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Research Article

ANTIMICROBIAL SUSCEPTIBILITY OF EXTENDED SPECTRUM BETA LACTAMASE (ESβL) PRODUCING E. COLI FROM URINARY TRACT INFECTED PATIENTS FROM HOSPITAL AND DIAGNOSTIC CENTRES IN KALABURAGI, KARNATAKA, INDIA

Raghavendra Ramachar., Shivannavar C.T* and Gaddad S.M

Department of Studies and Research in Microbiology, Gulbarga University Kalaburagi-585106

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A B S T R A C T

ESβL producing *E. coli* isolates from clinical samples were investigated in this study. The clean-catch mid-stream urine samples showing symptoms of urinary tract infections were collected from different hospitals and diagnostic centres of Kalaburagi city. The isolation of etiological agent was done by semi-quantitative method of inoculating the samples on the selective and differential media Eosin Methylene Blue (EMB) and MacConkey Agar respectively. The isolated pathogen was identified by Gram staining, motility and biochemical tests. The antibiogram studies were carried out by Kirby-Bauer disc diffusion technique and ESβL production by double disk-diffusion test (DDDT) as per CLSI guidelines 2007. Out of 200 isolates screened 64 *E. coli* isolates were multidrug resistant with ESβL production. In this study 80% isolates were resistant to all cephalosporins. All ESβL producing *E. coli* isolates were resistant to ceftazidime and exhibited higher level of resistance to Cephalothin, Erythromycin, Cotrimoxazole and Aztreonam. Finally it was found that nitrofurantoin, ofloxacin and tetracycline were most effective antibiotics and may be considered as drug of choice for the treatment. The data from present study will help in forming good regimens for treating *E. coli* infections more efficiently and avoiding the emergence of MDR strains.

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common infectious diseases ranking next to upper respiratory tract infection and is an important cause of morbidity and mortality in humans. Bacteria are the major causative organisms and are responsible for more than 95% of UTI cases among which *Escherichia coli* is the most prevalent causative organisms of UTI and is solely responsible for more than 80% of the infections (Paterson D.L., 2001).

It is estimated that about 150 million cases of UTI occur per annum worldwide (Stamm *et al*., 2001). *E. coli* is the most common organism causing UTI. It has the ability of producing Extended Spectrum Beta Lactamases (ESβLs), which confer multiple drug resistance and it creates difficulty in treating urinary tract infection (Kariuki *et al*., 2007). ESβL producing bacteria are typically resistant to penicillins, first and second generation Cephalosporins as well as the third generation oxyiminicephalosporins (e.g., Ceftazidime, Ceftriaxone) and Monobactam (Aztreonam) except cephamycins and carbapenems (Rawat *et al*., 2010).

Corresponding author:* **Shivannavar C.T
Department of Studies and Research in Microbiology, Gulbarga University Kalaburagi-585106

The persistent exposure of the bacterial strains to a multitude of β-lactams has induced a dynamic and continuous selective pressure which produces mutation of β-lactamases in bacteria. ESβLs are plasmid borne and they have evolved from point mutations which altered the configuration of the active site of the original and long known β-lactamases which have been designated as TEM-1, TEM-2 and SHV-1 (Nathisuwan *et al*., 2001).

Broad Spectrum β-lactamase producing organisms are a growing worldwide problem (Livermore, 2001). It was first observed in Germany 1983 in isolates of *Klebsiella pneumoniae* (Knothe *et al*.,1983), ESβLs spread rapidly to Europe, United States and Asia and are now found all over the world (Suganya *et al*.,2014) It is difficult to by regular disc diffusion techniques. ESβLs are inhibited by β-lactam inhibitors like clavulanic acid. Sulbactam and tazobactam and this property of specific inhibition can be used for detecting and confirming ESβLs. Delay in identifying and reporting ESβL production contributes to their uncontrolled spread. A heightened awareness among the clinicians and enhanced testing by laboratories is the need of hour. Knowledge of antibiotic resistance pattern will help in the appropriate and judicial use of antibiotics. The extended spectrum β-lactamase (ESβL) producing strains have variable susceptibility rates for fluoroquinolones, aminoglycosides, and fourth generation

cephalosporins (Lautenbach, 2001 and Kariuki *et al*., 2007). The aim of the study was to detect the prevalence of ESβL producing *E. coli* isolates causing urinary tract infection which were collected from various hospitals and diagnostic centers and we found that ofloxacin, tetracycline and nitroforantoin may be considered as drug of choice for the treatment.

