

DOI: <http://dx.doi.org/10.18203/2320-1770.ijrcog20184961>

Original Research Article

Prevalence of multidrug resistance (MDR) and extended spectrum beta-lactamases (ESBLs) among uropathogenic *Escherichia coli* isolates from female patients in a tertiary care hospital in North India

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Received: 01 October 2018

Accepted: 27 October 2018

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ABSTRACT

Background: Urinary tract infection (UTI) is a common bacterial infection in women. It is very common during pregnancy and may present as asymptomatic bacteriuria. *Escherichia coli* is the primary urinary pathogen accounting for 74-90% of uncomplicated urinary tract infection. The aim of the study was to evaluate in-vitro antibiotic susceptibility among urine isolates of *Escherichia coli* and prevalence of resistance marker like ESBL producer and MDR status.

Methods: Mid-stream urine samples of 205 female patients were processed for culture and sensitivity. All the samples were inoculated on culture media and growth showing significant bacteriuria ($>10^5$ cfu/ml) were subjected to identification and antibiotic sensitivity testing on Automated system BD Phoenix 100. ESBL status was detected by the system and MDR status was evaluated by standard guidelines.

Results: Out of 205 urine samples, 47 samples (22.93%) showed growth of organism. Out of the 47 growth positive samples, 36 (76.60%) samples showed growth of *Escherichia coli* and out of which 13 (36.11%) were ESBL producer and 23 (63.89%) were ESBL non-producer. *Escherichia coli* was mostly isolated from younger age group (21-40 years) (61.11%). Antimicrobial susceptibility showed very good sensitivity towards Amikacin, Tigecycline, Carbapenems, Fosfomycin, Piperacillin-tazobactam and Nitrofurantoin. All the isolates of ESBL producer are MDR (100%) and 21.7% of ESBL non-producer are MDR. There is evidence of transfer of resistance genes for non-β-lactam antibiotics along with ESBL resistance marker.

Conclusions: *Escherichia coli* was the predominant uropathogen isolated from female patients and Amikacin, Nitrofurantoin and Fosfomycin can be used as first line drug.

Keywords: Asymptomatic bacteriuria, Extended spectrum beta-lactamase, Multidrug resistance, Urinary tract infection, Uropathogen

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infection in women and responsible for significant morbidity and health care costs. It is a common problem in pregnancy due to morphological and physiological changes that takes place in genito-urinary

tract during pregnancy. Asymptomatic bacteriuria (ASB) is the presence of actively multiplying bacteriuria of more than 10^5 /ml of urine without any symptom. *Escherichia coli* is the primary urinary tract pathogen accounting for 74 to 90% of uncomplicated urinary tract infection. Other bacteria include *Staphylococcus saprophyticus*, *Klebsiella spp*, *Proteus spp*, *Enterococcus spp* and

*Enterobacter spp.*¹⁻³ About 50% of all females experience at least one episode of UTI during their life time. Asymptomatic bacteriuria and urinary tract infections (UTI) have a higher prevalence during pregnancy and in very sexually active females. UTI is an infection that affects any part of the urinary tract from bladder to the kidney. Symptoms include frequent and/or painful urination, urgency, fever and flank pain. The urine may contain pus or blood. UTI is a risk factor for pyelonephritis, preterm delivery and miscarriage among pregnant women.^{4,5}

Extended spectrum β -lactamases (ESBLs) are defined as β -lactamases capable of hydrolyzing oxyimino-cephalosporins and are inhibited by β -lactamase inhibitors. The primary organism causing urinary tract infection (UTI) like *Escherichia coli* produce ESBLs. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infections difficult to treat. Inappropriate and wide spread use of antibiotics has lead to emergence of resistance markers like production of Extended spectrum β -lactamases (ESBLs) and concomitant multidrug resistance (MDR).^{6,7}

The aim of this study was to determine in-vitro antibiotic susceptibility among urine isolates of *Escherichia coli* and prevalence of resistance markers like ESBL production and MDR status in female patients to guide the clinicians in patient care.

