Extended spectrum β lactamase producing *Escherichia coli* in urinary tract infections tip-off to evaluate treatment practice

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Abstract

Urinary tract infection (UTI) is one of the commonest infections worldwide. Although, the spectrum of etiological agents causing UTI have not changed but the antimicrobial susceptibility profile among them is changing over time and area specifically along with increase in antimicrobial resistance. Hence, this study was done to analyse the etiological agents and susceptibility pattern of *E. coli*, the most common UTI pathogen. Antibiotic susceptibility pattern and bacteria isolated from urine of patients who visited hospital between January 1 to December 31, 2015 was done. Bacteria were identified by standard microbiological methods and susceptibility test was done according to Kirby Bauer disc diffusion method. Of 107 urine samples, *E. coli* 73 (67.7%) was the most common isolate followed by *Staphylococcus* and *Klebsiella* spp. Most of *E. coli* isolates were sensitive to amikacin and nitrofurantoin. Out of total *E. coli* isolates, 57 were ESBL producers and 28 were MBL producers. The result indicates increase in multidrug resistant strains of *E. coli*. Further, study indicates the need for periodic monitoring of drug susceptibility pattern in a way to prevent the spread, and development of antimicrobial resistant strains, eventually.

Keywords: *E. coli*, urinary tract infection, antibiotic resistance

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Introduction

Urinary tract infections (UTIs) are the most common bacterial infections in developing and developed countries with *Enterobacteriaceae* being most frequent cause.[1] UTIs involves bacterial invasion and multiplications of the pathogen in the organs of the urinary tract system, which based on organ involved, is classified into uncomplicated and complicated infections. The status of infection (uncomplicated and/or complicated) also affects the choices of treatment.[2-3] It is estimated that about 35% of healthy women suffer from symptoms of urinary tract infection at some point in their life.[4] The incidence is more common in women than men due to shortness of female urethra, absence of prostatic secretions, easy contamination with fecal flora and pregnancy.[5]

Despite the widespread availability of antibiotics, UTI is one of the most important causes of nosocomial infection and high morbidity.[6] Gram negative bacteria like *E. coli, Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp. and Gram positive bacteria such as *Streptococcus* spp. and *Staphylococcus* spp. are commonly involved in causing UTI.[7-8] *E. coli* is the most common etiological agent in both outpatients and inpatients. However, etiology depends on factors like age, diabetes, urinary catheterization etc. Moreover, the spectrum of bacteria causing complicated UTI is much broader than of uncomplicated ones.[3] Number of studies have pointed towards high incidence rate of UTI associated with *E. coli* and antibiotic resistance. The emergence of multi drug resistant variant of *E. coli* has been reported.[9-10] Keeping in view the high incidence rate and associated drug resistance, area specific surveillance for the type of pathogens involved and their resistance pattern is of utmost importance. In Gram negative bacteria, the assorted antibiotic susceptibility patterns of pathogens from time to time and place to place has become matter of concern due to the emergence of beta-lactamase, extended spectrum beta-lactamases (ESBL), Amp-C beta-lactamases and metallo-beta—lactamases (MBL) resistance worldwide.[9,11-13] These enzymes collectively can hydrolyze almost all β-lactam drugs, which are used most frequently for the treatment of serious infections.[14] In developing countries, indiscriminate and empirical use of antimicrobial agents, has led to the emergence of resistant microorganisms.[4,15] Therefore, knowledge in change in the current bacterial etiology and susceptibility pattern is required for the selection of correct treatment regimes for UTI. Hence, in the present study was conducted to ascertain the correct etiology and their antibiotic susceptibility pattern in our hospital setting, which caters to a large population of the area.

Materials and Methods

Bacteriological examination: Fresh urine samples were collected and processed from patients attending

Subbaiah Institute of Medical Sciences, Shimoga between the period January 1 to December 31, 2015. Confidentiality of the patients was kept intact as per ethical guidelines. For microbiological analysis, clean-catch midstream urine specimens were collected using sterile wide mouth container. Urine samples were plated on MacConkey agar and blood agar plates using calibrated wire loops. Plates were incubated aerobically at 37°C for 24 h. Identification of uropathogens was done based on standard bacteriological procedures like colony morphology, Gram staining and biochemical reactions.[16] A significant bacterium was considered if culture yields $\geq 10^5$ C.F.U./mL.

Antibiotic susceptibility: Antibiotic susceptibility testing of isolated microorganisms was done by routinely used antibiotic susceptibility method i.e. Kirby disc diffusion method. Briefly, Mueller Hinton agar plate was divided in two parts. On one part standard strain was plated while on other half, test strain was plated. Antimicrobial discs of different antibiotics were placed on both sides. Plates were incubated overnight for growth at 37ºC. Resistance data were interpreted according to Clinical Laboratory Standards Institute.[17] Isolates resistant to the third generation cephalosporins were tested for ESBL production and isolates showing resistance to imipenem were tested for MBL production.

Detection of ESBL: Test was performed as phenotypic confirmatory test according to the recommendations of CLSI. The ceftazidime (30 μg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 μg disc) were used. An increase in zone of inhibition of ≥ 5mm in combination discs in comparison to the ceftazidime disc alone was considered ESBL producer.

Detection of MBL: Test was performed by Imipenem EDTA combined disc method. Two (10 μg) imipenem discs were placed on a plate inoculated with the test organism, and 10 μl of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of ≥ 7 mm was interpreted as a positive result for the MBL production.