MATERIALS AND METHODS

Sources of samples

During the period from September 2014 to January 2015, a total of 200 clinical samples were collected from patients visiting various government and private hospitals, clinics and diagnostic centers in Kalaburagi city, Karnataka.

Data collection

The detailed information was collected from the patient which included age, sex, locality of the patient, history of illness, and whether the patient was on prior antibiotic administration before the sample collection.

Collection of urine samples

Clean catch, Midstream urine samples were collected from the patients in a sterile wide-mouth container. The samples were labeled, transported and processed in the laboratory immediately. Samples were maintained at 4˚C for 4 hr if delayed for processing.

Isolation and Identification of the isolates

Isolation of the etiological agent was done by inoculating the samples on MacConkey's agar, Blood agar and Eosin Methylene Blue agar respectively. Plates were incubated at 37˚ C for 24hrs. Identification of all the isolates were done on the basis of routine biochemical tests i.e fermentation of lactose, ability to produce indole, reaction on triple sugar iron agar (TSI) medium , hemolysis on blood agar, citrate utilization and motility of organism (Cheesbrough, 1989). The organism were maintained at 4^{o}C on agar slants and used for further studies.

Antimicrobial susceptibility

The fifteen antimicrobial agents used in this study were Amikacin (AK), Gentamicin(GEN), Penicillin-G (P^{10}), Tetracycline (TE), Cotrimoxazole (COT), Aztreonam (AT), Ofloxacin (OF), Ciprofloxacin (CIP), Erythromycin (E), Imipenem (IPM), Nitrofurantoin (NIT), Cephalothin (CEP), Cefoxitin (CX), Ceftazidime (CAZ), and Cefotaxme (CTX). All the chemicals and discs were procured from Hi-media Laboratory Pvt. Ltd., Mumbai. Susceptibility test were done on Mueller-Hinton agar by using Kirby-Bauer's disc diffusion method (Bauer, 1996). The AST was conducted as per CLSI guidelines (2007).

Phenotypic detection of ESβL production by double disc diffusion test (DDDT)

All the isolates showing resistance to one or more third generation cephalosporins(3GCs) were tested for ESβL production by the double disc diffusion test (DDDT) using Cefotaxime and Ceftazidime at a distance of 20mm from a disk of Cefotaxime+Clavulanic acid (30/10 µg) and Ceftazidime+ Clavulanic acid(30/10 µg) respectively on a lawn culture of *E. coli* (0.5 McFarland inoculums size) on Mueller-Hinton agar. After overnight incubation at 37 ˚C ESβL production was confirmed if there was ≥ 5mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanate versus its zone when tested alone. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively.

RESULTS AND DISCUSSION

Data depicted in the Table-1 shows number and percentage of ESβL and Non ESβL producing *E. coli***.** Out of 200 urine samples 64 positive isolates of *E. coli* were obtained with a prevalence of 32.8% which is similar to the reports of DMBT Dissanayake *et al*.,(2012) and Basavaraj *et al*.,(2011) who reported 29% and 32% of prevalence of *E. coli* respectively. With relation to age group, it was observed that a highest of 80% is in the age group 50 and above, followed by 48.14% in 41-50, 45.5% in 1-10 years and 26.82% in 31-40 years age group. However, a lowest of 10.7% was observed in the age group of 10-20 years.

