

Determination of ESBL Production & Antibiotic Resistance Profile of *Escherichia coli* Isolated from Patients with Urinary Tract Infections: A Study from Tertiary Care Hospital

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Abstract :

Urinary tract infection (UTI), is defined as a disease caused by invasion of urinary tract by microorganisms. Majority of UTI cases are due to bacterial infection constitute about 95% of total UTI cases. About 80% of UTI cases are caused by *E. coli* producing extended spectrum β -lactamase (ESBL) producing isolates. In recent years limitations in treating infections caused by multidrug resistant organisms has increased. This study aims to determine ESBL production of *E. coli* cases from a tertiary care hospital. **Methodology:** A total 358 midstream urine samples were collected by total consecutive sampling method during March 2015 to June 2018. Identification, antibiotic sensitivity testing, performed according to standard protocol following Clinical and Laboratory Standard Institute (CLSI) guidelines, 2013. Screening for ESBL producing *E. coli* isolates performed using ceftazidime further confirmation done by phenotypic disc diffusion test using combined disc method using ceftazidime (30 μ g) & ceftazidime/ clavulanic acid (30/10 μ g) as per CLSI guidelines. **Results:** Total 358 specimens processed for urine culture. Gram negative bacilli isolated from 123(34.35 %), out of which 68 (55.28%) were *E. coli*, 19 (15.44%) *K. pneumoniae*, 15 (12.19%), *Pseudomonas spp.* 08 (6.50%), *Citrobacter spp* and *Acinetobacter spp*, 03 (2.43%), *Proteus mirabilis*, 01 (0.81%) *Proteus vulgaris* and *Enterobacter* respectively. Out of 68 isolates of *E. coli*, 65 (95.58%) were MDR, ESBL was detected in 31 (47.69%) out of these 65 isolates. Out of these 31 cases 19 (61.29%) were female and 12 (38.70%) were male cases. **Conclusion:** This study concludes 47.69%

ESBL producing MDR *E. coli* were isolated from UTI cases with female predominance.

Key Words: Extended spectrum Beta-lactamase, Urinary Tract Infection, *E. coli*, *K. pneumoniae*, *Pseudomonas*, *Acinetobacter*, Antibiotic Sensitivity, Antimicrobial Drug Resistance, Multidrug Resistance.

Introduction:

Urinary tract infection (UTI) is a common infective syndrome in all age groups and both genders.⁽¹⁾ Urinary tract infection is defined as a disease caused by invasion of urinary tract by microorganisms.⁽²⁾ This invasion may extend from renal cortex of kidney to urethral meatus⁽²⁾. The rate of UTI is high in females than in males. Females are more susceptible, due to conditions like anatomy of urinary tract, hormonal changes, pregnancy and behavioural patterns⁽¹⁾. Majority of UTI cases are due to bacteria which constitute about 95 % of total UTI cases.⁽³⁾ More than 80% of UTI is caused due to *E. coli*.⁽³⁾

A significant change in proportion and number of isolation of multi drug resistant (MDR) pathogens has increased over last decade. Global organizations such as World Health Organization (WHO), Centre for Disease Control and Prevention (CDC), and European Centre for Disease Prevention and Control (ECDPC), consider MDR isolates as a global threat to public health worldwide.⁽⁴⁾ The MDR isolates are associated with increased morbidity and mortality, such agents usually fails to respond to conventional treatment so become more tedious.⁽⁵⁾ So although rational use of antibiotics is necessary, development of new antimicrobial agent is in pipeline.⁽⁴⁾

Considering this background it becomes inevitably important to mention that β -lactamases production by gram negative bacilli is the most leading cause of resistance to the β -lactam antibiotics.⁽⁶⁾

The ESBL production is either chromosomal or plasmid mediated.⁽⁷⁾ Chromosomally mediated ESBL production is due to expression of AmpC gene, either constitutive or inducible.^(8,9) It can also be due to other clinically important genes like VEB, PER, BEL-1, BES-1, SFO-1, TLA and IBC-II.^(9,10) Plasmid mediated ESBL production is due to TEM-1, TEM-2 and SHV-1, point amino acid substitution in these classical plasmid mediated β -lactamases can increase spectrum of activity in 1st generation β -lactam and 3rd generation cephalosporins and even in monobactams.⁽¹⁰⁻¹³⁾

