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Screening for ESβL producing *E. coli* isolated from clinical urine samples collected from different places of Kalaburagi city

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ABSTRACT

Bacteria are capable of invading and infecting humans, leading to disease and sometimes death. Different body tissues, organs and systems are vulnerable to different organisms. This study was aimed to isolate and identify the bacteria causing Urinary Tract Infection (UTI) in different age groups and also screened for ESβL production. The mid-stream urine samples showing symptoms of urinary tract infections were collected in a wide container from different hospitals and diagnostic centres of Kalaburagi city. The isolation of uropathogen was done by semi-quantitative method of inoculating the samples on the selective and differential media such as Eosin Methylene Blue (EMB) and MacConkey (MAC) agar media respectively. The isolated pathogen was identified by conventional methods like cultural, morphological and biochemical tests. The antibiotic susceptibility test was carried out by Kirby-Bauer disc diffusion technique and ESβL production by double disk-diffusion test (DDDT) as per CLSI guidelines (2013). Out of 550 samples screened, a total of 288 bacteria were isolated of those half of them (146) were *E. coli* isolates and all were found to be multidrug resistant and half of them (50%) were of ESβL producers. All ESβL producing *E. coli* isolates were resistant to ceftazidime and exhibited higher level of resistance to Cephalothin, Erythromycin, Cotrimoxazole and Aztreonam. As ESβL producing organisms limits the available treatment options, so, the timely administration of sensitive antibiotic and avoiding antibiotic abuse will help in curing the disease without going for the costly drugs such as Carbapenems. Our study revealed that nitrofurantoin, ofloxacin and cefoxitin may be considered as drug of choice for the treatment of UTI patients. The

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ESβL production in uropathogens should be continuously monitored in the clinics and hospitals as to avoid the emergence of more multi drug resistance strains.

***Keywords*:** Uropathogenic *E. coli,* Green Metallic Sheen, Antibiogram, ESβL, Phenotypic detection, Double-disc diffusion test

1. INTRODUCTION

Escherichia coli is a common pathogen causing community-acquired urinary tract infections (UTIs) affecting people of all ages. Urinary Tract Infection (UTI) is the commonest infection seen in clinical practice. Estimation says that 10% of the patients visiting the hospitals suffer from UTI (Taslima, *et al*., 2007). Both sexes of all age groups are vulnerable to UTI. Women are more prone to UTI than males and 20% of women will suffer from UTI at least once in their lifetime (Ramprasad *et al*., 1993). UTI is a major cause among hospital acquired infections too. More than 70% of community acquired urinary tract infections (UTI) are due to *E. coli* infections. Originally ESβL (extended spectrum beta lactamases) producing *E. coli* was isolated from hospital settings along with *Kelbsiella* sps. now it is frequently isolated from community infections. The existence of enzymes Extended Spectrum Beta Lactamases (ESβLs) producing organisms that are resistant to majority of the available β-lactam antibiotics (Philippon *et al*., 1989) and most of them are emerged as multiple drug resistant strains.

ESβLs are plasmid mediated enzymes that confer resistance to beta-lactam antibiotics including penicillins, cephalosporins and the monobactam aztreonam commonly found in the family Enterobacteriaceae mainly *E. coli* and *Klebsiella pneumoniae.* Infections with ESβL producing organisms have been associated with poor clinical outcomes. Infections caused by ESβL-producing bacteria often involve immuno-compromised individuals making it difficult to treat them in high-risk wards, such as intensive care units (ICUs).

Constant increase in resistance to second and third generation cephalosporins observed in medical institutions as a result of acquisition and expression of extended spectrum beta lactamases and posed serious public health problems. Community and Hospital acquired ESβL-producing Enterobacteriaceae are prevalent worldwide. (Ben *et al*., 2009). Reliable identification of ESβL-producing organisms in clinical laboratories is challenging, so their prevalence is likely underestimated.