Result

Among the 108 isolates, 82 (75.9%) were Gram negative bacteria with *Enterobacteriaceae* as the major one. The majority age group involved was 18-45years followed by age group of 1-18 years. *E. coli* (67.7%) was the major pathogen associated with UTI followed by *Staphylococcus* and *Klebsiella* spp. Amongst 73 *E. coli* isolates, 18 (24.6%) were from male and 55 (75.4%) were from female patients. (Table 1).

Table 1: Age and gender wise distribution of patients with UTI caused by *E. coli*

Age (years)	No. of isolates, No. (%)	Male, No. (%)	Female, No. (%)
1 – 18	6 (8.2%)	2 (2.7%)	4 (5.4%)
18 -45	37 (50.7%)	5 (6.8%)	32 (43.9%)
> 45	30 (41.1%)	11 (15.1%)	19 (26.1%)
Total	73 (100%)	18 (24.6%)	55 (75.4%)

The antibiotic susceptibility pattern of *E. coli* showed that amikacin was the most effective drug followed by nitrofurantoin. Out of total 73 isolates, 57 found to be ESBL producer and 28 found to be MBL producers (Table 2-3).

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

AMP	AMC	G	AK	NET	CIP	COT	NIT	CAZ	CTR	CTX	CXM	CN	CPM	AT	MRP
10 (13.6)	16 (21.9)	55 (75.3)	69 (94.5)	62 (84.9)	21 (28.7)	34 (46.5)	65 (89.1)	16 (21.9)	16 (21.9)	16 (21.9)	16 (21.9)	33 (45.2)	26 (35.6)	16 (21.9)	45 (61.6)

AMP – Ampicillin, AMC – Amoxyclav, G – Gentamycin, AK – Amikacin, NET – Netilmicin, CIP – Ciprofloxacin, COT – Cotrimoxazole, NIT – Nitofurantoin, CAZ – Ceftazidime, CTR – Ceftriaxone CTX – Cefotaxime , CXM – Cefuroxime, CN – Cefoxitin, CPM – Cefepime, AT – Aztreonam, MRP – Meropenem

Table 3: Number of ESBL and MBL producers in *E. coli* isolates

No. of *E. coli* isolates	ESBL, No. (%)	MBL, No. (%)
73	57 (78.1)	28 (38.3)

Discussion

Urinary tract infection is emerging as an important community acquired and nosocomial bacterial infection.[4] It is the second most common clinical indication for empirical antimicrobial treatment in primary and secondary care.[18] If remains untreated, UTI can proceed via ureters, to the kidneys, and hence may cause pyelonephritis leading to irreversible kidney damage, renal failure, and death.[19-20] Although, the main etiological agents causing UTI have not changed much over the years but the spectrum of antibiotics have changed drastically with increase in drug resistance.[15] *E. coli* is the most common pathogen responsible for urinary tract infections.[9,21-22]

In the current study, *E. coli* was isolated in 73 (67.7%) of urinary tract infections. Number of studies have reported *E. coli* as major bacterial pathogen associated with UTI.[9,13,21,23] It has also been reported that bacterial causes of UTI may show geographic variations, and may even vary over time within a population with vast antibiotic sensitivity pattern.[20,24] In this analysis, the growth was predominant 37 (50.7%) in age group 18-45 years and in female gender 55 (75.4%). Similar results were also reported by Bhattacharyya et al., which showed that female's especially younger age group showed more infection rate with mean age of 31.1 years.[25] The result of antimicrobial sensitivity showed that ampicillin, amoxicillin, and cotrimoxazole, which are used as a single agent for empirical treatment, may not be effective in near future. Maximum sensitivity of *E. coli* uro-isolates was observed to amikacin 69 (94.5%) and nitrofurantoin 65 (89.1%). Similar results have also been reported, previously.[1,8] The antimicrobial sensitivity and resistance pattern varies from community to community and from hospital to hospital. This pattern may vary time to time in the same setting. It is because of emergence of new resistant strains as a result of indiscriminate use of antibiotics.[26-27]

In another study, nitrofurantoin was represented as most active drug against *E. coli* isolates. However, this is not a drug of choice for serious upper UTIs or for those with systemic involvement.[3] Development of anti-microbial resistance constitutes one of the most serious problems in the control of infectious diseases.[28] The high level of drug resistance seen among *E. coli* is mediated by β-lactamases, which hydrolyze the β-lactam ring, and hence inactivates the antibiotic.[1,11] Overall resistance to third generation cephalosporins was high on account of the production of extended spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBL), which are chromosomally encoded or plasmid mediated.[29] In the present study, prevalence of ESBL and MBL producers was found to be 57 (78.1%) and 28 (38.3%), respectively. In this situation, concurrent administration of a β- lactamase inhibitor such as clavulanate or sulbactam, markedly expands the spectrum of activity. The results of current study indicated that *E. coli* is still a major pathogen associated with UTI and amikacin and nitrofurantoin are considered as most appropriate antimicrobials for the empirical treatment of UTI.

Conclusion

UTI is most common bacterial infection, and *E. coli* is the most common pathogen associated with it, worldwide. However, indiscriminate use of antibiotics had raised the concern over antimicrobials used for the treatment of UTI. Moreover, recognition of antimicrobial resistance and more specifically towards β- lactam drugs has raised the concern over availability of antimicrobials since drug resistance is an evolving process. Therefore, routine surveillance and monitoring is required to choose correct treatment options. Further, area specific monitoring adds to knowledge about the variety of pathogens involved and treatment options available to clinician. The results of this study indicate the need for periodic monitoring of drug susceptibility pattern, and later development of treatment guidelines based on local susceptibility profile. This would certainly prevent the spread, and development of antimicrobial resistant strains, eventually.

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