Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 102 (2018) 193-200 EISSN 2392-2192

SHORT COMMUNICATION

Antibiogram studies and screening for ESβL (Extended Spectrum Beta Lactamase) producing *E. coli* strains isolated from clinical urine samples

Raghavendra Ramachar*, Channappa T. Shivannavar, Subhashchandra M. Gaddad

Department of Studies and Research in Microbiology, Gulbarga University, Kalaburagi, 585106, Karnataka State, India

*E-mail address: raghudream.mathad@gmail.com

ABSTRACT

Bacteria are capable of invading and infecting humans, leading to disease and sometimes death. Different body organs and tissues are vulnerable to different organisms. This study was aimed to isolate and identify the aerobic bacteria causing Urinary Tract Infection in different age groups. The 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 (MAC) 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 2016. Out of 600 isolates screened 146 *E. coli* isolates were isolated which were multidrug resistant with 50% isolates being ESβL producers. All ESβL producing *E. coli* isolates were resistant to ceftazidime and exhibited higher level of resistance to Cephalothin, Erythromycin, Cotrimoxazole and Aztreonam. ESβL producing organisms limits the available treatment options. So, the prudent use of antibiotics will help in curing the disease without going for the costly drugs such as Carbapenems. We suggest nitrofurantoin, ofloxacin and tetracycline may be considered as drug of

(Received 14 June 2018; Accepted 29 June 2018; Date of Publication 29 June 2018)

choice for the treatment in UTI patients. The ESβL production should be continuously monitored in the clinics and hospitals as to avoid the emergence of drug resistance.

***Keywords*:** Uropathogenic, *Escherichia coli*, Green Metallic Sheen, Indole production, Antibiogram, ESβL, Phenotypic detection, Double-disc diffusion test

1. INTRODUCTION

Escherichia coli is a common pathogen causing community-acquired urinary tract infections (UTIs) in adults as well as in pediatric age groups. Community acquired urinary tract infection (UTI) due to *Escherichia coli* is one of the most common form of bacterial infections, affecting people of all ages. Originally ESβL (extended spectrum beta lactamases) producing *E. coli* was isolated from hospital setting but lately this organism has begun to disseminate in the community. Urinary Tract Infection (UTI) is the commonest infection seen in clinical practice. An estimation says that 10% of the patients visiting the hospitals suffer from UTI (Taslima Taher Lina et al., 2007). Both sexes of all age groups are vulnerable to UTI. Women are especially prone to UTI and 20% of women will suffer from UTI in their lifetime (Ramprasad et al., 1993). UTI is a major cause among hospital acquired infections.

Apart from socioeconomic reasons such as illiteracy, ignorance and insaniataion 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. UTI presents protein manifestations and may be asymptomatic (Hanif S, 2006). There is an occurance of different types of organisms in different areas (Mandal P et al., 2001).

In India, community presence of ESβL producing organisms has been well documented. However, various epidemiological factors associated with producing strains has 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.

A community UTI when complicated may be potentially a life threatening condition. 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 number of reports on ESβL producing *E. coli* strains. Hence, the following study was undertaken.

2. MATERIALS AND METHODS

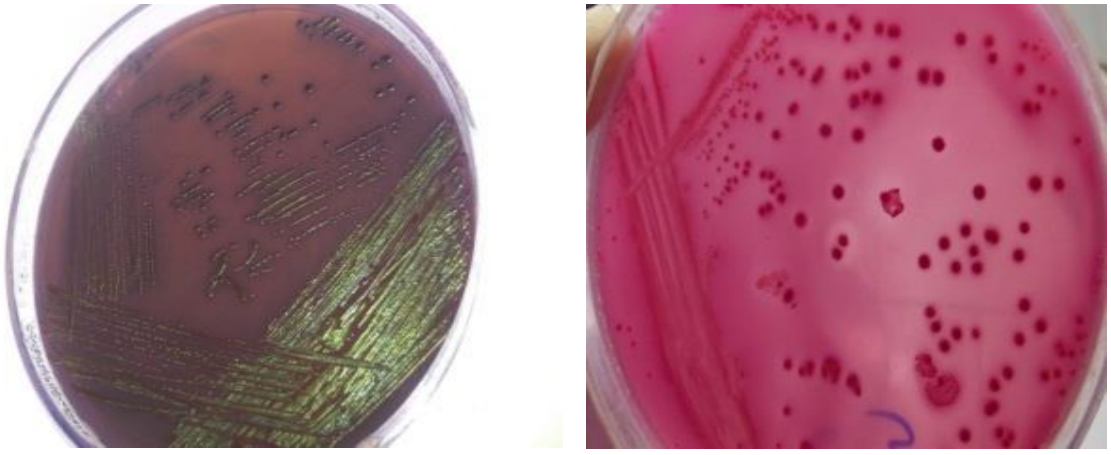
This study was conducted in Department of Studies and Research in Microbiology, Gulbarga University, Kalaburagi, India from February 2012 to January 2017. Six hundred midstream urine samples (non-repeatative) were collected in sterile containers, from patients from whom consent was obtained, with a suggestive history of UTI. The study group included patients showing signs and symptoms of UTI in outpatient clinic, or emergency room or patients diagnosed within 48 hrs after hospitalization. These patients were labeled as patients having a community UTI. A diagnosis of symptomatic UTI was made when a patient had atleast one of the following signs or symptoms: fever ≥38.8 °C, urgency, frequency, dysuria or suprapubic tenderness and a positive urine culture (i.e. $\geq 10^5$ microorganisms/ml of urine (Dong SL et al., 2010). Various epidemiological factors for each patient were recorded on

