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Original Research Article

Antibiotic susceptibility profile and extended spectrum β-lactamases production by uropathogenic *Escherichia coli* from tertiary care hospital of rural settings

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ABSTRACT

Background: *Escherichia coli* are the most common cause of urinary tract infections in community as well as hospital settings. Emergence of drug resistance in *Escherichia coli* due to various mechanisms makes the treatment options very limited. This study was undertaken to detect ESBLs in uropathogenic *Escherichia coli* isolates and to determine their antimicrobial susceptibility pattern in rural setting.
Methods: A prospective study was done on 502 *E. coli* isolates from clinically suspected cases of urinary tract infections (UTI) patients of all age groups. All samples were inoculated on Cysteine Lactose Electrolyte Deficient Agar (CLED). Organisms grown in pure culture were identified by standard biochemical tests. Antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar. ESBL detection was done as per CLSI guidelines.
Results: Of the 502 isolates of *Escherichia coli*, nitrofurantoin (82%) was found be most sensitive antimicrobial followed by amikacin (73%), gentamycin (71%) and imipenem (64%). Common empirically used antibiotics like fluroquinolones and Cotrimoxazole drugs showed alarming rate of resistance. 60% isolates were found to be multidrug resistant. ESBL production was detected in 31% isolates. ESBL producing strains were found to be more drug resistant than non ESBL producing strains.
Conclusions: So, drug resistance due to production of ESBLs in *Escherichia coli* is a serious threat for clinicians. Strict infection control measures and early detection of beta lactamase producing isolates are the need of the hour to contain the emergence of this type of resistance.

Keywords: Antibiotic resistance, ESBL production, *Escherichia coli*, Urinary tract infection

INTRODUCTION

The urinary tract infections (UTIs) are one of the most common infectious diseases encountered in hospital as well as community settings. It is the third most common infection found in India which affects people of all age groups.[1]

At some point of their lives, 10-20% women and 12% men experience an acute episode of symptomatic UTI.[2] UTI is much more common in women than in men, due to anatomic and physiological reasons.[3] It is a serious illness in humans due to recurrence and difficulty in eradication due to emergence of drug resistance.[4]

Urinary tract infection (UTI) is caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus.[3] *Escherichia coli* is the most common Gram negative bacteria causing both community acquired and hospital acquired UTI followed by *Klebsiella spp. Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter spp*. Among Gram positive bacteria, *Enterococcus* sp. is the most common organism responsible for UTI followed by *Staphylococcus aureus* and coagulase negative staphylococci especially *Staphylococcus saprophyticus* in sexually active females.[5,6] *Escherichia coli* are solely responsible for more than 80% of the infections.[3]

Emergence of antibiotic resistance in uropathogens is increasing worldwide in both outpatients as well as hospitalized patients. It varies according to geographical locales and it is directly proportional to the injudicious use of antibiotics. Emergence of antimicrobial resistance is of critical importance as the changing rate of antibiotic resistance has a large impact on the empirical therapy of urinary tract infections.[7] Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory.[8]

The various mechanisms responsible for drug resistance in Gram-negative bacilli include extended spectrum beta lactamase (ESBL) production; AmpC lactamase production, efflux mechanisms and porin deficiency.[9]Production of beta-lactamases confer multidrug resistance, making infections difficult to treat.[10] Clinicians often face problems in choosing appropriate antibiotic therapy for treating UTIs caused by multi-drug-resistant (MDR) bacteria.[11]

ESBLs are plasmid mediated β-lactamases that confer resistance to broad spectrum β-lactum antibiotics including third and fourth generation cephalosporins, azetronam, and extended spectrum penicillin. These plasmids often encode mutations which confer resistance to other broad-spectrum agents including aminoglycosides, co-trimoxazole and fluoroquinolones, resulting in organism resistant to most broad spectrum antibiotics.[12]

The detection of ESBLs is a serious challenge for routine clinical microbiology laboratories in resource limited settings which clearly leads to underreporting as well as missing of ESBL isolates resulting therapeutically failure that leads to prolonged hospital stay, increased morbidity, increased motility and high health care costs.[13,14]

