# Prevalence of ESBL Producing *E. coli* and *Klebsiella* Species in a Tertiary Care Hospital, Telangana

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## ABSTRACT

### BACKGROUND

Extended Spectrum Beta - Lactamase producing organisms causing urinary tract infections (ESBL- UTI) are increasing in incidence and pose a major burden to healthcare. We wanted to assess the prevalence of ESBL producing *E. coli* and *Klebsiella* spp. in a tertiary hospital using a phenotypic detection procedure based on the combined disk diffusion method.

## METHODS

This is a prospective study done among 600 midstream urine samples in the Department of Microbiology at Osmania Medical College and General Hospital, Hyderabad, Telangana over a period of one-year, between March 2019 and February 2020.

## RESULTS

A total of 600 urine samples were studied and 385 (64 %) showed significant growth. A higher prevalence of ESBL production was observed in *Klebsiella* species (70.3 %) followed by *E. coli* (50 %). Out of 385 isolates, 229 isolates of *E. coli* and *Klebsiella* spp. passed ESBL screening test but only 36 (15.7 %) were confirmed ESBL producers phenotypically.

## CONCLUSIONS

Hospitals should develop strategies to minimize the spread of ESBL producing organisms by observing universal precautions and minimizing contact among hospitalized patients. This might reduce the spread of ESBL producing organisms in the community. Adherence to recommended hand washing techniques or use of hand rubs may help to prevent transmission of these infections from one patient to the other.

## **KEYWORDS**

E. coli, Klebsiella Species, ESBL

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## BACKGROUND

Urinary Tract Infection (UTI) is a spectrum of diseases caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus.1 UTI is an extremely common condition that occurs in both males and females of all ages. The prevalence and incidence of infection are higher in women than in men, which could be due to several clinical factors including anatomical differences, hormonal effects and behaviour patterns.<sup>2</sup> Enterobacteriaceae including Escherichia coli and Klebsiella are the leading cause of UTI.<sup>3</sup> ESBLs are encountered globally and are found in a significant percentage of Escherichia coli and Klebsiella pneumoniae strains in certain countries. They are also seen in other Enterobacteriaceae strains and Pseudomonas aeruginosa. Microbes having these  $\beta$  - lactamases pose numerous therapeutic challenges to the clinician.<sup>4</sup> Extended Spectrum Beta - Lactamase producing organisms causing urinary tract infections (ESBL - UTI) are increasing in incidence and pose a major burden to health care. Risk factors for UTIs caused by ESBL producers are many which include recent hospitalization and antibiotic treatment, especially the use of 2<sup>nd</sup> or 3<sup>rd</sup> generation Cephalosporins, Quinolones and Penicillins, men over 60 years of age, diabetics, recent Klebsiella pneumoniae infections.5 Increase in extended spectrum  $\beta$  - lactamases (ESBL) producing microbes in recent years has led to limitations of treatment options.<sup>6</sup>

We wanted to assess the prevalence of ESBL producing *E. coli* and *Klebsiella* species at a tertiary hospital using a phenotypic detection procedure based on the combined disk diffusion method. We also wanted to evaluate the urinary tract infections caused by ESBL producers and determine the antibiotic susceptibility patterns at Osmania General Hospital, Hyderabad.

## METHODS

This is a prospective study done in the Department of Microbiology at Osmania Medical College and General Hospital, Hyderabad, over a period of one year from March 2019 to February 2020. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from all the cases included in the study. A total of 600 midstream urine samples were collected and analysed in the study.

A total of 600 midstream urine samples were collected. All Midstream urine samples (MSU) were cultured on routine culture media. 1  $\mu$ L of urine was inoculated on MacConkey and blood agar plate by streaking semi-quantitatively and incubated aerobically for 18 - 48 hours at 37 °C. Growth of 100 colonies or more, i.e. 10<sup>5</sup> colony forming units (CFU) / mL urine, was considered as culture positive. Isolation and identification of isolates was done following their morphology in Gram's staining, cultural characteristics and biochemical properties.