METHODS

This is a retrospective study conducted in Department of Microbiology, Sarvodaya Hospital and Research Centre, Sector-8, Faridabad, Haryana, India. Urine samples of female patients received from Obstetrics and Gynecology dept for bacteriological culture and sensitivity testing from September'17 to February'18 were analyzed.

A total of 205 mid-stream urine samples collected in wide mouthed sterile container for aerobic culture and sensitivity were analyzed. Samples were processed within one hour of collection. For routine examination of urine (wet film preparation), 50 μ l of well mixed uncentrifuged urine was taken on a slide with coverslip placed on it and viewed under high power objective of microscope. The presence of one pus cell per 7 high power field was considered as significant pyuria. For urine culture, 1 μ l of urine sample was inoculated on HiCrome UTI agar media and MacConkey agar media (HiMedia, Mumbai, India) using a standard loop of internal diameter 1.34mm (semi-quantitative method). The plates were read after 24 hours of aerobic incubation at 37 $^{\circ}$ C. They were further incubated for another 24 hours before reported as negative.⁸ A single organism of colony count > 1,00,000 cfu/ml was subjected to identification and antibiotic sensitivity testing by Automated system BD Phoenix 100 (Becton Dickinson). The Extended Spectrum β -Lactamase production is also detected by this automated system. Multidrug resistance (MDR) is defined as

antibiotic resistance to one or more agent in each of three or more groups of antibiotics.⁷ MDR status was evaluated by calculating the resistance to each drug group. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 used as quality control.

RESULTS

A total of 205 urine samples received in Microbiology Lab from Obstetrics and Gynecology dept. for aerobic culture and sensitivity, out of which 47 (22.93%) samples yielded positive growth, whereas 158 (77.07%) samples yielded no growth (Figure 1).

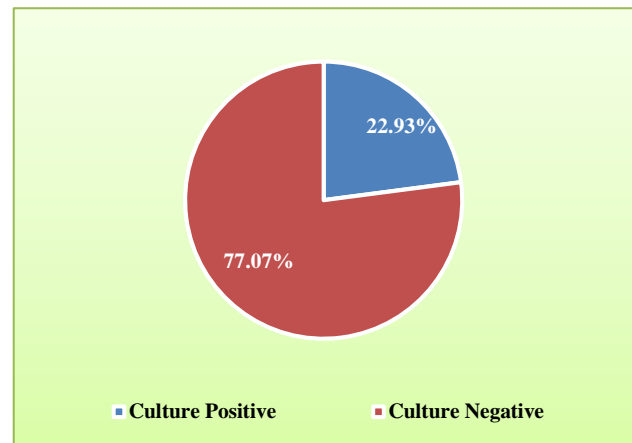


Figure 1: Culture positivity of urine samples in female patients.

Among the 47 positive isolates the predominant gram-negative bacteria was *Escherichia coli* (76.60%) out of which 36.11% was ESBL producer and 63.89% was ESBL non-producer. This was followed by *Klebsiella pneumoniae* (4.25%), *Pseudomonas aeruginosa* and *Proteus mirabilis* (2.13% each). The predominant gram-positive organism was *Enterococcus faecalis* (6.38%) followed by *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* (2.13% each). The percentage of *Candida spp.* was 4.25% (Table 1).