Table 1 Comparison of resistance rates to various antibiotics in ESβL and non ESβL producing *E. coli* isolates

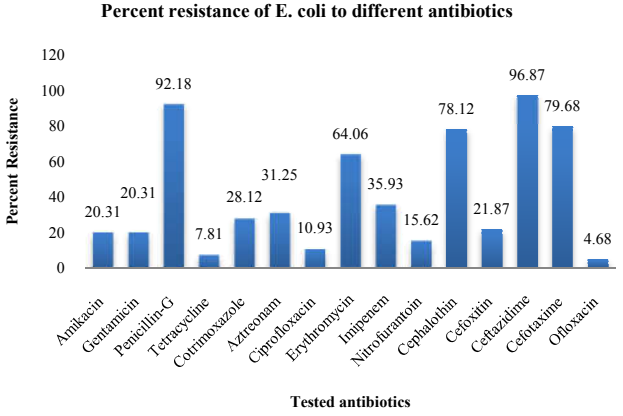
Antibiotics	ESβL producers (n=21) (32.82%)		Non ESβL producers (n=43) (67.18%)	
	Resistant	Sensitive	Resistant	Sensitive
Amikacin	2 (9.5)	19 (90.4)	11 (25.5)	32 (74.4)
Gentamicin	12 (57.1)	9 (42.8)	3 (6.9)	40 (93.0)
Penicillin-G	20 (95.2)	1 (4.7)	37 (86.0)	6 (13.9)
Tetracycline	4 (19.0)	17 (80.9)	0 (0.0)	43 (100.0)
Cotrimoxazole	17 (80.9)	4 (19.0)	1 (2.3)	42 (97.6)
Aztreonam	16 (76.1)	5 (23.8)	6 (13.95)	37 (86.0)
Ciprofloxacin	5 (23.8)	16 (76. 1)	2 (4.6)	41 (95.3)
Erythromycin	18 (85.7)	3 (14.28)	26 (60.4)	17 (39.53)
Imipenem	2 (9.5)	19 (90.4)	21 (48.8)	22 (51. 16)
Nitrofurantoin	3 (14.2)	18 (85.7)	8 (18.0)	35 (81.39)
Cephalothin	19 (90.4)	2 (9.5)	31 (72.0)	12 (27.90)
Cefoxitin	13 (61.9)	8 (38.0)	2 (4.6)	41 (95.3)
Ceftazidime	21 (100)	0 (0.0)	31 (72.0)	12 (27.90)
Cefotaxime	3 (14.2)	18 (85.9)	28 (65.11)	15 (34.8)
Ofloxacin	17 (80.9)	4 (19.0)	26 (60.4)	17 (39.53)

In the present study significant differences were observed with respect to the susceptibility of the isolates to flouroquinolones, tetracycline and aminoglycosides for both ESβL and non ESβL producing *E. coli* isolates (ESβL producing *E. coli* isolates have shown complete resistance to ceftazidime (100%), penicillin (95.2%), cephalothin (90.4%) and erythromycin (60.4%) respectively. Lowest resistance was observed for amikacin, imipenem with 9.5% each and nitrofurantoin (14.2%) which is much lower than the value reported by Behroozi *et al*., (2010).

96.87% resistance observed in *E. coli* for the antibiotic ceftazidime followed by 92.18% to penicillin-G, 79.68% cefotaxime, 78.12% cephalothin. Most of the isolates were sensitive to ofloxacin (4.6%) and tetracycline(7.8%) and Imipenem (90.4%) which is nearly equal to Daryl *et al*.,(2014). Overall multidrug resistance for the *E. coli* isolates were observed to be 89.06% with highest of 14.06% of isolates were resistant to 5, 6 and 7 antibiotics.

ESβL detection by double disk-diffusion test (DDDT) was performed for all the 64 *E. coli* isolates (Figure-1). Out of which 21 were found to be ESβL producers indicating an incidence rate of 32.80% and 43 isolates were Non ESβL producers. All the ESβL producing isolates have been found to be MDR (multi drug resistant) and also shown higher

resistance compared to that of non- ESβL producing *E. coli* isolates (Table-1)



Graph 1 Percent resistance of *E. coli* to different antibiotics.