Antibiotic susceptibility testing was done by modified Kirby - Bauer disc diffusion method and interpretation was done following Clinical and Laboratory Standard Institute (CLSI) guidelines of 2018. Strains showing zone of inhibition of  $\leq$ 25 mm for ceftriaxone and / or  $\leq$ 17 mm for cefpodoxime and / or  $\leq$ 27 mm for cefotaxime were considered for confirmational test using combined disc method.

Comparison of the zone of inhibition was made for the cefotaxime (30 µg) discs alone versus that of the cefotaxime discs containing clavulanic acid (10 µg), when placed 25 mm apart (center to center). Isolates showing an increase in zone diameter of  $\geq$ 5 mm around either of the clavulanate combined discs compared to that of the disc alone was considered ESBL producer.

### RESULTS

The present study had a total of 600 midstream urine samples. Significant growth was seen in 385 (64 %) samples. The ratio of number of inpatients to outpatients was 1:1.5.

Age (in years)	Males (n)	Females (n)	Total (%)	
20 - 30	48	85	133 (34.5 %)	
31 - 40	42	83	125 (32.4 %)	
41 - 50	11	39	50 (12.9 %)	
50 - 60	28	49	77 (20 %)	
Total	129	256	385 (100 %)	
Table 1. Age and Gender Distribution of				
ESBL Producing Isolates				

The prevalence was higher among 21 - 40 years age group as most isolates, accounting for 66.9 %, were isolated from this group. In the present study there was a slight female preponderance and the male to female ratio was 1 : 1.9. It was also observed that females were higher in all age groups.

Туре	No. of Samples	ESBL Producing		
Culture Positives		385		
E.coli	68	34 (50 %)		
Klebsiella species	277	195 (70.3 %)		
Table 2. Distribution of Bacterial Isolates				

*Klebsiella* species was the most common organism isolated. A higher prevalence of ESBL production was observed in *Klebsiella* pneumoniae, (70.3 %) followed by *E. coli* (50 %). Present study of 385 isolates of *E. coli* and *Klebsiella* spp, 229 isolates passed ESBL screening test but only 36 (15.7 %) were confirmed ESBL producers phenotypically.



## DISCUSSION

Identifying ESBL producing organisms is a major challenge in clinical settings due to the selective pressure caused by heavy use of expanded - spectrum cephalosporins, lapses in effective infection control measures and affinity of these enzymes for different substrates and due to the rise in outbreaks. ESBL - producing strains are creating significant therapeutic problems since these pathogens are resistant to a wide range of  $\beta$  - lactams, including third generation cephalosporins as well as have potential for plasmid mediated quinolone and carbapenem resistance.

The prevalence of ESBL producing *Enterobacteriaceae* varies greatly among countries and among the hospitals within the country. Less than 1 % to greater than 70 % ESBLs producers are reported worldwide. In the present study, the ratio of number of inpatients to outpatients was 1 : 1.5. In the study by Shakya et al<sup>6</sup> the ratio of number of inpatients to outpatients to outpatients was 1 : 2.96.

In our study, the prevalence of ESBL production was higher in 21 - 40 years age group and was almost 67 %. Self-medication practice which is high in this age group, could have further accounted for higher prevalence. In the study by Somily et al<sup>7</sup> there was a slight female preponderance in the outpatient group, and the majority of the hospitalized patients were older than 18 years of age. Similar observation was reported by Shakya et al<sup>6</sup> where the male to female patient ratio was 1 : 2.2 and females were higher in all age groups. Taha et al<sup>8</sup> studied patients who were older than 12 years and were diagnosed with Gram negative UTI.