individual forms. That included age, presence of diabetes mellitus, renal calculi, pregnancy, history of urinary instrumentation, recurrent UTI (more than 3 UTI episodes in the preceding year) and antibiotic intake (use of beta-lactam in the preceding 3 months) (Azap OK et al., 2010; Ravikant et al., 2016)).

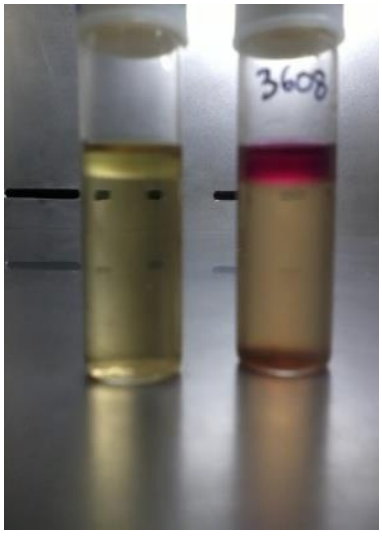
These patients were from 1 to 75 years of age: and of sex, 20 with essential hypertension, and 52 with diabetes mellitus. Pregnant women, women having thyrotoxicosis, genitouritary procedure, carcinoma, vaginitis, prostitis, recipient of renal transplant and with a history of previous or recent hospitalization were excluded from the study.

Midstream urine samples were collected aseptically and with all sterile precautions from patients symptoms like; fever, chills, frequency and urgency of urination, dysuria and suprapubic pain were inoculated onto MacConkey agar and Eosin Methylene Blue Agar and incubated at 37 °C for 18-24 hours for isolation. The incubation period was extended if there is an absence of cultural growth for a period of 48 hours. Identification of the aerobic bacteria was performed by various biochemical reactions (Collee JG et al., 2013, Cheesbrough, M 1989), (**Figure 1**).

A.



B.



Yellow = negative, Cherry red ring = positive for Indole production.

C.

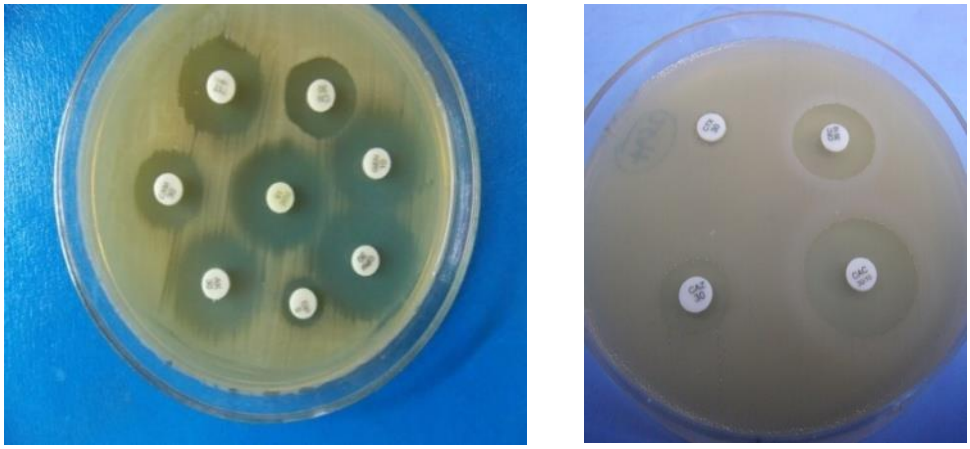


Figure 1. (**A**) Plates showing growth of *E. coli* on MacConkey agar (MAC) and Eosin Methylene Blue Agar respectively; (**B**) Tubes showing Indole producing *E. coli*; (**C**) Mueller-Hinton agar plates showing Antibiogram and ESβL production.

