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# PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM BETA LACTAMASES ISOLATED FROM PATIENTS WITH UTI.

Medical Microbiology

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## ABSTRACT

Complicated UTIs with extended spectrum beta lactamase producing bacteria have become a global concern, due to multidrug resistance. In order to achieve a proper treatment option identification of the prevalent species which generate ESBL as well as their antibiotic susceptibility pattern is essential. This project evaluates the incidence of ESBL producing bacteria and analyses the antimicrobial susceptibility pattern for those strains. **Method:** Total 2800 urine specimens were processed. 2100 urine samples reported as no growth and remaining 700 urine specimens were processed for routine antibiotic sensitivity (Kirby bauer method).Out of these 700 urine specimens 438(264 males,174 females) urine specimens were selected for ESBL study as these 438 specimens were suggestive of organism having multi drug resistance. **Result:** For the entire data set there were significant findings of prevalence of ESBL producing gram negative bacteria. High prevalence rate of ESBL producing strain tested positive by screening tests were Klebsiella spp.(107, 60.1%) followed by E.coli (108, 56.46%) and Pseudomonas and Proteus spp. Collectively (27, 46.55%). ESBL producing organisms were most susceptible to antibiotics Meropenam(10mcg), Ceftazidime + Clavulanic acid and Pipperacillin + Tazobactum(100+10mcg). **Conclusion:** Rate of ESBL incidence among E.coli and Klebsiella spp are more and they are found to be susceptible to Meropenam, combination of drugs (ceftazidime + Clavulanic acid, pipperacillin + tazobactum)

## KEYWORDS

ESBL, UTI, Antibiotic susceptibility, E Coli, Klebsiella spp, Pseudomonas spp, Proteus sp

## INTRODUCTION

Urinary tract infection (UTIs) has been reported to affect up to 150 million individuals annually worldwide. [1]. A complicated UTI is an infection either due to a structural or functional abnormality of the genitourinary tract, or the presenceof an underlying disease that interferes with the host defence mechanism which increases the risk of acquiring infection or of therapeutic failure [2]. The World health organizations have recognized the importance of studying the emergence and determinants of acquired antimicrobial resistance and the need to devise appropriate strategies for their control [3, 4, 5]. The Extended Spectrum beta Lactamase (ESBL) producing bacteria (ESBLs) are emerging worldwide [6, 7, 8]. ESBLs are mostly Gram-negative bacilli, especially Enterobacteriaceae family[9]. ESBL-producing Enterobacteriaceae cause a variety of hospital and community-acquired infections such as bloodstream, wound infections, respiratory tract, and urinary tract infections [10]. Urinary tract infections (UTIs) are very common infectious diseases that occur in a high proportion of the population and are a serious concern in the healthcare system[11].

The prevalence of ESBL-producing isolates depends on some factors including species, geographic region, hospital/ward, group of patients and type of infection, and extensive overuse of antibiotics [12]. Most of the ESBLs are inhibited by Clavulanic acid and Tazobactum [13] However, antibiotics combining these inhibitory drugs with penicillin can be clinically used safely in the treatment of UTI. The aim of this study was to determine the prevalence of ESBL-producing bacteria and their antibiotic susceptibility pattern among uropathogens isolated from patients.

## MATERIALS AND METHODS

### Study Population And Samples Processing:

This observational, prospective study was conducted at the Department of Nephrology and Research Lab, Seth G.S.Medical College and K.E.M.Hospital, Parel, Mumbai-400012. Ethical approval was obtained from the KEM Institutional Ethics Committee (Approval no EC522010) prior to the study commencement. Written informed consent was obtained from the patients involved in the study.

### Inclusion And Exclusion Criteria:

Patients above 18years of age and with UTI were included in the study. Pregnant women and Patients who have allergy to antimicrobial agents used in the study were excluded.

### Procedure:

The patients were instructed to void clean catch mid-stream urine sample in a wide mouth sterile urine container.Mid-stream clean catch voided urine samples were inoculated in MacConkey Agar and Blood Agar plate using a 1 microliter calibrated sterile loop. Following overnight incubation at 37°C, plates were examined and bacterial colonies were counted. Uropathogens were identified according to the biochemical characteristics.

### Antimicrobial Susceptibility Testing:

Uropathogen in numbers greater than $10^5$ CFU/ml, bacterial isolates were identified and susceptibility testing for antimicrobials was performed using Kirby Bauer's Method on Mueller Hinton agar.

### Methods For Phenotypic Confirmation Of ESBL:

Screening for extended-spectrum beta lactamases was done according to the guidelinesrecommended by the Clinical and Laboratory Standard Institute (CLSI 2010) using Disk Diffusion Method, Control strains E. coli ATCC 25922 (beta lactamase negative) and Klebsiella pneumoniae ATCC700603 (ESBLpositive) were used for quality control.

### (CLSI 2010) Clinical And Laboratory Standard Institute's Disk Potentiation Method:

Disks containing Ceftazidime (30mcg) and Cefotaxime (30mcg) with and withoutclavulanic acid (30/10 mcg) were placed on inoculated Muller Hinton Agar plate.(Indian Jmed Res 128 Aug 2008 pp209-211)

### Interpretation:

A >5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone is consideredas presence of (ESBL) Extended Spectrum beta lactamase. (Ceftazidime=16mm, and ceftazidime + clavulanic acid zone=21mm)

### Statistical Analysis:

The data was tabulated for the entire data set (438 urine specimens). For comparative statistics following statistical tests were used 1. Chi-

square test; 2. Student T tests. All tests were done using SPSS version 16 software. The data was categorized for analysis according to
**1**. Prevalence of ESBL producing organism
2. Antibiotic susceptibility pattern of ESBL producing bacteria;