Among the list of ESBL producer gram negative bacilli *E. coli* and *Klebsiella pneumoniae* are significantly higher in global prevalence.^(1, 6,14-17)

ESBL production due to plasmid is mediated by conjugation and hence seeks attention in view of infection control, clinical and therapeutic implications. Hence the present study aims to determine ESBL production and antibiotic resistance profile of *Escherichia coli* from cases of UTI in tertiary care hospital in Ahmednagar, Maharashtra state.

Material & Methods:

Place of Study: This is a hospital based study carried out in the Department of Microbiology, Dr. Vithalrao Vikhe Patil Foundation's Medical College and Hospital, which is a 750 bedded tertiary care hospital situated in Ahmednagar district of Maharashtra state in India.

Period Of Study: This study was carried out between March 2015 to June 2018.

Study Population: The study was carried out on the symptomatic patients attending the hospital for treatment.

Ethics Statement: The study was approved by Institutional Ethical Committee of DVVPF's Medical College, Ahmednagar.

Inclusion criteria:

1. Subjects having symptoms of UTI's and referred by clinicians to carry out urine culture and antibiotic sensitivity testing.
2. Subjects of all the age groups and all the genders were included.
3. Subjects with comorbid aetiologies were included.
4. Subjects belonging to the OPD, IPD, ICU.
5. Only those specimens which were collected in containers provided by the laboratory or IPD were accepted.

Exclusion criteria:

1. Specimens not collected and labelled properly as per

standard guidelines.

2. Any specimen which was collected by the patient in a self-arranged container.
3. Subjects not willing to participate in the study.

Sampling method: Total consecutive sampling method was followed.

Sample size: 358

Type of study: It is a cross sectional study.

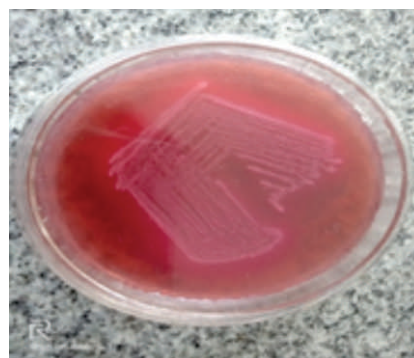
Methodology: Urine specimens received in Dept. of Microbiology from March 2015 to June 2018 were included in study and subjected for following examinations.

1. Macroscopic Examination: The specimens were macroscopically observed for its natural colour, turbidity and presence of blood.⁽¹⁸⁾

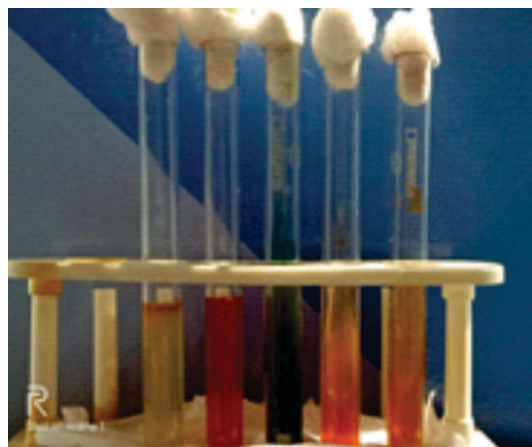
2. Microscopic Examination:⁽¹⁸⁾

1. Wet mount were prepared using a clean grease free slide with one drop of urine placed in centre of slide and a cover slip kept on it.
2. Wet mount preparations of the specimens were observed microscopically at 10X and 40X objective lenses for presence of pus cells, bacteria, fungi, parasites and any other casts and crystals.