Table 1: Distribution of organism isolates obtained from urine samples.

| Organism | Number (%) (n=47) |
|-------------------------------------|-------------------|
| <i>Escherichia coli</i> | 36 (76.60%) |
| ESBL producer <i>E. coli</i> | 13 (36.11%) |
| ESBL non-producer <i>E. coli</i> | 23 (63.89%) |
| <i>Klebsiella pneumoniae</i> | 2 (4.25%) |
| <i>Pseudomonas aeruginosa</i> | 1 (2.13%) |
| <i>Proteus mirabilis</i> | 1 (2.13%) |
| <i>Enterococcus faecalis</i> | 3 (6.38%) |
| <i>Staphylococcus saprophyticus</i> | 1 (2.13%) |
| <i>Staphylococcus haemolyticus</i> | 1 (2.13%) |
| <i>Candida spp.</i> | 2 (4.25%) |

The age group wise distribution of *Escherichia coli* isolates showed that highest percentage of isolates were from 21-40 years of age group (61.11%) followed by 41-60 years (22.22%), whereas ESBL producer *Escherichia coli* was highest from 41-60 years of age group (38.46%) followed by 21-40 years (30.77%) (Table 2).

Table 2: Age group wise distribution of *Escherichia coli* isolates along with ESBL status.

| Age group distribution | Total <i>E. coli</i> isolates No (%) (n=36) | ESBL producer <i>E. coli</i> No (%) (n=13) |
|------------------------|---|--|
| 0 - 20 years | 2 (5.56%) | 1 (7.69%) |
| 21 - 40 years | 22 (61.11%) | 4 (30.77%) |
| 41 - 60 years | 8 (22.22%) | 5 (38.46%) |
| 61 - 80 years | 4 (11.11%) | 3 (23.08%) |

The overall antimicrobial susceptibility pattern of *Escherichia coli* showed that the sensitivity was highest in Amikacin and Tigecycline (100%) followed by

Meropenem and Fosfomycin (97.2% each), Imipenem (94.4%), Ertapenem (91.6%), Piperacillin-tazobactam (88.9%), Nitrofurantoin (86.1%), Gentamicin (80.5%), Tobramycin (72.2%), Norfloxacin (58.3%), Ciprofloxacin and Cefepime (52.8% each), Cefazolin, Ceftazidime, Ceftriaxone, Aztreonam and Trimethoprim-sulfamethoxazole (50% each), Amoxicillin-clavulanic acid (47.2%) and lastly Ampicillin (27.8%). The distribution of antimicrobial susceptibility pattern in ESBL producer and ESBL non-producer shows that most of the antibiotic gain resistance along with ESBL resistance marker except Amikacin and Tigecycline where the sensitivity remains the same (100%). In case of carbapenems (Meropenem, Imipenem and Ertapenem) and Piperacillin-tazobactam, the sensitivity increases in ESBL producers. The antimicrobial susceptibility pattern in case of most of non- β -lactam antibiotics like Norfloxacin, Trimethoprim-sulfamethoxazole, Gentamicin, Fosfomycin, Nitrofurantoin shows that the sensitivity decreases in ESBL producers (Table 3).

Table 3: The antimicrobial susceptibility pattern of *Escherichia coli*.

| Antibiotics | Total sensitivity No. (%) (n=36) | Sensitivity in ESBL non-producer No. (%) (n=13) | Sensitivity in ESBL non-producer No. (%) (n=23) |
|-------------------------------|----------------------------------|---|---|
| Amikacin | 36 (100%) | 13 (100%) | 23 (100%) |
| Tigecycline | 36 (100%) | 13 (100%) | 23 (100%) |
| Meropenem | 35 (97.2%) | 13 (100%) | 22 (95.6%) |
| Fosfomycin | 35 (97.2%) | 12 (92.3%) | 23 (100%) |
| Imipenem | 34 (94.4%) | 13 (100%) | 21 (91.3%) |
| Ertapenem | 33 (91.6%) | 12 (92.3%) | 21 (91.3%) |
| Piperacillin-tazobactam | 32 (88.9%) | 12 (92.3%) | 20 (86.9%) |
| Nitrofurantoin | 31 (86.1%) | 11 (84.6%) | 20 (86.9%) |
| Gentamicin | 29 (80.5%) | 8 (61.5%) | 21 (91.3%) |
| Tobramycin | 26 (72.2%) | 5 (38.4%) | 21 (91.3%) |
| Norfloxacin | 21 (58.3%) | 3 (23.1%) | 18 (78.2%) |
| Ciprofloxacin | 19 (52.8%) | 2 (15.3%) | 17 (73.9%) |
| Cefepime | 19 (52.8%) | 0 (0%) | 19 (82.6%) |
| Cefazolin | 18 (50%) | 0 (0%) | 18 (78.2%) |
| Ceftazidime | 18 (50%) | 0 (0%) | 18 (78.2%) |
| Ceftriaxone | 18 (50%) | 0 (0%) | 18 (78.2%) |
| Aztreonam | 18 (50%) | 0 (0%) | 18 (78.2%) |
| Trimethoprim-sulfamethoxazole | 18 (50%) | 2 (15.3%) | 16 (69.5%) |
| Amoxicillin-clavulanic acid | 17 (47.2%) | 0 (0%) | 17 (73.9%) |
| Ampicillin | 10 (27.8%) | 0 (0%) | 10 (43.4%) |