Beta-lactamases are enzymes that open the beta-lactam ring, and inactivating the antibiotic. The first plasmid-mediated beta-lactamase in gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient Temoniera from whom it was isolated (Bradford, 2001). Subsequently, a closely related enzyme was discovered and named as TEM-2. These two are the most common plasmid mediated beta-lactamases genes in gram-negative bacteria including Enterobacteriaceae; *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. TEM-1 and TEM-2 hydrolye penicillins and narrow spectrum cephalosporins such as; cephalothin or cefazolin.

However they are not effective against higher generation cephalosporins with an oxyimino side chain such as: cefotaxime, ceftazidime, ceftriaxone or cefepime. Consequently when these antibiotics were first introduced, they were effective against a broad group of otherwise resistant bacteria (Hima Bindu *et al*., 2015).

The patients and carriers are the sources of infections in both hospital and community. However, in hospital ESβL producing bacteria are spread mostly through hospital staff like doctors, nurses or other healthcare professionals. They are vastly responsible for causing infections such as UTI, diarrhea, skin infection and pneumonia. Since these bacteria are highly resistant to many antibiotics should be administered only after performing an antibiotic sensitivity testing according to CLSI guidelines.

The extended spectrum beta lactamase producing bacteria are increasingly causing urinary tract infections (UTIs) both in hospitalized and outpatients. The increase of drug resistance among these organisms has made therapy of UTI difficult and has led to greater use of expensive broad-spectrum antibiotics such as third generation cephalosporins. Detection of ESβL producing pathogens using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESβL production by Gram negative bacilli is associated with prolonged hospital stay, increased morbidity, and mortality and health care costs. Resistance has emerged even to newer, more potent antimicrobial agents (Taneja *et al*., 2008). It is estimated that about 15 million cases of UTI occur per annum worldwide (Stamm and Norbby, 2001). Apart from socioeconomic reasons such as illiteracy, ignorance and insanitation other factors are also known to predispose UTI which could be anatomical position of urethra, prostrate hypotrophy, renal calculi, structure of urethra, catheterization and diabetes (Anantanarayan,2 013, Ann Pallett 2010, Thomas MH 1996).

In India, community presence of ESβL producing organisms has been well documented (Baby padmini *et al*., 2004; Akram *et al*., 2007; Kothari *et al*., 2008; Siddiqui *et al*. 2013 and Singh *et al*. 2016). However, various epidemiological factors associated with ESβL producing strains have to be clearly documented. This will allow the clinicians to separate the patients with community UTI with those factors so that appropriate and timely treatment can be given. In addition, for deciding the correct empirical treatment for patients with UTI a thorough knowledge of local epidemiology is required. In our region there are only limited numbers of reports on ESβL producing *E.coli* strains in the UTI cases from general population. Therefore this study has been taken up with aim of prevalence of uropathogens specially ESβL producing *E.coli* and their antibiotics resistance patterns.

2. MATERIALS AND METHODS

The samples were collected from suscepted UTI patients attended different hospitals, clinics and diagnostic lab at Kalaburagi city during February 2012 to January 2017. A total of 550 individuals were included in this study and consist of both genders and different age groups. With consent of patients the samples of midstream urine were collected in sterile containers patients showing signs and symptoms of UTI in outpatient clinic, or emergency room or patients diagnosed within 48 hrs after hospitalization. A diagnosis of symptomatic UTI was made when a patient had at least one of the following signs or symptoms fever (≥38.8 °C), urgency, frequency, dysuria or suprapubic tenderness and microbial load (i.e. ≥ 10^5 microorganisms/ml of urine) (Dong *et al*., 2010). Various epidemiological factors for each patient were recorded on individual forms. That included age, gender, presence of diabetes mellitus, renal calculi, pregnancy, history of urinary cathetetization, recurrent UTI (more than 3 UTI episodes in the preceding year) and antibiotic intake in the preceding 3 months (Azap *et al*., 2010).

The collected urine samples were inoculated onto MacConkey (MAC) agar and Eosin Methylene Blue (EMB) agar by semi-quantitative method and incubated at 37 °C for 18-24 hours for isolation. The incubation period was extended if there is an absence of growth for a period of 48 hours. Identification of the isolated bacteria was performed by cultural, morphological and biochemical tests such as IMViC tests (Collee *et al*., 2013, Cheesbrough, 1989).