3. Culture: Urine culture was performed by standard procedures.⁽¹⁸⁾ There after specimens were stored. Bacterial isolates were confirmed to be *E. coli* by biochemical properties as per standard guidelines.⁽¹⁹⁾



Lactose fermenting colonies of *E. coli* on Mac Conkey's Agar



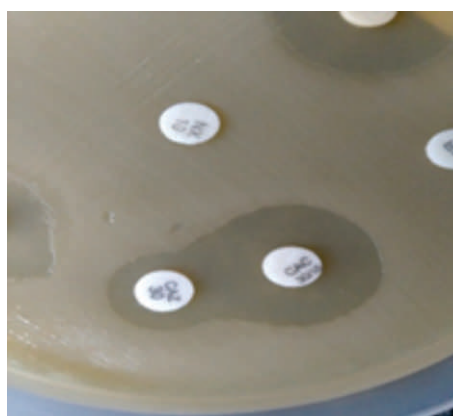
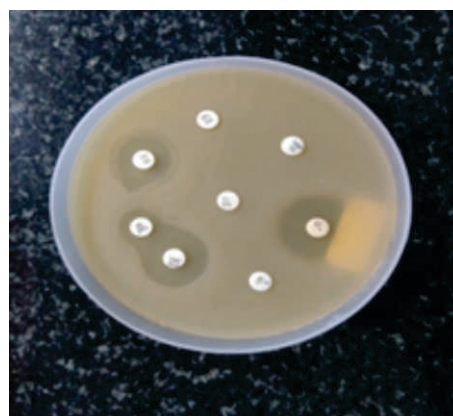
IMViC tests and TSI showing biochemical identification of isolates of *E.coli*

4. Antibiotic Sensitivity Testing: Antibiotic sensitivity testing of *E. coli* isolates was done by Kirby-Bauer disc diffusion method (Bauer, 1966) on Mueller Hinton agar plates. The procedure was carried out as per the guidelines provided by Clinical and Laboratory Standards Institute (CLSI).⁽¹⁰⁾ Briefly to explain, *E. coli* culture was grown overnight and suspension of it was prepared in 0.5ml peptone water. The density of suspension was adjusted to 0.5 Mc Farland's constant. A lawn culture of this suspension was made on Mueller Hinton agar plate. Antibiotic discs were seeded on the plate and incubated at 37°C for 24 hrs. Diameters of zone of inhibition were recorded. Based on the zone diameters, the isolates were classified as sensitive, intermediate sensitive and resistant as per the CLSI guidelines. There after the strains were screened for ESBL production.

5. Screening of ESBL-Producing Strains: According to CLSI guidelines, strains showing zone of inhibition of ≤ 22 mm for ceftazidime and/or ≤ 27 mm for cefotaxime were suspected for production of ESBL.⁽¹⁰⁾ The suspected strains were further confirmed for ESBL production.

6. Confirmation Of Extended Spectrum B-Lactamases (ESBL) Producers: ESBL production among potential ESBL-producing isolates was confirmed phenotypically using combined disc method.

Comparison of the zone of inhibition was made for the ceftazidime (30 μ g) vs. that of the ceftazidime disc containing clavulanic acid (30/10 μ g), when placed 15 to 20mm apart (center to center), Isolates showing an increase in zone diameter of ≥ 5 mm around the clavulanate combined discs compared to that of the disc alone was confirmed to be an ESBL producer.⁽¹⁰⁾



ESBL Production detection by phenotypic test done on MH agar using Ceftazidime and Ceftazidime / Clavulanic acid disc (Combination disc test).

Results:

Table No. 1: The distribution of specimens in detailed

Sr. no.	Culture particulars	No. of cultures (n=358) (%)
1	No growth	176 (49.16)
2	Insignificant bacteriuria	19 (5.30)
3	Contamination	07 (1.95)
4	<i>Candida spp.</i>	13 (3.63)
5	Gram negative bacilli	123 (34.35)
6	Gram positive cocci	20 (5.58)
Total		358 (100)

A total of 358 specimens were processed for culture. Out of which 123 were Gram negative isolates and 20 were Gram positive isolates. *Candida species* (spp) grown in 13 specimens out of 358. No growth observed in total 176 specimens out of 358 and insignificant bacteriuria observed in 19 specimens out of 358.