As urinary tract infections if not treated timely can contribute significant amount of morbidity and mortality. So, information on prevalent levels of antimicrobial resistance of a particular locale among common pathogens is useful in making an appropriate choice of empiric therapy.[15]

This study would help clinicians to be aware of the potential of treatment failures associated with ESBL production by the organism and will also guide for appropriate empirical antimicrobial therapy as well. So that is why accurate identification of pathogens in short turnaround time and generation of authenticated results is the primary responsibility of a clinical microbiology laboratory. Keeping in view the above facts in mind, we conducted this study to determine the antimicrobial susceptibility pattern and production of ESBL strains among *Escherichia coli* uropathogens at study centre.

METHODS

A prospective study was done on all the *E. coli* isolates from clinically suspected cases of urinary tract infections (UTI) patients of all age groups attending OPD and IPD of tertiary care hospital of rural setting in North India. The study was of one year duration from March 2015 to February 2016. Permission was taken from Institutional Ethical committee. Mid-stream specimen of urine (MSU) was collected in sterile universal container.

Samples were transported to the laboratory without delay. Microscopical examination of urine was done by wet film preparation to detect the indicators of urinary tract infections. All samples were inoculated on Cysteine Lactose Electrolyte Deficient Agar (CLED) and incubated at 37 ^{0}C for 18-24 hours, aerobically. Figure 1 showing growth of *Escherichia coli* on CLED agar.

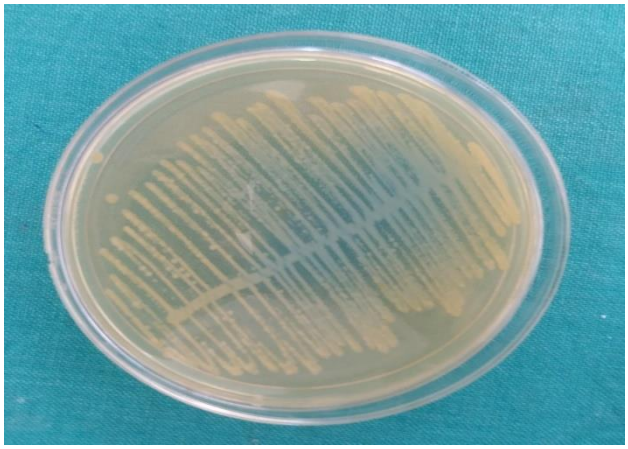

Figure 1: Growth of *E. coli* on CLED agar.

Organisms grown in pure culture were identified by standard biochemical tests.[16] Figure 2 Gram staining from culture of *Escherichia coli* depicting Gram negative bacilli.

Antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar. Antimicrobial agents tested were ampicillin, amoxy-clavulanic acid, gentamicin, amikacin, norfloxacin, ofloxacin, nitrofurantoin, cotrimoxazole, piperacillin-tazobactam, cefixime, cefuroxime, ceftriaxone,

ceftazidime, cefotaxime, imipenem recommended by the CLSI using *E. coli* ATCC 25922 as a standard strain for routine quality control.[17,18]

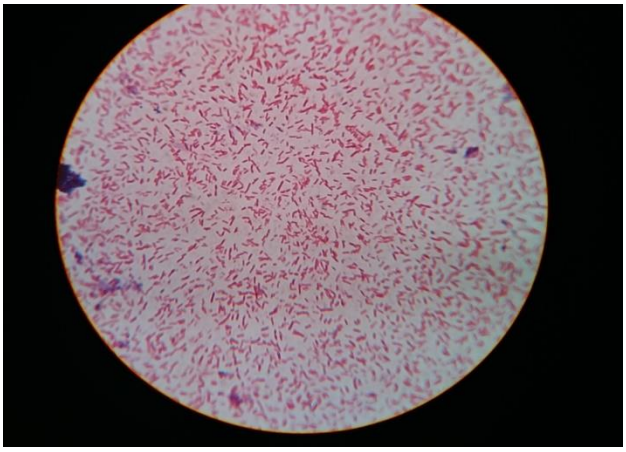

Figure 2: Gram negative bacilli of *E. coli* isolates.

