

Original Research Article

Prevalence of Beta-Lactamases in Uropathogenic *Escherichia Coli* and *Klebsiella pneumoniae* Isolates at Tertiary Care Hospital

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ABSTRACT

Objectives: Resistance to third generation cephalosporins in *E. coli* and *K. pneumoniae* are due to various factors. Present study was an attempt to detect resistance mediated by beta-lactamases production.

Methods: A total of 300 uropathogenic *E. coli* and *Klebsiella pneumoniae* isolates were further subjected to detection of beta-lactamases by disc diffusion method.

Result: A total of 300 urinary isolates were studied, out of these 189 isolates were beta-lactamases producer. Out of 189, *E. coli* shown to have beta-lactamases production in 144 isolates while 45 isolates of *K. pneumoniae* were beta-lactamases producer.

Conclusion: The prevalence of resistance mediated by beta-lactamase production is increasing day by day and varies from different geographical areas; hence it should be mandatory to detect beta-lactamases on routine basis.

Keywords: *E. coli*, *K. pneumoniae*, beta-lactamases.

INTRODUCTION

Urinary tract infection forms the largest single group of hospital acquired infections and accounts for about 45-50% of total nosocomial infections.^[1] It results in significant morbidity and high medical cost.^[2]

Escherichia coli account for approximately 80% of the Urinary tract infections. *Klebsiella species* accounts for approximately 17% of the nosocomial urinary tract infections.^[3] Urinary tract infections are often treated with broad spectrum antibiotics.^[4,5] Cephalosporins are used in the treatment of urinary tract infections.^[6] Gram negative organisms exhibit resistance to antimicrobial agents through various mechanisms like target site modification, altered PBP, poor diffusion or altered porins, active efflux mechanism, and

producing inactivating enzymes.^[7] Among different mechanisms of resistance beta-lactamase induced resistance play a predominant role and threatens the therapeutic efficacy of antibiotics.^[8]

ESBL represent a major group of beta-lactamases currently being identified in large numbers and are now found in significant percentage in *Escherichia coli* and *klebsiella pneumoniae* strains.^[9] Microorganisms responsible for urinary tract infections such as *Escherichia coli* and *klebsiella* species have ability to produce ESBL in large quantities.^[10]

ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* show multidrug resistance.^[11] Both global and Indian figures showed marked increase in beta-lactamase producing organisms. Many

ESBL producing organism also express AmpC. In India ESBL producing strains of enterobacteriaceae have emerged as challenge in hospitals and community based patients. [12]

Routine susceptibility test by clinical laboratory fails to detect ESBL positivity and show false positive in vitro susceptibility. [13,14] The high incidence of beta lactamase production due to multiple mechanism in clinical isolates is alarming and urgent actions needs to be taken from therapeutic and infection control perspective. [9]

Present study was undertaken to evaluate prevalence of beta-lactamases in isolates of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* from a tertiary care hospital.

MATERIALS AND METHODS

Present study was conducted at department of microbiology, during the period of August 2010 to March 2013 after the clearance from ethical committee.

All Isolates of *E. coli* and *K. pneumoniae* from clinically diagnosed patients of urinary tract infection attending various clinical services in our hospital were included in this study. Repetitive urinary isolates of *E. coli* and *K. pneumoniae* from the same patient were excluded from the study. All relevant clinical data were collected.

All the reagents, media, and antimicrobial discs used in the present study were supplied by Hi-Media Laboratory, Mumbai. Readymade Plates of 5% sheep blood agar were used and other culture media were prepared in house form powder according to manufacturer's instructions.

All urine samples were inoculated on 5% sheep Blood agar and McConkey's agar plates using calibrated loop and incubated aerobically at 37°C. After overnight incubation, plates were observed for bacterial growth. The organisms were identified by their colony morphology, gram staining characters, motility and other

relevant biochemical tests as per standard laboratory methods of identification. [7,15] Clinically significant isolates of *E. coli* and *K. pneumoniae* were subjected to detection of beta-lactamases. [16]

DETECTION OF BETALACTAMASES: [9]

The disc placement was designed in a novel fashion to assess ESBL and AmpC. The Ceftazidime and Ceftazidime+ Clavulanic acid discs were kept 15-20 mm apart from each other (center to center). Imipenem, an inducer, was placed in the center and on either side of it, at a 15mm distance, were placed Ceftazidime and Cefotaxime (indicators of induction). In addition, another inducer Cefoxitin was placed at 15 mm from Cefotaxime (indicator). This was placed opposite to that of Ceftazidime+Clavulanic acid to avoid any effect of inducible -lactamase on the zone of inhibition of the latter.