To evaluate Multidrug Resistance (MDR), sensitivity to each drug group was evaluated and resistance to one or more antibiotic in a group was considered as resistance to the group. It was observed that resistance was highest in case of Aminopenicillin group (72.2%) followed by Cephalosporins, Folate Pathway Inhibitor and Monobactam (50% each), Beta-lactam-beta-lactam Inhibitor (47.2%), Fluoroquinilones (44.4%) and Aminoglycosides (27.8%). In ESBL producers highest

resistance was showed by Aminopenicillin, Cephalosporins, Monobactam and Beta-lactam-beta-lactam Inhibitor group (100% each), followed by Folate Pathway Inhibitor (84.6%), Fluoroquinilones (76.9%) and Aminoglycosides (61.5%). In case of ESBL non-producers highest resistance was observed in Aminopenicillin group (56.5%) followed by Folate Pathway Inhibitor (30.4%) and Fluoroquinilones (26.0%) (Table 4).

Table 4: Percentage of resistance of *Escherichia coli* against various groups of antibiotics.

| Groups of antibiotics | Class of antibiotic | Antibiotic used | Total resistant isolates (n=36) No. (%) | Resistant isolates in ESBL producer (n=13) No. (%) | Resistant isolates in ESBL non- producer (n=23) No. (%) |
|-----------------------|---|---|---|--|---|
| Group 1 | Aminoglycoside | Amikacin, Gentamicin Tobramycin | 10 (27.8%) | 8 (61.5%) | 2 (8.7%) |
| Group 2 | Carbapenem | Imipenem Meropenem Ertapenem | 3 (8.3%) | 1 (7.7%) | 2 (8.7%) |
| Group 3 | Fluoroquinolone | Ciprofloxacin Norfloxacin | 16 (44.4%) | 10 (76.9%) | 6 (26.0%) |
| Group 4 | Cephalosporin- - 1 st Generation | Cefazolin | 18 (50%) | 13 (100%) | 5 (21.7%) |
| | 3 rd Generation | Ceftriaxone Cefotaxime | | | |
| | 4 th Generation | Cefepime | | | |
| Group 5 | Folate pathway inhibitor | Trimethoprim-sulfamethoxazole | 18 (50%) | 11 (84.6%) | 7 (30.4%) |
| Group 6 | Furadantin | Nitrofurantoin | 2 (5.6%) | 1 (7.7%) | 1 (4.3%) |
| Group 7 | Beta-lactam-beta-lactam Inhibitor | Amoxicillin-clavulanic acid Piperacillin-tazobactam | 17 (47.2%) | 13 (100%) | 4 (17.4%) |
| Group 8 | Monobactam | Aztreonam | 18 (50%) | 13 (100%) | 5 (21.7%) |
| Group 9 | Aminopenicillin | Ampicillin | 26 (72.2%) | 13 (100%) | 13 (56.5%) |
| Group 10 | Phosphonic acid derivative | Fosfomycin | 1 (2.8%) | 1 (7.7%) | 0 (0%) |
| Group 11 | Tetracycline | Tigecycline | 0 (0%) | 0 (0%) | 0 (0%) |

