiotics among ESBL producers in comparison with ESBL non producers in our study was as follows: Piperacillin Tazobactam (77.5vs50), gentamycin (71.25vs55), amikacin (58.75 vs 45), cotrimoxazole (62.5 vs 35), norfloxacin (93.75 vs 80), amoxiclavulanic acid (86.25 vs 65%), imipenem (75 vs 50), levofloxacin (93.75vs 80), nitrofurantoin (0 vs 0), fosfomycin (0 vs 0), ciprofloxacin (93.75vs 80), aztreonam (50 vs 50) and correlated well with other studies. In the present study more number of UPEC isolates producing ESBL showed resistance to norfloxacin, ciprofloxacin and levofloxacin.

Similar study done by Ponnuswamy et al showed antibiotic resistance pattern between ESBL and non ESBL producers as follows: Piperacillin tazobactam (35 vs 35), gentamycin (47 vs 42), amikacin (56 vs 40), cotrimoxazole (49 vs 46), norfloxacin (37 vs 35), amoxiclavulanic acid (86 vs 87), imipenem (8 vs 6), levofloxacin (17 vs 11), nitrofurantoin (53 vs 38).<sup>26</sup> Results are not in accordance with present study.

Another study done by Mandira et al showed antibiotic resistance pattern between ESBL and non ESBL producers as follows: gentamycin (50vs22), amikacin (22.2 vs 5.6), cotrimoxazole (94.5 vs 0), levofloxacin (27.8 vs 27.8), nalidixic acid (100vs0), ciprofloxacin (100 vs 0).<sup>19</sup> Results

are not in accordance with present study.

Study done by Bajpai et al showed antibiotic resistance pattern between ESBL and non ESBL producers as follows: Piperacillin Tazobactam (27.1 vs 41.2), gentamycin (31.3 vs 51.5), amikacin (12.5 vs 36.8), cotrimoxazole (98 vs 78), norfloxacin (83.4 vs 78), levofloxacin (68.8 vs 73.6), nitrofurantoin (0 vs 22.2), aztreonam (93.4 vs 67.71).<sup>29</sup>

All the above mentioned studies show that there is increase percentage of antibiotic resistance among ESBL producers when compared to ESBL non producers. The present study also indicate that multidrug resistant ESBL isolates are widely prevalent. Multidrug resistance (MDR) due to ESBL which is a transferable drug resistance mediated via plasmids, resistance genes to other agents like fluoroquinolones, aminoglycosides and cotrimoxazole are also transferred by conjugation.<sup>26</sup> This multidrug resistance leads to a change in the choice of empirical antimicrobial agents. Disadvantages of ESBL strains are it causes restriction of usage of beta-lactam and its usage in conditions like pregnancy where choice of antimicrobials is limited to beta-lactam antibiotics like cephalosporins ampicillin, which causes treatment failure.<sup>29</sup>

This study confirms that ESBL producing *E.coli* strains are notable cause of community onset infections especially in predisposed patients. This study proves statistically ( $p < 0.01$ ) that there is significant correlation between ESBL production and antibiotic resistance among the isolates.

The resistance pattern of the isolates to various antibiotics among biofilm producers in comparison with biofilm non producers in our study was as follows: Gentamycin (73.41% vs 52.38%), cefotaxime (87.34% vs 76.19%), ceftazidime (87.34% vs 76.19%), amikacin (69.6% vs 57.14%), cotrimoxazole (72.15% vs 66.66%), norfloxacin (88.60 % vs 85.71%), imipenem (78.48% vs 71.42%), nitrofurantoin (0 vs 0), fosfomycin (0 vs 0), ciprofloxacin (75.94 VS 57.14), aztreonam (74.68 VS 66.66).

Study done by Shravani tadepalli et al showed antibiotic resistance pattern among biofilm producers in comparison with biofilm non producers as follows: Ciprofloxacin(75% vs 45%), norfloxacin (75% vs 45%), gentamcin (52% vs 26%) amikacin (19% vs 5%), cotrimoxazole (68% vs 42%), amoxicillin (92% vs 87%), ceftazidime (76% vs 40%), cefotaxim (76% vs 35%), ceftriaxone (67% vs 28%).<sup>27</sup>

Study done by Bajpai et al showed antibiotic resistance pattern among biofilm producers in comparison with biofilm non producers as follows: Ciprofloxacin(81.8% vs 87.5%), Norfloxacin (81.8% vs 87.5%), gentamcin (9% vs 37.5%) amikacin (9% vs 12.5%), cotrimoxazole (63.6% vs 87.5%), ceftazidime (81.8% vs 87.5%), cefotaxim (72.7% vs 87.5%).<sup>29</sup> In the present study antibiotic resistance was seen more among biofilm producing isolates when compared to biofilm non producing isolates. Multidrug resistance pattern of the biofilm producing isolates was seen in present study.

Biofilm is considered pathogenic determinant which allows strains to persist for long time in urinary tract and interfere with bacterial eradication which causes recurrent infections.<sup>30</sup> Antibiotic resistance is mainly by limitation of antibiotic diffusion through matrix, transmission of resistance genes, inactivation by change in concentration of metallic ions by the biofilm formation.<sup>27,31</sup> This study proves statistically ( $p < 0.01$ ) that there is significant correlation between biofilm production and antibiotic resistance among the UPEC isolates.

## 5. Conclusion

Our study has demonstrated significant production of biofilm and extended spectrum betalactamase (ESBL) among the UPEC isolates from UTI patients of tertiary care hospital, SIMS, Shimoga. It also showed significant correlation between biofilm formation, ESBL production and antibiotic resistance. The present study established Biofilm producing UPEC and ESBL producing UPEC has led to the emergence of antibiotic resistance. Multidrug resistant strains leads to treatment failure, delayed clinical response, high morbidity and mortality.

Most of the UPEC which were isolated in the study were found to be resistant to routinely used antibiotics. Data from our study shows fosfomycin and nitrofurantoin as an important treatment option for uncomplicated UTIs in the current era of increasing fluoroquinolone resistance among uropathogens. All the biofilm producers and ESBL producers which were isolated were multidrug resistant. Therefore, this is an important issue which has to be addressed by the policy makers, to formulate a strict antibiotic prescription policy.

Because the patterns of sensitivity of the microorganisms to the antibiotics vary over time and among different geographical areas, the empiric antibacterial therapy of the infections should be based on a local experience of the susceptibility and the resistance profile.

## 6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

## 7. Source of Funding

None.

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