Antibiotic sensitivity test was done by disc diffusion method (modified Kirby-Bauer method) on Mueller-Hinton agar plates using fifteen antibiotics, Amikacin (AK) , Aztreonam (AZT), Tetracycline (T), Penicillin-G (P^{10}), 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 chemical discs were procured from Hi-media Laboratories Pvt. Ltd, Mumbai, India and the antibiotic sensitivity test was performed as per the CLSI guidelines (2016).

2. 1. Combination disc method for ESβL detection

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 20 mm from a disc of Cefotaxime + Clavulanic acid (30/10 µg) and Ceftazidime + Clavulanic acid (30/10 µg) (Procured from Hi-media Laboratories, Mumbai, India) respectively on a lawn culture of *E. coli* (0.5 McFarland inoculums size) on Mueller-Hinton agar plates. After overnight incubation at 37 °C ESβL production was confirmed if there was ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanete versus its zone when tested alone. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively

3. RESULTS AND DISCUSSION

Total of 600 midstream urine samples were collected aseptically for the isolation of uropathogen. Out of 600 samples 288 samples (48%) yielded bacterial growth. Out of 600 urine samples 146 isolates of *E. coli* were obtained with a prevalence of 50.69% (**Table 1**) which is less when compared to the report of Mahesh et al., (2010) and Chaudhary et al., (2013) (**Table 2**) who reported 56.2% and 54.5% of prevalence of *E. coli* respectively.

Table 1. Bacteria isolated from urine

Bacteria	Number of isolates	% isolation
Eshcherichia coli	146	50.69
Klebsiella spp	64	22.22
Proteus spp	6	02.14
S. epidermis	2	0.69
S. aureus	2	0.69
Enterobacter spp	22	7.63
Pseudomonas spp	41	14.23
S. typhi	5	1.73

Table 2. Uropathogenic *E. coli* from other workers

S. No	Studies	Year	Prevalence
1	Chaudhary et al.,	2013	54.5%
2	Ravindranath Gangane et al.,	2017	61%
3	Singh N et al.,	2016	82.6%
4.	Dugal S et al.,	2013	24.4%
5.	Datta et al.,	2014	21.4%
6.	Mahesh et al.,	2010	56.2%
7.	**Present study**	**2018**	**50.69%**

With relation to age group, it was observed that highest of 54.54% is in the age group 1-10, followed by 44.44% in the age group 51 and above, 20.83% in 11-20, 14.96% in 41-50, 14.18% in 31-40 and 13.0% in the age group 21-30 respectively (**Table 3**).

In the present study, significant differences were observed with respect to susceptibility of the isolates to fluoroquinolones, 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-G (92.46%), Cephalothin (78.76%) and Erythromycin (61.64%) respectively. Lowest resistance wa observed for Ofloxacin 4.79%, Imipenem and Amikacin with19.17%, Tetracycline and Nitrofurantoin with 6.84% and 15.75% respectively which is much lower than the value reported by Behroozi A et al., (2010). 93.15% resistance observed in *E. coli* for the antibiotic ceftazidime followed by 92.46% penicillin-G, 90.41% cefoaxime, 78.76% cephalothin. Most of the isolates were sensitive to ofloxacin (4.79%) and tetracycline (6.84%) and Imipenem (19.17%) which is nearly equal to Daryl et al. (2014), (**Table 4**).

Table 3. Incidence of *E. coli* from UTI among male and female of different age groups

Age (in years)	Number of samples		Number of isolates		*E. coli* isolation rate	
	Male	Female	Male	Female	Male	Female
1-10	4	7	2	4	50 %	57.14%
11-20	26	22	5	5	19.23%	22.72%
21-30	67	56	8	8	11.94%	14.28%
31-40	71	70	10	10	14.08%	14.28%
41-50	78	69	10	12	12.82%	17.39%
51 and above	80	82	41	35	42.68%s	27.04%
Total	294	306	70	76	23.80%	24.83%

Table 4. Resistance rate of *E. coli* isolates to different antibiotics

Sl. No	Name of antibiotics	Concn µg/disc	No. of resistant isolates	Resistance rate (%) (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

ESβL detection by double disc-diffusion test (DDDT) was performed for all the 146 *E. coli* isolates (**Figure 1**), out of which 74 were found to be ESβL producers indicating an incidence rate of 50.69% and 72 isolates were non- ESβL producers. Majority of the isolates are Multi-drug resistant.