The remaining discs were placed as shown in figure [9]: 1-Imipenem, 2- cefotaxime, 3-Cefoxitin, 4-Ceftazidime, 5- Ceftazidime + Clavulanic acid, 6- Aztreonam, 7-Ceftriaxone.

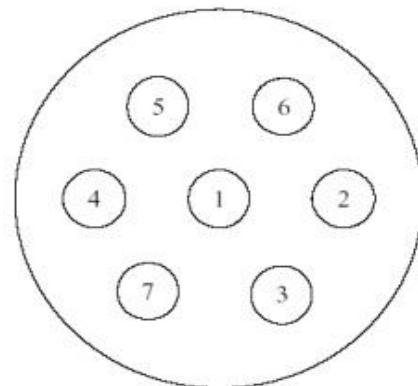


Figure: A novel scheme of disc placement to assess ESBL and AmpC production.

1 - Imipenem, 2 - Cefotaxime, 3 - Cefoxitin, 4 - Ceftazidime, 5 - Ceftazidime + Clavulanic acid, 6 - Aztreonam, 7 - Ceftriaxone

Image courtesy: C. Rodrigues et al [9]

Following criteria was used for deciding an organism to be either ESBL producer, inducible Amp C producer or a depressed mutant.

Screening test for detection of ESBL (CLSI): [17] An isolate was suspected to be

an ESBL producer by the screening method if it had the zone sizes for the cephalosporins like Aztreonam \geq 27mm, Cefotaxime \geq 27mm, Cefpodoxime \geq 21mm, Ceftazidime \geq 22mm and Ceftriaxone \geq 25 mm.

Criteria for detection of ESBL Producer

1. Zone diameters for various 3rd generation cephalosporins as mentioned above
2. Susceptible to Cefoxitin
3. Increase in zone size with addition of an inhibitor by 5 mm

Phenotypic confirmation of ESBL was performed as per CLSI guidelines. ATCC strains of *Klebsiella pneumoniae* (ATCC 706003) and *Escherichia coli* (ATCC 25922) were used for the study. [17]

Criteria for detection of inducible AmpC producer:

1. Blunting of zone towards inducer
2. No increase in zone size with addition of an inhibitor
3. Susceptible to Cefepime.

Criteria for detection of derepressed mutants (DD):

1. Resistant to Cefoxitin and Cefotaxime

Table-2: Distribution of Beta-lactamase & Non Beta-lactamase producing *E. coli* & *K. pneumoniae*.

Name of the organism	β lactamase producers	Non- β lactamase producer	Total isolates
<i>E. coli</i> (n=228)	144 (63.15%)	84 (36.85%)	228
<i>K. pneumoniae</i> (n=72)	45 (62.5%)	27 (24.32%)	72
Total isolates	189	111	300

Table-3: Different mechanisms of beta-lactamase production by *E. coli* & *K. pneumoniae*

Type of Mechanism	<i>E. coli</i> (n=144)	<i>K. pneumoniae</i> (n=45)	Total isolates
ESBL	68 (29.82%)	27 (37.5%)	95
AmpC	5 (2.19%)	2 (2.77%)	7
MM	53 (23.24%)	13 (18.05%)	66
DM	18 (7.89%)	3 (4.16%)	21
Total	144	45	189

DISCUSSION

All the 300 hundred isolates were subjected to detection of beta-lactamases. We observed that out of 300 isolates, 189 isolates (63%) were beta-lactamase producers. Amongst beta-lactamases producing isolates, 95(63.70%) isolates were plain ESBL producers and beta-lactamase productions through multiple mechanisms were seen in 66 (34.92%) isolates. Derepressed mutants were found in

2. No increase in zone size with addition of an inhibitor

Criteria for detection of ESBL production due to multiple mechanisms (MM):

1. Resistant to Cefoxitin
2. Blunting of zone towards inducer
3. Increase in zone size with addition of an inhibitor by 5 mm

A total of 300 urinary isolates were studied and data was analyzed by Excel computer based program.

OBSERVATION AND RESULTS

A total of 300 urinary isolates were studied, out of these 228 isolates were *E. coli* and 72 isolates were *K. pneumoniae*. These isolates were further subjected to detection of beta-lactamases as per CLSI guidelines. [17]

Table-1: Distribution of urinary isolates of *E. coli* & *K. pneumoniae* (n=300)

Patients	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>
IPD	123 (53.9%)	41 (56.94%)
OPD	105 (46.05)	31 (43.05%)
Total	228	72

21(11.11%) isolates and only 7(3.70%) isolates were AmpC producers.