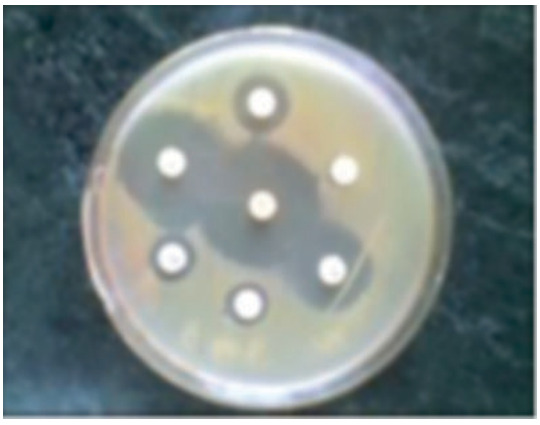

**Fig 1** ESBL positive

## RESULTS

Total 2800 urine specimens were processed. Out of which 2100 urine specimens were reported as no growth and remaining 700 specimens reported with significant growth were processed for routine susceptibility test (kirby bauer method). Fromthese 700 specimens, 438 urine specimens were multidrug resistant, were selected for ESBL study. Table 1 shows in 438 urine specimen Commonest oraganism found was E. coli (203, 46.3%), followed by Klebsiella spp (177, 40.4%) Pseudomonas spp(55,12.6%), and Proteus spp. (3, 0.7%) . Out of the 438 samples 242 samples (55%) were ESBL positive.

**Table 1 Distribution Of ESBL Positive UTI**

| M. organisms | Total No. Of positive specimens (%) | ESBL positive specimens (%) |
|---|---|---|
| E.Coli | 203(46.3) | 108(56.46) |
| Klebsiella spp. | 177(40.4) | 107(60.1) |
| Pseudomonas spp. | 55(12.6) | 27 (46.55) |
| Proteus spp. | 3(0.7) | |
| Total | 438(100) | 242 |

Table 1 shows that 108/203 of E.coli were ESBL positive strains. 107/177 of Klebsiella spp were ESBL positive strains. 27/58 of pseudomonas and proteus spp were ESBL positive strains.

**Table 2 Susceptibility Profile Of E.Coli Producing ESBL (n=108).**

| Drug | Sensitive (%) | Intermediate % | Resistant% |
|---|---|---|---|
| Amoxy-clav | 45(42.05) | 3(2.8) | 59(55.1) |
| Cefta-clav | 58(54.2) | 2(1.8) | 47(43.9) |
| Cefo-clav | 64(59.8) | 4(3.7) | 39(26.4) |
| Meropenum | 78(72.8) | 2(1.8) | 27(25.2) |
| Piperacillin+tazobactum | 73(68.2) | 0(0) | 34(31.7) |

**Table3 Susceptibility Profile Of Klebsiella Spp Producing ESBL (n=107)**

| Drug | Sensitive% | Intermediate % | Resistant % |
|---|---|---|---|
| (Amoxy-clav | 55(50.9) | 2(1.85) | 51(47.2) |
| Cefta-clav | 62(57.4) | 5(4.6) | 41(37.9) |
| Cefo- clav | 68(62.9) | 3(2.7) | 37(34.2) |
| Meropenem | 90(83.3) | 2(1.8) | 16(14.8) |
| Piperacillin+tazobactum | 87(80.5) | 3(2.7) | 18(16.6) |

**Table4 Susceptibility Profile Others* Producing ESBL (n=27)**

| Drug | Sensitive% | Intermediate % | Resistant % |
|---|---|---|---|
| Amoxy-clav | 11(40.7) | 2(7.4) | 14(51.8) |
| Cefta-clav | 15(55.5 | 0(0) | 12(44.4) |
| Cefo-clav | 18(66.6) | 2(7.4) | 7(25.9) |
| Meropenum | 20(74.0) | 0(0) | 7(25.9) |
| Piperacillin+tazobactum | 16(59.2) | 2(7.4) | 9(33.3) |

Others*Pseudomonas and proteus spp

Table 2,3 and 4 show susceptibility pattern of ESBLs to Meropenem and combination of drugs (Ceftazidime + Clavulanic acid (10+20mcg) and Pipracillin + Tazobactum (100+10mcg).

## DISCUSSION

In the present study total 2800 urine specimens were processed. Out of which 2100 urine specimens were reported as no growth andremaining 700 urine specimens reported with significant growth were processed forroutine antibiotic susceptibility test (Kirby Bauer Method). From these 700 urinespecimens 438 urine specimens were selected for ESBL study because these 438 urinespecimens were suggestive of organism having multi-drug resistance.Out of 438 clinical isolates 264 were of males (60.27%) and 174 were of females (39.72%) in the age group of 18- 90years. In this study, E coli followed by Klebsiella spp were identified as man causative agent of UTI. These results are similar to earlier studies conducted by other researchers [14, 15]. Prevalence of ESBL positive cases were 242(55%) obtained from the present study.In our study among ESBL producers predominantly seen was Klebsiella spp (60.1%) followed by E coli (56.46%). This is in accordance with other studies where Klebsiella spp was prominent ESBL isolate followed by E.coli. [16, 17]

## CONCLUSION:

In this project following conclusions have been reached
1.) From this study, mainly E.coli and Klebsiella spp.have been reported as the most common organisms causing UTI.
2)Prevalence of Extended Spectrum Beta-lactamse (ESBL) positive cases were 55% and
Klebsiella spp (60.1%) followed by E. coli (56.46%) were prominent ESBLs
3) From the susceptibility profile of ESBL producing organisms, ESBLs were susceptible to Meropenem andcombination drugs (Ceftazidime+clavulanic acid10+20mcg); Pipracillin+ Tazobactum 100+10mcg).

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