Table No. 2: The details of isolates of Gram negative bacilli

Sr. no.	Gram negative bacilli	No. of isolates (n=123)	(%)
1	<i>E. coli</i>	68	55.28
2	<i>Klebsiella pneumoniae</i>	19	15.44
3	<i>Pseudomonas aeruginosa</i>	15	12.19
4	<i>Citrobacter</i>	08	6.50
5	<i>Acinetobacter</i>	08	6.50
6	<i>Proteus mirabilis</i>	03	2.43
7	<i>Proteus vulgaris</i>	01	0.81
8	<i>Enterobacter</i>	01	0.81
Total		123	100

The Gram negative bacilli were isolated from 123 specimens. *E. coli* was isolated from total 68 samples. *K. pneumoniae* isolated from total 19 specimens. *Pseudomonas aeruginosa* isolated from total 15 out of 123. *Citrobacter* & *Acinetobacter species*. were recovered from 8 specimens respectively from total 123 samples. *Proteus mirabilis* isolated from 03 specimens out of 123. While 01 isolate of *Proteus vulgaris* & *Enterobacter spp.* respectively.

Table No. 3: Distribution of total number of *E. coli* isolates and details of strains with Multidrug resistance and ESBL productions

Sr. No.	Gram negative bacilli	Total no. of isolates (n=123) (%)	No. of MDR strains (n=68) (%)	ESBL Confirmed (n=65) (%)
1	<i>E. coli</i>	68 (55.28)	65 (95.58)	31 (47.69)

Out of 123 isolates of gram negative bacilli 68 (55.28%) isolates were of *E. coli*. Out of 68 *E. coli* isolates 65 (95.58%) were MDR isolates. ESBL enzymes could be detected from 31 (47.69%) out of 65 MDR *E. coli*. Total number of *E. coli* isolates, total number of MDR *E. coli* isolates and total number of ESBL producer out of MDR isolates details given in table no. 3.

Table No. 4: Sex wise distribution of ESBL producing isolates of *E. coli* from total number of isolates

Sr. No.	Sex	<i>E. coli</i> isolates (%) (n=68)	MDR (n=65) (%)	ESBL producer (n=31) (%)
1	Male	22 (32.35)	22 (33.84)	12 (38.70)
2	Female	46 (67.64)	43 (66.15)	19 (61.29)
Total		68 (100)	65 (100)	31 (100)

Table No. 5: Antibiotic resistance pattern of ESBL producing MDR isolates of *E. coli*

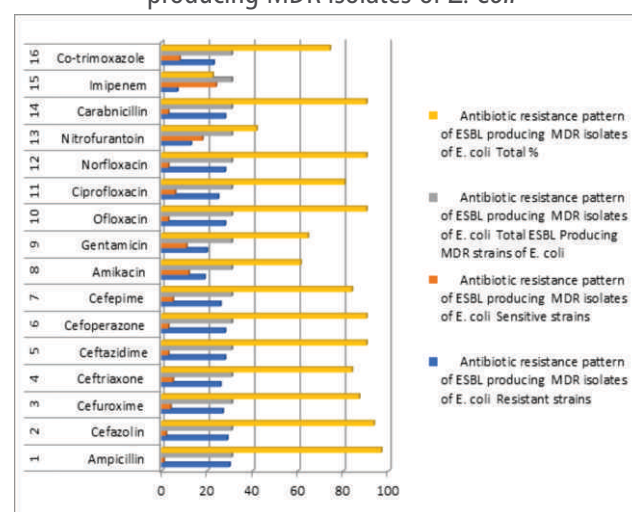


Table No. 5: Antibiotic resistance pattern of *E. coli* isolates

Sr. No.	Name of antibiotic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
1	Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	31
2	Cefazolin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	29
3	Cefuroxime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	27
4	Ceftriaxone	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	26
5	Cefepime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	26
6	Cefoperazone	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	26
7	Ceftazidime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	26
8	Amikacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	19
9	Gentamicin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	20
10	Ofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	28
11	Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	28
12	Norfloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	28
13	Nitrofurantoin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	13
14	Carabimicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	31
15	Imipenem	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	7
16	Co-trimoxazole	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	31