In conclusion, the isolation rate of *E. coli* as uropathogen is similar to the early reports from India (**Table 2**). Among *E. coli* isolates, 1/2nd of them were ESβL producers. Policy makers in Indiahave taken initiation by making "National Policy for Conainment 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.

4. CONCLUSIONS

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 resitance patterns among the general population also accounts for the emergence of resistant pathogens.

This study highlights the needs for an antibiotic policy for their rationale 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. 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 Gentamicin, Nitrofurantoin and Imipenem can be considered as the drug of choice and the present data will help the physicians to opt for the correct empirical treatment regimen.

ACKNOWLEDGEMENTS

Authors would like to thank Gulbarga University Kalaburagi for providing financial assistance through "University Research Studentship for Meritorius Students (GUG/DEV-III/2012-13/433). We thank Nandan Diagnostic center, Pooja Diagnostic Lab, Mediscan Diagnostics and Shridhar Diagnostic Center for providing the clinical samples for our studies.

References

[1] Taslima Taher Lina et al., (2007). Multiple antibiotic resistances medited by plasmids and integrons of uropathogenic *Eshcherichia coli* and *Klebsiella pneumoniae*. *Bangladesh J Microbiol.* 24: 19-23.

[2] Ramprasad AV et al., (1993). Urine culture sensitivity pattern in a private laboratory setup. *Indian J Path Microbiol*. 36(2): 119-23.

[3] Hanif S. (2006). Frequency and pattern of urinary complaints about pregnant women. *JCPSP* 16(8): 514-17.

[4] Mandal P et al., (2001). Uropathogenic *Eshcherichia coli* causing urinary tract infections. *Indian J Med Res*. 114: 207-11.

[5] Collee JG et al., *Mackie and McCartney: Practical Medical Microbiology*-14th Ed. Elsevier, 2013.

[6] Carter MW et al., (2000). Detection of Extended Spectrum beta-lactamases in Klebsiella with the Oxoid combination disc method. *J. Clin. Microbiol.* 38: 4228-4332.

[7] Cheesbrough, M. Medical Laboratory Manual Tropical Countries, Vol. II, Microbilogy. Cambridge, Great Britain, 1989, pp. 248-263.

[8] Kariuki et al., (2007). *Eshcherichia coli* from commonly-acquired urinary tract infections resistant to fluoroquinolones and extended spectrum beta lactams. *J. Infect. Developing. Count*. 1: 257-262.

[9] Clinical and Laboratory Standards Institute (2016). Performance standards for Antimicrobial Susceptibility Testing: Twenty-sixth Informaional Supplement CLSI *document.*M100-S26. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA.

[10] Bauer et al., (1966). Antibiotic susceptibility testing by a standardized single disc method. *American journal of clinical pathology* 22(3), 172.

[11] Behroozi A et al. (2010). Frequency of extended spectrum beta lactamase (ESβLs) producing *Eshcherichia coli* and *Klebsiella pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital. *Africal Journal of Microbiology Research,* 4(9), 881-884.

[12] Datta, P et al., (2014). Community Urinary Tract Infection due to ESβL producing *E. coli:* Epidemiology and Susceptibility to oral antimicrobials including Mecillinam. *Nepal Journal of Medical Sciences* 3(1), 5-7.

[13] Paterson DL, Bonomo RA (2005). Extended Spectrum Beta Lactamases: a clinical update. *Clinical Microbiology Reviews* 18(4): 657-686.

[14] Ravindranath Gangane and Javeria Firdous (2017). Isolation and Antibiotic Sensitivity Pattern of Extended Spectrum Beta Lactamases (ESβL) producing *Eshcherichia coli* Isolated from Urinary Tract Infection. *Int. J. Curr. Microbiol. App. Sci.* 6(6), 279-286.

[15] Raghavendra Ramachar et al., (2017). Antimicrobial Susceptibility of Extended Spectrum Beta Lactamase (ESβL) producing *E. coli* from urinary tract infected patients from hospital and diagnostic centers in Kalaburagi, Karnataka, India, *International Journal of Current Advanced Research* 06(12), 8467-8470.

[16] Ravikant, Parveen Kumar, Swapnil Ranotkar, Shubhranshu Zutshi, Mangala Lahkar, Chimanjita Phukan & Kandarpa K Saikia. Prevalence and identification of extended spectrum β-lactamases (ESBL) in *Escherichia coli* isolated from a tertiary care hospital in North-East India. *Indian Journal of Experimental Biology* Vol. 54, February 2016, pp. 108-114