ESBL detection

Screening and a phenotypic confirmatory test was carried out to assess the detection of ESBL, recommended by the CLSI.

Initial screening test

Initial screening tests recommended by the CLSI was done by Disc diffusion method by putting disc of ceftazidime, ceftriaxone and cefotaxime on Muller-Hinton agar for antibiotic sensitivity testing. Zone sizes of above antibiotics measured as per CLSI guidelines.

- Ceftazidime zone ≤22 mm
- Cefotaxime zone ≤27 mm
- Ceftriaxone zone ≤ 25 mm

Zones above may indicate ESBL production so confirmed by following test.

Phenotypic confirmatory test

A phenotypic confirmatory test recommended by the CLSI was done by combined disc diffusion method. ESBLs was detected by the confirmatory method of CLSI using ceftazidime (30mg) and a disc of ceftazidime plus clavulanic acid (30/10mg) were placed at a distance of 20 mm on a lawn culture (0.5 McFarland inoculum size) of suspected ESBL producing clinical isolate on Mueller-Hinton Agar (MHA, Hi-Media, Mumbai). *Escherichia coli* ATCC 25922 were used as the negative control and *Klebsiella pneumoniae* ATCC 700603 were used as the ESBL positive control.

ESBL production was inferred if the inhibition zone increased by 5mm towards the ceftazidime plus clavulanic acid disc in comparison to ceftazidime disc alone.[17] Figure 3 showing ESBL production by *Escherichia coli* isolate.

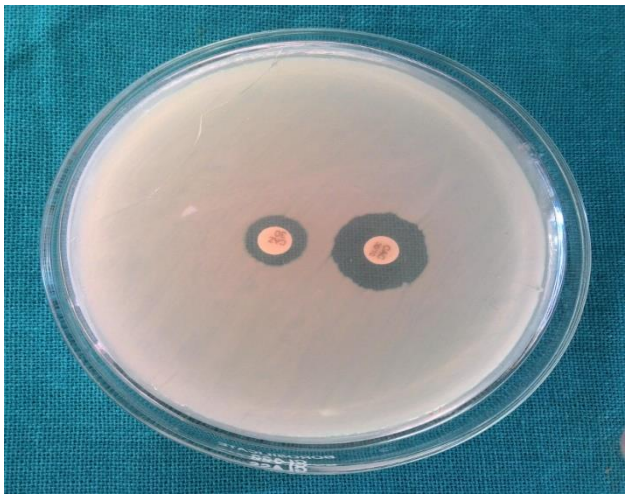

Figure 3: Extended spectrum β-lactamase production by *Escherichia coli* isolate.

RESULTS

A total of 3625 urine samples were received for culture and sensitivity during the study period. Among these, 798 samples (22.01 %%) yielded significant bacteriuria; 2696 samples (74.3%) showed no growth and 131 samples (3.6%) showed mixed growth. The various organisms isolated from urine culture are shown in Table 1.

Table 1: Rate of uropathogens isolated from urine samples.

Name of the organism	Number and percentage
Escherichia coli	502 (62.9)
Klebsiella species	132 (26.29)
Citrobacter species	16 (2)
Pseudomonas species	12 (1.50)
Proteus species	4 (0.5)
Enterococcus faecalis	92 (11.52)
Staphylococcus aureus	25 (3.13)
Staphylococcus saprophyticus	4 (0.5)
Candida species	11 (1.38)
Total	798

E. coli was the commonest organism accounting for 62.90 per cent of the uropathogens. A total of 502 *Escherichia coli* isolates were isolated in significant numbers from patients with symptomatic UTIs. These isolates were subjected to antibiotic susceptibility testing, ESBL screening and phenotype confirmation tests.