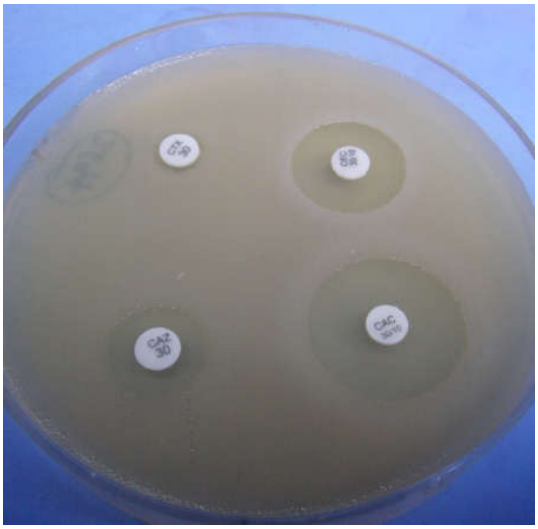


Figure 1 A ≥ 5mm increase in zone of inhibition for Cefotaxime + Clavulanic acid versus its zone diameter when tested alone by Cefotaxime, and Ceftazidime + Clavulanic acid versus its zone diameter when tested alone by Ceftazidime confirmed an ESβL producer.

Table 2 Multidrug resistance patterns of *E. coli* isolated from clinical samples

Number of Antibiotics	Number of isolates resistant	Incidence of MDR (%) (n=64)	Overall MDR carriage rate (%) (n=200)
3	7	10.93	3.5
4	4	6.25	2.0
5	9	14.06	4.5
6	9	14.06	4.5
7	9	14.06	4.5
8	7	10.93	3.5
9	6	9.37	3
10	3	4.68	1.5
11	1	1.56	0.5
12	0	0	0
13	1	1.56	0.5
14	1	1.56	0.5
15	0	0	0.5
	MDR isolates 57	89.06	28.5

In conclusion, the isolation rate of *E. coli* as uropathogen is very low compared to the early reports from India and other parts of the world (Table-3). Amongst *E. coli* isolates, one third of them were ESβL producers. Policy makers in India have taken initiation by making "National Policy for Containment of Antimicrobial Resistance" in 2011. This has to be achieved by monitoring antibiotic resistance pattern of newly and emerging pathogens like *E. coli,* that will helps in forming good regimens for treating *E. coli* infections more efficiently and avoid emergence of MDR strains. In this study around 80% of isolates were resistant to all cephalosporins except cefoxitin (22% resistance). All the ESβL producing *E. coli* isolates were resistance to ceftazidime and also exhibited higher level of resistance to most antibiotics tested except Imipenem, nitrofurantoin, amikacin and cefotaxime, showing less than 15% resistance.

Table 3 Various studies showing the prevalence of ESβL producing *E. coli* isolated from UTI patients

	Studies	Year	Prevalence
1	Mahesh *et al*.,	2010	56.2%
2	DMBT Disanayake *et al.,*	2012	29%
3	Dugal S *et al*.,	2013	24.4%
4	Chaudhary *et al*.,	2013	54.5%
5	Datta *et al*.,	2014	21.4%
6	Singh N *et al*.,	2016	82.6%
7	Ravindranath Gangane *et al*.,	2017	61%
8.	Present Study	2017	32.8%

Finally we conclude ofloxacin, tetracycline and nitrofurantoin are the most effective antibiotics, and thus these may be the drug of choice for treatment of *E. coli* infections especially UTIs. The incidence of ESβL producing *E. coli* poses not only a therapeutic problem but also a serious concern for inection control management. Therefore, it is adviced for contiuous monitoring of antibiotic resistant patterns of pathogenic ESβL producing *E. coli* that may helps in preventing emergence of superbugs.

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