In the present study, a total of 600 Midstream urine samples were collected from patients for urine culture. About 385 samples (64 %) showed significant growth. Shakya et al<sup>6</sup> in their study reported that a total of 2209 nonrepetitive MSU samples were collected from patients for urine culture. Only 451 (20.4 %) samples showed significant growth. Chowdhury et al<sup>5</sup> in their study analysed a total of 120 urine samples from patients of suspected urinary tract infections, and in 71 (59.17 %) cases, bacterial strains were isolated. Somily et al<sup>7</sup> in their study, found a total of 17,105 strains of Enterobacteriaceae, of which 12224 strains were of E. coli and 4,881 strains were of K. pneumoniae. There were 1076 isolates. Urinary tract infections caused by Extended Spectrum Beta - Lactamase producing organisms (UTIESBL) are increasing in incidence and pose a great threat to modern medicine. In Dissanayake et al<sup>9</sup> study, a total of 2303 urine samples from adult patients were studied of which 626 showed significant positive cultures.

In the present study, of 385 isolates of E. coli and Klebsiella species, 229 isolates passed ESBL screening test but only 36 (15.7 %) were confirmed ESBL producers phenotypically. In the study by Taha et al<sup>8</sup> out of the 427 urine cultures screened for ESBL, 163 (38.4 %) were confirmed to be ESBL producers. In Somily et al<sup>7</sup> study, there were 1076 (6.3 %) ESBL isolates including 808 (75 %) E. coli and 268 (25 %) K. pneumoniae. In Dissanayake et al<sup>9</sup> Thirty three percent (n = 94) of these isolates produced extended - spectrum  $\beta$  - lactamases (ESBLs). Half (50 %) of the Klebsiella isolates were ESBL producers while 29 % of

the *E. coli* produced ESBLs. In Shakya et al<sup>6</sup> study of the 451 isolates, the most predominant isolate was *E. coli* accounting for 365 (80.9 %) cases. Out of total, 168 (85.2 %) isolates passed screening test for ESBL but only 36 (21.4 %) of them were ESBL producers. *E. coli* was the highest ESBL producers i.e. 33 (91.7 %) and followed by *K. pneumoniae* i.e. 3 (8.3 %). Among 451 isolates, 236 (52.3 %) were multi drug resistant (MDR) and *E. coli* was with the most MDR share i.e. 188 (79.7 %).

In the present study, a higher prevalence of ESBL production was observed in by *Klebsiella* species (70.3 %) followed by E. coli (50 %). The findings are in agreement with this study. ESBL - producing strains are creating significant therapeutic problems since these pathogens are resistant to a wide range of  $\beta$  - lactams, including third generation cephalosporins as well as have potential for plasmid mediated quinolone and carbapenem resistance. In Shakya et al<sup>6</sup> study, the prevalence of ESBL producing *E. coli* was higher in female patients, 27 (81.8 %) compared to male patients, 6 (18.2 %). Similarly, the prevalence of ESBL producing Klebsiella spp. was 2 (66.7 %) in females and 1 (33.3 %) in males. In Somily et al<sup>7</sup> study, the overall frequency rates of ESBLs among E. coli and K. pneumoniae were 6.6 % and 5.5 %, respectively. In Chowdhury et al<sup>5</sup> study, among the 71 bacterial isolates, majority were E. coli 45 (63.38 %), followed by *Klebsiella* species 14 (19.72 %), Enterococci species 05 (07.04 %), Acinetobacter species 03 (04.22 %), Proteus species 02 (02.82 %) and Pseudomonas species 02 (02.82 %). Taha et al<sup>8</sup> in their study reported that E. coli was the most frequent uropathogen. K. pneumoniae produced the highest rate of ESBL (54.9 %), followed by E. coli (42.5 %), and Proteus mirabilis (7.14 %).

Dissanayake et al<sup>9</sup> observed in their study that *Escherichia coli* and *Klebsiella* species accounted for 46 % (n = 286) of the total isolates with the majority (n = 228, 80 %) being *E. coli*.

## CONCLUSIONS

Hospitals should develop and execute strategies to minimize the spread of ESBL producing organisms by observing universal precautions and minimizing contact among hospitalized patients. This might reduce the spread of ESBL producing organisms in the community. Adherence to recommended hand washing techniques or use of hand rubs may help to prevent transmission of these infections from one patient to other.

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