Of the 502 *Escherichia coli* isolates, 186 were from male and 316 were from female patients. Maximum number (56%) of isolates were isolated from the age group of 15-45 years followed by (18%) each in 40-60 years and more

than 60 years of age group. 71% of patients were from rural areas whereas rest 29% belongs to urban. Higher percentages of UTI are found among the patients attending OPD (67%) as compare to IPD (33%). The antibiotic susceptibility profile of *E. coli* showed nitrofurantoin (82%) the most sensitive antimicrobial followed by amikacin (73%), gentamycin (71%) and imipenem (64%).

Among fluroquinolones, norfloxacin showed sensitivity to only 41 % isolates while ofloxacin showed only 11% susceptibility. Sensitivity to commonly used empirical drug i.e. cotrimoxazole was merely 12%. Even imipenem showed only 64% susceptibility. Table 2 showing antibiotic susceptibility pattern of *Escherichia coli*.

Table 2: Antibiotic susceptibility profile of *E. coli* isolates.

Name of antibiotics	Percentage of susceptible isolates
Ampicillin	8%
Gentamicin	71%
Amikacin	73%
Norfloxacin	41%
Ofloxacin	11%
Sulphafurazole	18%
Cotrimoxazole	12%
Nitrofurantoin	82%
Cefepime	19%
Cefixime	21%
Cefuroxime	17%
Ceftriaxone	51%
Ceftazidime	23%
Imipenem	64%
Amoxyclav	7%
Piperacillin tazobactam	35%

Of the 502 isolates, 301 (60%) isolates were found to be multidrug resistant. Of these 301 isolates, 175 (52.08%) were from OPD and 126 (76%) were from IPD. The most common risk factor associated with MDR in our study was renal pathologies like chronic renal failure, renal calculi, Diabetes mellitus of long duration and catheterization in IPD patients.

ESBL production was found in 31% of isolates. Most of ESBL producing isolates were found from IPD (58.07%) while 41.93% % were from OPD. The susceptibility pattern of *Escherichia coli* isolates-ESBL producers and ESBL non-producers to various antibiotics is being shown in the Figure 4. The most effective drug found in ESBL producers was ceftazidime clavulanic acid (100%) followed by nitrofurantoin (93.54%). Among ESBL non-producers, most susceptible antibiotic was nitrofurantoin (76.81%) followed by imipenem (71.01%). Also, multidrug resistance was common to ESBL producing strains than non ESBL producing strains.

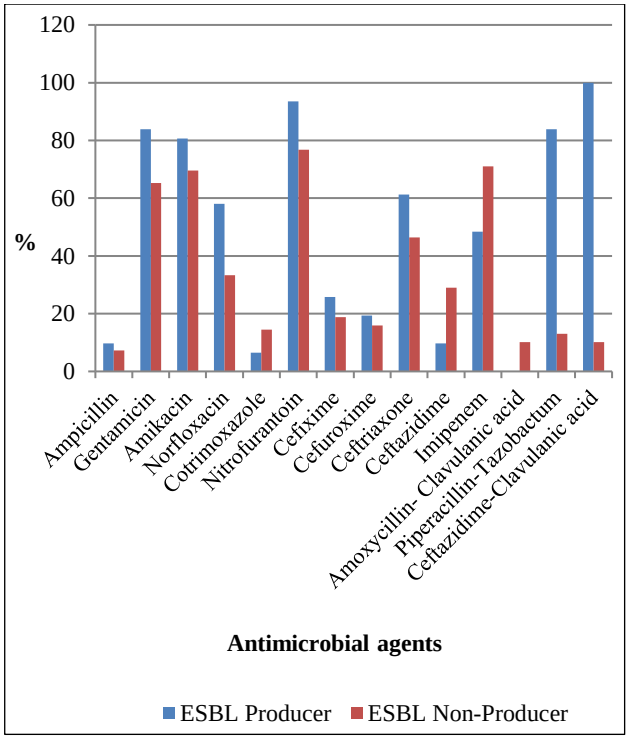

Figure 4: Antibiotic susceptibility profile of ESBL producing and ESBL non-producing *E. coli* isolates (%).

