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RESEARCH ARTICLE

THE STUDY OF DRUG RESISTANCE AND ESBL PRODUCTION IN ESCHERICHIA COLI CAUSING URINARY TRACT INFECTIONS

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ABSTRACT

Urinary tract infection is one of the most common cause of bacterial infections and *Escherichia coli* is the predominant urinary pathogen isolated from community based and hospitalized patients.[1,2] The treatment has been posing as a challenge due to the emergence of multidrug resistant *E .coli* especially in nosocomial infections, Extended Spectrum β-lactamases (ESBL) bearing the major brunt of the problem limiting the therapeutic options.[3] The present study was carried out to determine the antimicrobial susceptibility pattern and to detect ESBL production in *E. coli* causing Urinary tract infections. Total of 100 *E.coli* isolates from clinically suspected UTI cases from patients attending Victoria hospital & Vani Vilas hospital attached to BMC & RI, Bangalore were studied from October 2010 to September 2012. Mid stream urine samples were collected after taking informed consent from all patients and were processed. All samples were cultured by semi quantitative method. The identification of *E.coli* was done using standard biochemical tests. *E.coli* thus identified were studied for the drug resistance patterns. The isolates were further screened for ESBL production by disc diffusion method and confirmed by phenotypic confirmatory method. Considering high morbidity and poor clinical response to serious infections in UTI the occurrence of drug resistance and ESBL production in Uropathogenic *E.coli* (UPEC) strains strengthens the association with pathogenecity. Hence the screening using above mentioned simple methods can be routinely done in clinical laboratory.

INTRODUCTION

Urinary tract is the most common site of bacterial infection both in community and hospitalized patients. UTI is an important cause of morbidity and mortality with *E. coli* being the most common pathogen, accounting for 85% of community acquired infections .[1] It is the most frequent urinary pathogen isolated from 50-90% of all uncomplicated UTI.[4] Some strains of *E.coli* can diverge from their commensal cohorts taking on a more pathogenic nature and the ability to cause disease both within the intestinal tract and elsewhere in the host. These pathogenic strains are broadly categorised as diarrhoegenic *E.coli* or extra intestinal pathogenic *E.coli* (*ExPEC*).[5] ExPEC have the capacity to disseminate and colonise other host niches including blood, CNS and urinary tract resulting in diseases.[5]Among ExPEC certain strains were consistently associated with uropathogenecity and were designated as Uropathogenic *E.coli* (UPEC). UTI is usually treated empirically without culture but it contributes to 10-15% prolongation of hospital stay due to its ability to exhibit resistant bacteria in hospital.[3] In the past, most *E. coli* isolates were highly susceptible to a broad range of antimicrobial agents including beta lactams accounting for 60% of antibiotic use. The prevalence of resistance to first generation cephalosporin and trimethoprim-sulfamethoxazole is increased to about 23.4% and 33.7% respectively. Resistance of *E. coli* to fluoroquinolones has increased over the last decade. The prevalence of co-resistance to more advanced cephalosporin (second, third and fourth generation), monobactams, piperacillin-tazobactam and aminoglycosides is increasing.[13] The most common cause of resistance to β lactams antibiotics is the production of β lactamases especially ESBL.

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These β-lactamases are transmissible, plasmid encoded that can be exchanged between bacteria and are inhibited by clavulanic acid, tazobactam and/or sulbactam.[14] The ability of these enzymes to spread to other bacteria has led to dramatic increase in their prevalence worldwide in a very short span of time. Hence regular monitoring of antibiotic resistance is crucial to prescribe appropriate therapy and also to prevent the spread of resistant strains in the hospital as well as in the community.[3, 15] The present study was undertaken to evaluate the antibiotic susceptibility pattern of *E. coli* isolated from UTI and to detect ESBL production in community and hospitalised patient population attending hospitals attached to Bangalore Medical College and Research Institute.

MATERIALS AND METHODS

This study was conducted from October 2010 to September 2012 in the Department of Microbiology attached to Victoria Hospital, Minto Institute of Ophthalmology and Vani Vilas Hospitals of Bangalore Medical College & Research Institute, Bangalore, India. Urine samples from suspected cases of UTI were processed by microscopy and culture. A total of 100 isolates of *E.coli* isolated from such cases were studied for the antimicrobial susceptibility patterns and ESBL production.

Method of collection

The study population included both out patients and inpatients attending different clinical departments of Victoria and Vani Vilas hospital, BMC &RI with suspected UTI [i.e., with dysuria, frequency, urgency, fever]. The patients were asked to collect a midstream sample of urine in a sterile container. In case of catheterised patients the urine samples were collected after 30 minutes of clamping the catheter, through a syringe and needle inserted proximal to the site of

clamping under all aseptic precautions. The specimens were immediately transported to the laboratory and processed.