Thus characterized *E. coli* isolates were also stored 4 °C for further use. For all *E. coli* isolates antibiotic sensitivity test was performed by disc diffusion method (modified Kirby-Bauer method) on Mueller-Hinton agar plates using fifteen antibiotics namely Amikacin (AK), Aztreonam (AZT), Tetracycline (T), Penicillin-G, Cotrimoxazole (COT), Ciprofloxacin (CIP), Cefoxitin (CX), Cefotaxime (CTX), Ceftazidime (CAZ), Erythromycin (E), Gentamicin (GEN), Cephalothin (CEP), Nitrofurantoin (NIT), Ofloxacin (OF) and Imipenem (IMP). All the antibiotic discs used in this study procured from Hi-media Laboratories Pvt. Ltd., Mumbai, India and the antibiotic sensitivity test was performed as per the CLSI guidelines (2013).

2. 1. Combination disc method for detection of ESβL producing *E. coli*

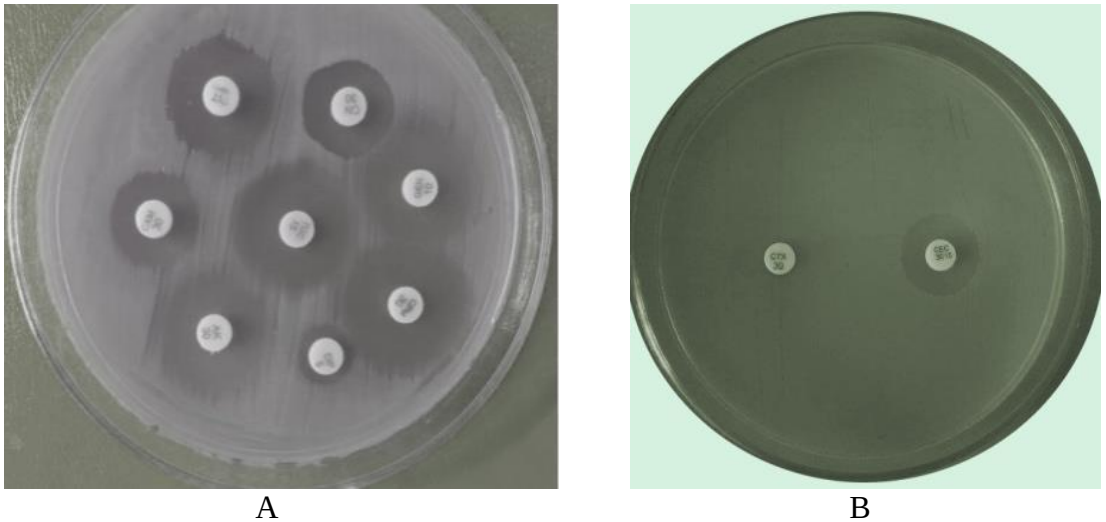
All the *E. coli* isolates showing resistance to one or more of third generation Cephalosporins (3GCs) were tested for ESβL production by the double-disc diffusion test (DDDT). In that Cefotaxime and Ceftazidime were placed 20 mm apart from a disc of Cefotaxime + Clavulanic acid (30/10 µg) and Ceftazidime + Clavulanic acid (30/10 µg) respectively on a lawn culture of *E. coli* (0.5 McFarland standard as a inoculums) on Mueller-Hinton agar plates. After overnight incubation at 37 °C ESβL production was confirmed, if there is an increase in zone diameter (≥5 mm) for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone. (Carter *et al*., 2000). *Klebsiella pneumoniae* ATCC 700603 and *E.coli* ATCC 25922 were used as positive and negative controls respectively.