Table 5: Percentage of multidrug resistant (MDR) and all drug group sensitive isolates of *Escherichia coli*.

| MDR (Multi-drug Resistant) Antibiotics | Total resistant isolates (n=36) No. (%) | Resistant isolates in ESBL producer (n=13) No. (%) | Resistant isolates in ESBL non- producer (n=23) No. (%) |
|--|--|---|--|
| Resistance to 3 drug groups | 0 (0%) | 0 (0%) | 0 (0%) |
| Resistance to 4 drug groups | 0 (0%) | 0 (0%) | 0 (0%) |
| Resistance to 5 drug groups | 7 (19.4%) | 4 (30.8%) | 3 (13.0%) |
| Resistance to 6 drug groups | 2 (5.6%) | 1 (7.7%) | 1 (4.3%) |
| Resistance to 7 drug groups | 7 (19.4%) | 7 (53.8%) | 0 (0%) |
| Resistance to 8 drug groups | 1 (2.8%) | 0 (0%) | 1 (4.3%) |
| Resistance to ≥ 9 drug groups | 1 (2.8%) | 1 (7.7%) | 0 (0%) |
| Total MDR | 18 (50%) | 13 (100%) | 5 (21.7%) |
| All group sensitive antibiotics | Total Sensitive isolates (n=36) No. (%) | Sensitive isolates in ESBL producer (n=13) No. (%) | Sensitive isolates in ESBL non- producer (n=23) No. (%) |
| Sensitive to all 11 drug groups | 8 (22.2%) | 0 (0%) | 8 (34.8%) |

Total number of multidrug resistant (MDR) *Escherichia coli* isolates were 18 (50%). All the isolates of ESBL producers are MDR (100%) and 21.7% of ESBL non-producers are MDR. All the MDR isolates were resistant to 5 or more groups of drugs. The highest percentage of MDR are resistant to 5 and 7 drug groups (19.4% each).

Whereas ESBL isolates also showed high resistance to these two drug groups (30.8% and 53.8% respectively).

Resistance to 8 and ≥ 9 drug groups are less (2.8% each). But there were 8 isolates (22.2%) which were sensitive to all the 11 drug groups and all of them were ESBL non-

producers, which again constitutes 34.8% of all the ESBL non-producers (Table 5).

DISCUSSION

Escherichia coli, an Enterobacteriaceae, is a normal intestinal flora and is the most commonly reported cause of UTI, being a common fecal contaminant. Due to short length of urethra in females and closeness of female anus and vagina to the urethra, the organism gets inoculated into the urethra during process of anal cleaning after defecation or during sexual intercourse.

Washing of genitals from back to front is more likely to lead to spread of anal or vaginal flora into urethra. Health education on the direction of washing and advice to micturate shortly after sexual activity can reduce the chances of getting UTI. If asymptomatic bacteriuria (ASB) is left undiagnosed, there is risk of developing acute pyelonephritis in pregnant women which is associated with preterm labour. Early diagnosis and treatment of ASB can reduce the incidence of pyelonephritis and prevent preterm labour.^{3,4}

The present study revealed *Escherichia coli* as the predominant isolate from urine samples of female patients, which well correlates with many studies.^{3-5,8-12} The age group wise distribution showed that *Escherichia coli* isolates were mostly prevalent in adult age group (21-40 years), which also correlates well with the studies conducted by Patnaik M et al, Oli A N et al and Arora G et al.^{2,4,13}

The antibiotic sensitivity pattern of *Escherichia coli* in this study showed very good sensitivity towards Amikacin, Tigecycline, Meropenem, Fosfomycin, Imipenem, Ertapenem, Piperacillin-tazobactam, Nitrofurantoin, Gentamicin and Tobramycin, whereas showed moderate sensitivity towards Norfloxacin, Ciprofloxacin, Cefepime, Cefazolin, Ceftazidime, Ceftriaxone, Aztreonam and Trimethoprim-sulfamethoxazole and showed low sensitivity towards Amoxicillin-clavulanic acid and Ampicillin.