Laboratory procedures

Semi quantitative culture for urine

A calibrated nichrome wire loop of 4mm diameter that delivers 0.01ml of urine was used to culture urine sample semi quantitatively. Urine sample was mixed thoroughly, the calibrated loop sterilized by red hot method was inserted vertically into the urine and a loopful of sample so removed was streaked on MacConkey's agar, Blood agar (BA) and CLED agar (Cysteine Lactose Electrolyte Deficient) agar. The plated cultures were incubated at 37^0C overnight. The number of colonies grown on BA were counted and interpreted as CFU/ml next morning. The presences of 100 or more colonies were considered as Significant Bacteriuria. Lactose fermenting large, moist, smooth, colonies resembling *E coli* was identified by standard biochemical tests.

Identification[1, 2]

Lactose fermenting, motile, Gram negative bacilli were identified by the following biochemical tests as *E coli.*

Oxidase non producer, Catalase producer, Indole producer, Methyl Red + Voges Proskauer - TSI agar showing A/A with gas and without H_2S

Urease not produced, Citrate not utilized. Glucose, Lactose, and Mannitol fermented with production of gas and Sucrose not fermented

Isolates were confirmed as *E coli* and antibiotic susceptibility testing was carried out.

Antibiotic susceptibility testing (AST)

AST was carried out by Kirby Bauer disc diffusion method on Mueller Hinton agar (Hi media). The media was prepared as per the instructions of the manufacturer by suspending 38g of dehydrated media in 1000ml of distilled water, autoclaved at 121^o C for 15 min and poured into petridishes to a depth of 4 mm. A broth culture of the isolate with turbidity adjusted to 0.5 McFarland turbidity standards was lawn cultured on the Mueller Hinton agar and allowed to dry. The antibiotic discs were taken out from the refrigerator brought to room temperature and were placed on the surface seeded with *E coli* and incubated at 37^oC overnight. The *E coli* isolates were tested for susceptibility to following antibiotics using commercial antibiotic disks (HiMedia).

Ampicillin (10 µg)
Cotrimoxazole (1.25 / 23.75 µg)
Amikacin (30µg)
Gentamicin (30µg)
Cefotaxime (30 µg)
Ofloxacin (5µg)
Norfloxacin (10µg)
Nitrofurantoin (300 µg)

The antibiotic susceptibility was interpreted as sensitive or resistant by comparing inhibitory zones produced by the test isolate with that of standard ATCC *E coli* 25922. All these isolates were further subjected for detection of ESBL.

DETECTION OF ESBL

Screening by standard disk diffusion method:

Screening for ESBL was done according to CLSI guidelines. Cefpodoxime (10µg), Ceftazidime (30µg), Aztreonam (30µg), Cefotaxime (30µg) and Ceftriaxone (30µg) were used for in-vitro testing by Kirby-Bauer disc diffusion method. Zone diameters were interpreted according to CLSI recommendations. An inhibition zone ≤17mm for Cefpodoxime, ≤22mm for Ceftazidime, ≤27mm for Aztreonam & Cefotaxime and ≤25mm for Ceftriaxone, indicated a probable ESBL production, requiring phenotypic confirmation.

ESBL confirmation

ESBL production was confirmed by CLSI described phenotypic confirmatory method. Briefly, a lawn of test organism was made on Mueller Hinton agar (MHA) after adjusting the inoculum to 0.5 McFarland. Cefotaxime (CE, 30µg) and Ceftazidime (CA, 30µg) discs with and without Clavulanic acid (CL) were placed on MHA and incubated at 37^0C for 18 – 24 hrs. A ≥5mm increase in the zone diameter of the CE (and/or CA) alone and in combination with CL was confirmative of ESBL production.

RESULTS

The present study was carried out on 100 *E coli* isolated from cases of UTI at the Department of Microbiology, BMC& RI from October 2010 to September 2012. Antibiotic susceptibility testing was performed for all the isolates by Kirby – Bauer disc diffusion method. Screening of isolates for ESBL production was done and confirmation of the same done using standard CLSI guidelines. The observations made from the study are shown in the following tables. A total of 100 patients with UTI were included in the study of which 45 were males and 55 were females. Maximum no of cases were seen between 1-9 years and 20-40yrs of age. Maximum number of isolates showed sensitivity to Amikacin (87%) and Nitrofurantoin (81%) and resistance to Ampicillin (90%). Higher resistance to commonly used antibiotics like Ofloxacin (73%), Norfloxacin (67%) and cefotaxime (71%) was seen. The isolates resistant to Cefotaxime (71) were screened for ESBL production. Amongst the drugs used for screening ESBL maximum resistance was seen with Ceftazidime (97%) followed by Aztreonam (84%). Of the 71 isolates resistant to Cefotaxime which were screened for ESBL production only 58(81.7%) isolates were shown to produce enhancement with Clavulanic acid and confirmed as ESBL producers.