3. RESULTS AND DISCUSSION

The uropathogens were isolated and identified on the basis of cultural morphology on differential MacConkey agar and selective medium Eosin Methylene Blue (EMB) agar plates. The most prevalent bacteria was *E. coli* (50.69%) which produced characteristic dark colonies with green metallic sheen on Eosin Methylene Blue agar medium and pink, lactose fermenting colonies on MacConkey agar plate (Fig. 1). The *E.coli* and other bacterial isolates were identified and cofirmed on the basis of colony morphology and biochemical (IMViC) tests. Out of 550 urine samples 288 (52.36%) yielded bacterial growth of those 146 were *E. coli*, 64 *Klebsiella* spp, 6 *Proteus* spp, 2 *S. epidermidis*, 2 *S. aureus*, 22 *Enterobacter* spp, 41 *Pseudomonas* spp, and 5 *S. typhi* were identified (Table 1). In this study, we are reporting more than 50% of uropathogenic bacterial isolates were of *E. coli* and 22.22% *Klebsiella* sps. Our results correlated with report of Mallikarjun Reddy *et al*., (2014) who reported 50% of *E.coli and* 27.38% of *Klebsiella*; however ours is slightly less than the reports(53%) of Mandal *et al.,* (2001) and Ethel *et al.,* (2006) and more than that of Acharya *et al.,*(1980;30%) and Ram *et al.,* (2000; 45.5%).

A1 A2 B

Fig. 1. Cultural and biochemical characteristics of *E. coli*
A1- Characteristic colonies with green metallic sheen on Eosin Methylene Blue (EMB) agar.
A2 - Pink colored colonies (lactose fermenter) on MacConkey agar.
B - Yellow = negative, Cherry red ring = positive for Indole production.



A B

Figure 2. Antibiogram and detection of ESβL producing *E. coli*
A. Mueller-Hinton agar plate showing Antibiogram against *E. coli* isolate by Kirby-Bauer method.
B. Plate showing detection of ESβL producing *E. coli* by Double-Disc Diffusion method.

With relation to incidence of *E. coli* isolation rate in males and females in different age groups, observed to be highest of 50% and 57.14% in the age group 1-10 years. Followed by 54.41% and 48.61% in the age group 51 and above years, however it was 19.23% and 22.72% in the age group 11-20 years, 17.24% and 20.33% in the age group 41-50 years, 16.39% and 16.66% in the age group 31-40 years and 14.03% and 14.28% in the age group 21-30 years respectively. Overall 26.81% of *E. coli* were isolated in females which is slightly more

compared to the that of males (26.27%) (Table 2). Mallikarjun Reddy *et al.,* (2014) reported almost same prevalence rate in all the age group of females, which is moderately higher incidence (36%) than in the males (23%) that is in contrast with our observations (26.81% in females and 26.27% in males). Incidence is more in males than in females in older age groups may be due to prostate enlargement which helps in retention of 2-3 ml residual urine is likely to cause UTI.

Table 1. Isolation and prevalence rate of bacteria isolated from urine

Bacterial isolates	**Number of isolates**	**Isolation rate in % (Total no of bacterial isolates = 288)**	**Prevalence rate (No of samples = 550)**
Eshcherichia coli	146	50.69	26.54
Klebsiella spp	64	22.22	11.63
Proteus spp	6	02.08	1.09
S. epidermis	2	0.69	0.36
S. aureus	2	0.69	0.36
Enterobacter spp	22	7.63	4.0
Pseudomonas spp	41	14.23	7.45
S. typhi	5	1.73	0.90
Total bacterial isolates	288	-	52.36

With relation to incidence of *E. coli* isolation rate in males and females in different age groups, observed to be highest of 50% and 57.14% in the age group 1-10 years. Followed by 54.41% and 48.61% in the age group 51 and above years, however it was 19.23% and 22.72% in the age group 11-20 years, 17.24% and 20.33% in the age group 41-50 years, 16.39% and 16.66% in the age group 31-40 years and 14.03% and 14.28% in the age group 21-30 years respectively. Overall 26.81% of *E. coli* were isolated in females which is slightly more compared to the that of males (26.27%) (Table 2). Mallikarjun Reddy *et al.,* (2014) reported almost same prevalence rate in all the age group of females, which is moderately higher incidence (36%) than in the males (23%) that is in contrast with our observations (26.81% in females and 26.27% in males). Incidence is more in males than in females in older age groups may be due to prostate enlargement which helps in retention of 2-3 ml residual urine is likely to cause UTI.