Similar pattern of antibiotic sensitivity was also shown by Elzayat MAA et al, Shaifali I et al, Gupta K et al and Kumari A et al.^{3,8,9,14} In case of ESBL producers, the overall sensitivity decreases except Amikacin, Tigecycline, Carbapenems and Piperacillin-tazobactam.

In case of non- β -lactam antibiotics the sensitivity decreases in ESBL producer strains which may be due to transfer of resistance genes for these non- β -lactam antibiotics along with ESBL resistance marker. Such type of coexistence of ESBL producer and other non- β -lactam antibiotic resistance marker was reported by Babypadmini S et al, as ESBLs are encoded by plasmids, which carry resistance genes for other antibiotics also.⁶ The evaluation of multidrug resistance pattern showed that 50% of all the isolates were MDR and most of the

isolates were resistant to more than 5 drug groups while only 22.2% of isolates were sensitive to all the drug groups. All the ESBL producer isolates were MDR. Similar pattern of multidrug resistance was also showed by Mukherjee M et al.¹⁵ The correlation of ESBL producer and MDR in this study closely resembles the study conducted by Rajjini CY et al.⁷

The first line of antibiotic for treatment of uncomplicated urinary tract infection in females is Trimethoprim-sulfamethoxazole while in second line it is fluoroquinolones.¹⁶ There are reports of increasing resistance of Ciprofloxacin and Trimethoprim-sulfamethoxazole over the years due to overuse of these antibiotics and treatment failure with Trimethoprim-sulfamethoxazole in areas with high prevalence of resistance with this antibiotic has been reported.^{17,18} Study conducted by Gobernado M et al found Fosfomycin as the best drug against *Escherichia coli* in uncomplicated cystitis.¹⁹

Infectious Disease Society of America (IDSA) (2010 update) recommends Nitrofurantoin as the first choice followed by Trimethoprim-sulfamethoxazole, Fosfomycin etc.²⁰ Nitrofurantoin has shown very low level of resistance but may cause birth defects and should be cautiously used.²¹ Patients infected with ESBL producer *Escherichia coli* strains, cannot be treated with β -lactam antibiotics and monobactams.⁶

As in our study high sensitivity was shown by Amikacin, Nitrofurantoin and Fosfomycin, these antibiotics can be used as alternatives for empirical treatment of such patients at low cost, as Nitrofurantoin and Fosfomycin are available in oral form, provided Nitrofurantoin is cautiously used in pregnant women. Also measures to be taken to preserve higher end antibiotics like carbapenems and Piperacillin-tazobactam and should be used only in non-responsive cases.

CONCLUSION

In this study *Escherichia coli* was the predominant uropathogen isolated from female patients and mostly resistant to commonly prescribed drugs and clinicians are left with very few alternative drugs.

The antimicrobial resistance pattern showed that fluoroquinolones or cephalosporins may not be appropriate first line agents in empirical treatment of urinary tract infections. Instead Amikacin, Nitrofurantoin and Fosfomycin can be used as first line drug, out of which Amikacin is available in injectable form and Nitrofurantoin and Fosfomycin available as oral antibiotic.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Das B, Mittal N, Goswami R, Adhana D, Rathore N. Prevalence of multidrug resistance (MDR) and extended spectrum beta-lactamases (ESBLs) among uropathogenic *Escherichia coli* isolates from female patients in a tertiary care hospital in North India. *Int J Reprod Contracept Obstet Gynecol* 2018;7:5031-6.