Table 1. The antibiotic susceptibility of the 100 *E coli* from UTI cases

Name of the antibiotic	Sensitive %	Resistant %
Ampicillin	10	90
Amikacin	87	13
Gentamicin	42	58
Cotrimoxazole	36	64
Ofloxacin	27	73
Nitrofurantoin	81	19
Norfloxacin	33	67
Cefotaxime	29	71

Table 2. Distribution of antibiotics for ESBL screening

Antibiotics	Resistance (n=71)		Sensitivity (n=71)	
	No	%	No	%
Cefpodoxime	45	63.4	26	36.6
Ceftazidime	69	97.2	2	2.8
Aztreonam	60	84.5	11	15.5
Ceftriaxone	55	77.5	16	22.5

Table 3. Production of ESBL

Cefotaxime with Clavulanate	Number of patients	%
Produced	58	81.7
Not produced	13	18.3
Total	71	100.0

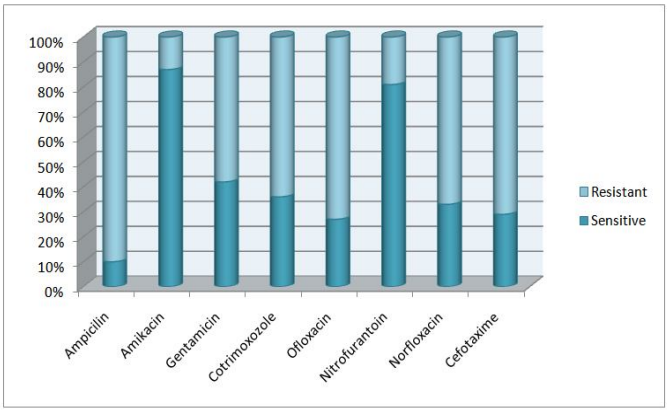

Figure 1. Antibiotic susceptibility pattern of *E.coli*

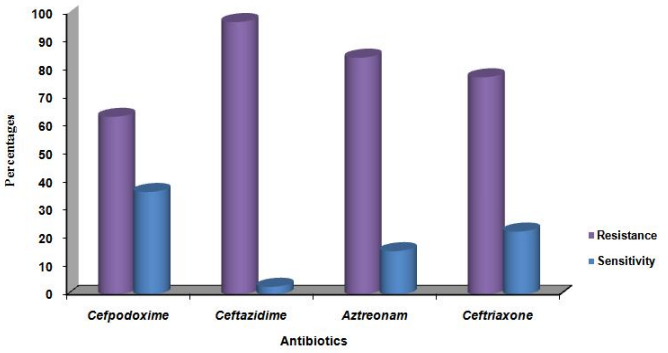

Figure 2. Distribution of antibiotics for ESBL screening

DISCUSSION

E. coli the most prevalent facultative Gram negative bacterium in the human fecal flora usually inhabits the colon as an innocuous commensal. It causes extra intestinal and intestinal infections.[10] UTI is the most common form of extra intestinal infection produced by *E.coli* and it is the most common cause of UTI. *E coli* is responsible for 50-90% of all uncomplicated UTIs. The treatment of *E coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by these organisms. In the present study, *E coli* showed more resistance to commonly used antibiotics such as ofloxacin, norfloxacin, cephotaxime. There is also increasing resistance to Gentamicin. Ampicillin resistance was seen in 90% of cases. High resistance to cephotaxime (71%) was seen. Amikacin resistance was seen in 13% of cases, similar to Biswas et al (11%) and Kausar et al (8%).[13,6] There was high resistance to fluoroquinolones (70%) which corroborates with earlier observations. Cotrimoxazole resistance was seen in 64%. Similar findings were reported by others. In the present study the most sensitive drug is Amikacin with 87%. This is correlated with Yaseem Kausar et al [6](2009) with 85-92% sensitivity. The least sensitive drug is Ampicillin with 10%. In the present study 58% of *E.coli* isolates showed ESBL production. In view of the emerging drug resistance among *E.coli*, it is important to advocate therapy only after antibiotic sensitivity has been performed .By doing so we can not only prevent indiscriminate use of antibiotics but also the further development of antibiotic resistance

Conclusion

UTI is the most common of bacterial infection both in community and hospitalized patients and *E.coli* is the most common bacterial agent causing it. The majority of *E.coli* isolates were between the age group 0-9 yrs and 20- 40yrs.Among the 100 isolates 55% isolates were from female and 45 % from males. These strains were subjected for ESBL detection. 58% were ESBL producers. AST of the isolates showed maximum sensitivity to Amikacin followed by Nitrofurantoin, Gentamicin, Cotrimoxazole, Norfloxacin, Ofloxacin and least for Ampicillin. Cephalosporins are the first line drugs used in the infections caused by E.coli. The extensive use of third generation cephalosporins has resulted in the increased prevalence of ESBL. In view of emerging drug resistance and multidrug resistance exhibited by *Escherichia coli*, periodic review and formulation of antibiotic policy are needed. In our study the use of simple screening for ESBL producers in combination with CLSI phenotypic confirmatory tests were simple highly sensitive and specific for identification of ESBL production which can be used in Microbiology laboratory and thus indiscriminate use of antibiotic must be discouraged and therapy should be advocated as far as possible after the culture and sensitivity reports are available.

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