When all the 146 *E. coli* were subjected for the detection of ESβL production by DDD Test (Fig. 2),74 isolates (50.69%) of *E. coli* isolates were showed presence of ESβL enzyme.

Table 2. Isolation of *E. coli* from Male and Female of different age groups

Age (years)	Number of Samples		Number of isolates		*E. coli* isolation rate	
	Male	Female	Male	Female	Male	Female
1-10	4	7	2	4	50.00%	57.14%
11-20	26	22	5	5	19.23%	22.72%
21-30	57	56	8	8	14.03%	14.28%
31-40	61	60	10	10	16.39%	16.66%
41-50	58	59	10	12	17.24%	20.33%
51 and above	68	72	37	35	54.41%	48.61%
Total	**274**	**276**	**72**	**74**	**26.27%**	**26.81%**

Our results are in nearly correlated with the reports of Chaudhary *et al*., (2013) and Mahesh *et al*., (2010) who reported 54.5% and 56.2% of prevalence of ESβL *E. coli* respectively. However, Ravindranath Gangane *et al.,* (2017) and Singh *et al.,* (2016) reported higher prevalence rate of 61% and 82.6% respectively when compared to our results. The lower prevalence of ESβL *E. coli* was reported by Datta *et al*., (2014), Dugal *et al.,* (2013) with 21.4% and 24.4% respectively. In our earlier report, we too reported only 32.80% of prevalence rate of ESβL producing *E. coli* (Raghavendra *et al.,* 2017).

In the present study, significant differences were observed with respect to susceptibility of the isolates to β-lactams, fluoroquinolones, tetracycline and aminoglycosides for both ESβL and Non-ESβL producing *E. coli* isolates. All isolates have shown multi drug resistance i.e resistance 3 or more than three antibiotics. The Table 3 shows the percent resistance *of E.coli* isolates against 15 antibiotics tested. Highest percent of resistance was seen against third generation cephalosporins ceftazidime 93.15% and cefotaxime 90.41% than to Penicillin (92.46%), which are nearly in correlation with the reports of Rajput and Sarsaiya, (2018) and Aruna *et al*., (2012). Erythromycin and cephalothin showed more than 60% resistance which is less compared to our earlier report with 85.7% and 90.4% respectively. Amikacin, gentamicin and imipenem showed same percent resistance of 19.17 which is less than the reported by Gangane *et al*., (2017) however it comparable with the results reported by Rajput and Sarsaiya, (2018). Less than 10% of the isolates were resistant to tetracycline (6.84%) and ofloxacin(4.79%), however nitrofurantoin showed 15.75% of resistance which is much lower than the value reported by Behroozi *et al*., (2010).

ESβL detection by double disc-diffusion test (DDDT) was performed for all the 146 *E.coli* isolates (Figure-2), out of which 74 were found to be ESβL producers indicating an incidence rate of 50.69% and 72 isolates were non- ESβL producers. According to age group, the carriage rate of ESβL producing *E. coli* was highest in the age group 1-10 with 27.27%,

followed by 22.85% in the age group 51 and above, and lowest 8.84% was found in the age group 21-30 years (Table 4).

Table 3. Resistance rate of *E. coli* isolates to different antibiotics.