DISCUSSION

Present study was conducted on uropathogenic *Escherichia coli* isolates as it is the most common organism isolated from community as well as hospital acquired urinary tract infections. Of the 502 isolates, 316 were isolated from female patients and maximum number i.e. 56% was in age group 15-45 years. Studies done by Ali I, Gupta V also showed predominance of *Escherichia coli* from female patients in the similar age group.[19,8] This predominance in female is due to sexual activity, anatomic and physiological reasons which are directly related to increased incidence of UTI in females.

Etiology of UTI depends on various demographic characteristics that include the place of study (rural/urban). In this study, positivity rate from rural areas was 71% as compared to patients from urban areas i.e. 29%. This may be because the present study was conducted in a rural area where maximum number of patients attending the OPD/IPD was from rural background and also majority of women are least conscious about their health as well as hygienic practices.

Emergence of antimicrobial resistance is believed to be a major health problem now a day. In developing countries, scenario is even more worrisome because of availability of antibiotics over the counter. As Cotrimoxazole is considered as first line empirical treatment for many years, increasing frequency of resistance to cotrimoxazole is troublesome.[2] In this study too only 12 % sensitivity

was observed to cotrimoxazole. According to the literature fluroquinolones are acclaimed as initial empirical therapy for uncomplicated UTI in areas where resistance to cotrimoxazole exceeds 20%.[19] In this study, *Escherichia coli* showed alarming reduced susceptibilities to fluroquinolones like norfloxacin and Ofloxacin. Studies done by Mandel J et al and Ali I et al, showed same alarming results for fluroquinolones.[19,20] This is a warning call for judicious use of fluroquinolones. Cephalosporins commonly prescribed drugs, even on an outpatient basis showed same scenario as fluroquinolones.[2] This results were consistent with study done by Farshad S.[21] Resistance shown by combination of drugs like amoxicillin-clavulanic acid; piperacillin-tazobactam was even more worrying. Studies done by Hena Rani et al and Varsha Gupta et al showed 83.6% and 90.6% resistance to amoxy clavulanic acid which is in concordance with this study. While both the above studies showed 1.9% and 6.6% resistance to piperacillin tazobactam which is very much less as compared to our study 35%, reason for this high resistance in this study could be more use of this drug in our set up.[22,23] In this study, highest sensitivity was shown by nitrofurantoin (82%) followed by amikacin (73%), gentamycin (71%). Higher percentage of susceptibilities to the above drugs shown by Hena Rani et al and Varsha Gupta et al was in concordance with this study.[22,23]

Rate of ESBL production by *Escherichia coli* in our study was 31%. ESBLs production cause therapeutic failure with cephalosporins and azetreonam when host organism appears to be susceptible to these agents in laboratory tests. Hence, CLSI recommends that laboratories should report ESBL producing isolates as resistant to all penicillins, cephalosporins (including cefepime and cefpirome), and azetreonam irrespective of *in-vitro* test results .[12]

ESBL producing *Escherichia coli* varies greatly among country and among the hospitals within the country. This Variation is because of range of factors including species, geographic locality, hospital/ward, group of patients and type of infection, and large variations have been reported in different studies.[24] Studies done by Tankhiwala SS et al showed lower percentage of ESBL isolates i.e. 18.5%.[25] Higher percentage of ESBL producing *Escherichia coli* was found by Neelam Taneja et al with a rate of 40.2 % while Gupta V showed 52.6%.[9,23]

Present study showed higher percentage of resistance in ESBL producing *E. coli* as compared to ESBL non-producers. Multidrug resistance in *Escherichia coli* were seen in most of the strains and majority of them were ESBL producer.

As indicated by the present findings together with previous findings, it appears to be mandatory to include ESBL detection in routine laboratory practice so as to limit the rapid spread of ESBL-producing organisms.

CONCLUSION

So, drug resistance due to ESBL production is a serious threat in UTI narrowing down the choice of antibiotics for treatment. So, there is dire need to introduce routine screening for ESBL production for all uropathogenic *Escherichia coli* causing urinary tract infection. Early detection of beta lactamase will avoid treatment failure, as often the isolates producing these enzymes show a susceptible phenotype in routine susceptibility test.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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