Out of 189 beta-lactamase producing isolates, 144 isolates were of *E. coli* and 45 isolates were of *K. pneumoniae*. Out of 144 beta-lactamase producing strains of *E. coli*, 68(22.66%) isolates were plain ESBL producers and 53(36.80%) isolates showed beta-lactamase resistance through multiple mechanisms. Eighteen (12.5%) isolates were derepressed mutants and only 5(3.47%) isolates were AmpC producers.

Out of 45 beta-lactamase producing isolates of *K. pneumoniae*, 27(60%) isolates were plain ESBL producers followed by 13(28.88%) isolates which show beta-lactamase resistance through multiple mechanisms. Three (6.66%) isolates were

derepressed mutants while only 2(4.44%) were AmpC producers.

C. Rodrigues et al [9] used the similar method for detection of beta-lactamase in all clinical gram negative isolates.

Our study differed from C. Rodrigues et al [9] who used similar method for detection of beta-lactamase in all clinical gram negative isolates. We observed a high prevalence of plain ESBL producing isolates of *E. coli* (29.82%) while they observed the high prevalence of *E. coli* isolates which were beta-lactamase producers through multiple mechanisms (59.3%).

In our study five isolates of *Escherichia coli* were AmpC producers while they did not find any AmpC producer.

Out of 72 isolates of *K. pneumoniae*, we observed a high prevalence of plain ESBL producing isolates (37.5%), while they observed the high prevalence of *K. pneumoniae* isolates which were beta-lactamase producers through multiple mechanisms (18.05%). In our study two isolates of *K. pneumoniae* were AmpC producers while they did not find any AmpC producer.

In our study number of isolate were more as compared to study by C Rodrigues et al [9] and also the prevalence of plain ESBL among the clinical isolates vary greatly worldwide and in geographical areas and its rapidly changing over time. And may be due to the use of third generation cephalosporins which is one of the risk factor for ESBL production

In the present study we observed that out of 228 isolates of *E. coli*, 68(29.82%) isolates were plain ESBL producers. Various studies reported prevalence of ESBL producers in *E. coli* in range (8% to 68%). Our study is in accordance with Parulagarwalet al [18] and Eshwarsingh et al [19] who observed 21% and 26.5% prevalence of plain ESBL producers in *E. coli* isolates respectively in their studies

In present study we observed that out of 72 isolates of *K. pneumoniae* 27(37.5%) were plain ESBL producers.

Various studies reported ESBL producing strains of *K. pneumoniae* in various range. Our study is in concordance with Akram et.al, [20] Baby Padmini et.al, [14] Iraj et.al, [21] and Shashirekha et.al, [13] they observed (34.42%), (40%), (40%) and (40.6%) prevalence of plain ESBL producing isolates of *K. pneumoniae* respectively in their studies.

Our study differed from Hasanejazet al [11] who observed very high prevalence (71.7%) and Kadar et.al [22] who observed very low prevalence (7.5%) of ESBL producing isolates of *K. pneumoniae*.

In our study we found *K. pneumoniae* as a predominant ESBL producer which is in concordance with other studies like Hasanejazet al, [11] Ana Khaftanzhieva et.al [23] and SubhaAnanthan et.al. [24]

In the present study out of 228 isolates of *E. coli*, 5(2.19%) were AmpC producer. This is in accordance with Avinashet al [25] who reported AmpC production in 11(7.14%) isolates of *E. coli*.

In the present study out of 72 isolates of *K. pneumoniae*, 2(2.77%) isolates were AmpC producer which is in accordance with Avinash et al [25] who reported AmpC production in 13(11.7%) isolates of *K. pneumoniae*.

In the present study prevalence of beta-lactamase resistance through multiple mechanisms in isolates of *E. coli* and in *K. pneumoniae* was (23.24%) and (18.05%) respectively. However, Garav Dalelaet al [26] and Avinash et al [25] found a low prevalence of beta-lactamase producing *E. coli* and *K. pneumoniae* this may be because the number of isolates of *E. coli* and *K. pneumoniae* were less in their study as compared to ours.

Multiple beta-lactamases conferring resistance in a single bacterial strain can be found and it is alarming. Our study differed from others this may be because of diversity of cephalosporin resistance pattern and beta-lactamase production in different geographical areas.

CONCLUSION

The prevalence of resistance mediated by beta-lactamase production is increasing day by day and varies from different geographical areas. Continuous monitoring of antimicrobial susceptibility pattern and analysis of beta-lactamases is necessary for judicial use of drugs and proper institution of the therapy.

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