Sl. No	Name of antibiotics	Concentration µg/disc	No. of resistant isolates	% Resistance (n = 146)	Class of Antibiotics
1	Amikacin (AK)	30	28	19.17	Aminoglycosides
2	Gentamicin (GEN)	10	28	19.17	Aminoglycosides
3	Penicillin-G(P^{10})	10 units	135	92.46	Penicillin
4	Tetracycline (T)	30	10	6.84	Tetracycline
5	Cotrimoxazole(COT)	25	40	27.39	Sulphonamides
6	Aztreonam (AZT)	30	30	20.54	Monobactams
7	Ciprofloxacin (CIP)	5	16	10.95	Quinolones
8	Erythromycin (E)	15	90	61.64	Macrolides
9.	Imipenem(IMP)	10	28	19.17	Carbapenems
10	Nitrofurantoin (NIT)	300	23	15.75	Nitrofurans
11	Cephalothin (CEP)	30	115	78.76	Cephalosporins-I
12	Cefoxitin (CX)	30	26	17.80	Cephalosporins-II
13	Ceftazidime(CAZ)	30	136	93.15	Cephalosporins-III
14	Cefotaxime(CTX)	30	132	90.41	Cephalosporins-III
15	Ofloxacin(OF)	2	7	04.79	Quinolones

Table 4. Age group wise incidence of ESβL and Non- ESβL producing *E. coli.*

Age group (years)	Number of samples	Number of *E. coli* isolates	ESβL producers	Non- ESβL producers	ESβL Prevalence rate
1-10	11	6	3(50%)	3(50%)	27.27 %
11-20	48	10	6(60%)	4(40%)	12.50 %

21-30	113	16	10(62.5%)	6(37.5%)	08.84 %
31-40	121	20	11(55%)	9(45%)	09.09 %
41-50	117	22	12(54.54%)	10(45.45%)	10.25 %
51 and above	140	72	32(44.44%)	40(55.55%)	22.85 %
Total	**550**	**146**	**74(50.68%)**	**72(49.31%)**	**13.45%**

4. CONCLUSIONS

In conclusion, the prevalence of ESβL producing *E. coli* as uropathogen is less (50.69%) compared to the early reports from India (Andrews *et al.*, 2018 (54.79%); Chaudhary *et al*., 013 (54.5%); Mahesh *et al*., 2010 (56.2%); Ravindranath Gangane *et al.*, (2017) (61%) and Singh *et al.*, 2013 (82.60%). Our study shows not much diversity in the isolation of aerobic uropathogens when compared to other parts of India. However, Ravindranath Gangane *et al.*, reported 61% of ESβL producing *E. coli* which higher than our repot (50.68%) from the same geographical area. It may due the techniques applied for the screening of ESβL producing *E. coli.* 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 pattern newly emerging pathogens like *E. coli* that will definitely help in forming good treatment regimens for treating *E. coli* infections more efficiently and avoid emergence of multi-drug resistant microbes.

It has been argued that there is a direct relation between the antibiotic used and the frequency and kind of antibiotic resistant strains in human beings. Misuse and self-medication is a major problem in the country and lack of awareness of resistance patterns of infecting agents among the general population also accounts for the emergence of resistant pathogens. This study shows that prevalevnce of ESβL producing *E. coli* isolates from urine samples collected from the different parts of Kalaburagi city was more than 50%. However, our earlier report was bit lower (i.e 32.3%) and in 2017 one of the study reported from Kalaburagi was more (61%). This may be due to the misuse and self medication of antibiotics by patients . These ESβL producers limit the available treatment regimens and forces the clinicians to go for costlier drugs such as: carbapenems (Imipenem) for the treatment. Hence, this study highlights the need for a strict antibiotic policy for their rational use in the country.

The policy just should not stress the prevention of infections but it must ensure proper selection of antibiotics and also minimal use of antibiotics should be stressed, because the overuse of antimicrobial agents exerts a high selective pressure by which the microbes change their genetic make-up and renders the antimicrobials as ineffective. Clinicians should depend more on laboratory guidance, while laboratories must provide resistance pattern data for the optimal management of patients more rapidly. There is a need of better strategies to prevent emergence and there is an urgent need to improve strict infection control programmes. Finally, we conclude that ofloxacin, Amikacin, Nitrofurantoin and Imipenem may be considered, as the drug of choice and the present data will help the physicians to opt for the correct empirical treatment regimen.

From the public health point of view, molecular epidemiology may be carried out with a larger sample size to obtain a more conclusive scenario and possibly extend its implications to other region of Kalaburagi. The results obtained can be confirmed using molecular studies to check whether the widespread resistance is due to a specific gene or multiple genes.

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