Antibiotic resistance pattern of the *E. coli* isolates: A high resistance was observed for Ampicillin in 30 (96.77%) isolates out of the 31 isolates of ESBL producing *E. coli*. Followed by Cefazolin 29 (93.54%), Ceftazidime, Cefoperazone, and Ofloxacin i.e. 28 (90.32%) respectively. Followed by Cefuroxime 27 (87.09%) and Ceftriaxone 26 (83.87%). Least resistance was noted for Imipenem that in only 7 (22.58%) isolates out of 31 isolates of ESBL producing *E. coli*. Followed by Nitrofurantoin 13 (41.93%), Amikacin 19 (61.29%) and Gentamicin 20 (64.51%).

Discussion:

In recent years the ESBL producing MDR *E. coli* strains due to their rapid spread have emerged as most important global pathogen both in hospital as well as in community acquired infections.⁽¹⁾ The present study noted *E. coli* as the most predominant gram negative bacilli isolated (55.28%) from 123 out of 358 urine specimens which were cultured. These results are almost similar with the findings of Nanoty VV, who reported 55.85% *E. coli* isolates as a main pathogen responsible for UTI in their study from Maharashtra.⁽⁴⁾ This is in accordance with Niranjana V and Malini A, who reported *E. coli* to be the commonest (56.8%) uropathogen.⁽²⁰⁾ Our findings are also fairly close to Bhattacharya S *et al* who also reported *E. coli* to be the most common (50%) urinary isolate.⁽²¹⁾

Out of 68 *E. coli*, 65 (95.58%) were MDR. Our findings are higher than Shakya *et al.* and Niranjana S and Malini A, who reported 79.7% and 76.5% MDR *E. coli* from urine respectively.^(1, 20)

Out of the 65 MDR *E. coli*, 31 (47.69%) were ESBL producers. Shakya *et al.* reported 91.7% ESBL producing MDR *E. coli* from urine and Andrews *et al.* reported 45.5% ESBL producing *E. coli* from various clinical specimens.^(1, 22)

Female preponderance (61.29%) as compared to males (38.70%) was observed in the present study. This is in contrast to Andrews *et al.* who reported 56.4% prevalence of ESBL producing *E. coli* in male and 43.5% in females.⁽¹⁶⁾ Women are known to be more prone to UTI due to reasons like hormonal influences, anatomical differences and behavioural patterns.⁽²³⁾

The results of antibiotic resistance pattern of our study are in well accordance with the reported resistance pattern by Niranjana V and Malini A which is described in the table below:

Sr. No.	Name of Antibiotic	High Resistance noted in present study	High Resistance reported by Niranjana V and Malini A (14)
1	Ampicillin	96.77%	88.4%
2	Cefuroxime	87.09%	72.2%
3	Ceftriaxone	83.87%	71.4%
4	Co-trimoxazole	74.19%	64.2%
Sr. No.	Name of Antibiotic	Least Resistance noted in present study	Sensitivity reported by Niranjana V and Malini V (14)
1	Imipenem	22.58%	98.9%
2	Nitrofurantoin	41.93%	82.1%
3	Amikacin	61.29%	82.6%

Our study observation records that Imipenem is the drug of choice for treating urinary tract infections caused by ESBL producing MDR *E. coli*, but as carbapenems is not an empirical antibiotic its choice should be reserved for life threatening infections hence must be used judiciously. Nitrofurantoin, Gentamicin and Amikacin can be used as an empirical antibiotic for treating sensitive cases.

Conclusion:

- The study concludes that there is 47.69% presence of ESBL producing MDR *E. coli* with female predominance from urine specimens in our settings.
- The study recommends judicious use of antibiotic to treat UTI cases.
- Antibiotics should be administered to the patients only when it is absolutely necessary and after performing the antibiotic sensitivity testing according to CLSI guidelines
- It also suggests a continuous surveillance system to be set up to monitor the increasing antibiotic resistance among bacteria and formulate an effective antibiotic policy for control and treatment of UTI cases in our setup.

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