ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Study of Extended - Spectrum β-Lactamases (ESBLs) and AmpC β-Lactamases in *Escherichia coli, Klebsiella* spp. *and Enterobacter* spp. in a Tertiary Care Hospital.

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**ABSTRACT**

Extended-spectrum β-lactamases (ESBLs) were reported for the first time in 1983 and plasmid-mediated AmpC β-lactamases were reported for the first time in 1988. ESBL and AmpC β-lactamase producers are responsible for majority of the cephalosporin resistance, limiting the therapeutic options. *Enterobacter* spp. are significant causes of nosocomial infections due to production of constitutive chromosome mediated AmpC β-lactamases. We conducted a study to assess the rate of ESBL and AmpC β-lactamases (plasmid and chromosome mediated) in *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. One hundred and thirty isolates (*Klebsiella* spp., *Escherichia coli* and *Enterobacter* spp.) from blood cultures and exudates were included in the study. ESBLs were confirmed by the combination disk method. Confirmation of AmpC β –lactamases was carried out by directly using the whole bacterial isolates, a technical variation of the conventional three dimensional extract test. Plasmid-mediated (derepressed, transferable) AmpC β –lactamases were detected by AmpC disk test. Chromosome mediated (Inducible) AmpC β-lactamase detection by Disk Antagonism Test (DAT). Of 130 isolates, 79 were ESBL-producers, 66 were plasmid-mediated (derepressed) AmpC β -lactamase producers and 08 were chromosome mediated (inducible) AmpC β -lactamase producers. Among the *Escherichia coli* and *Klebsiella* spp. none of them were positive for inducible AmpC β-lactamase producers. Of the *Enterobacter* spp. 20% were positive for inducible AmpC β –lactamases. A very high rate of ESBL, AmpC β-lactamase production among the isolates and co-existence of these two enzymes in *Enterobacter* spp. was observed in this study. Inducible/ chromosome mediated AmpC β-lactamases were not produced in *E. coli* and *Klebsiella* spp.

**Keywords**: ESBL, AmpC β –lactamase, plasmid mediated, Inducible

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ISSN: 0975-8585

## INTRODUCTION

Gram negative bacteria, mostly β-lactamase producing members of the family *Enterobacteriaceae* are responsible for increased resistance. Emergence and dissemination of resistance such antimicrobial resistance represent a serious threat to public health. [1] Extended-spectrum β-lactamases (ESBLs) were reported for the first time in 1983, similarly plasmid-mediated AmpC β-lactamases were reported for the first time in 1988. ESBLs are mutant, plasmid-mediated β-lactamases derived from β-lactamases, such TEM-1, TEM-2, SHV-1. ESBLs have an extended substrate profile that permits hydrolysis of oxyimino cephalosporins, penicillins, and aztreonam. [2] Plasmid-mediated AmpC β-lactamases are a result of transfer of chromosomal genes to plasmids to produce inducible AmpC β-lactamase. Plasmid-mediated AmpC β-lactamases are produced by *E. coli, Klebsiella pneumoniae, Salmonella* spp., *Citrobacter freundii, Enterobacter aerogenes, and Proteus mirabilis*. AmpC β-lactamase producers are different from ESBL producers, being usually susceptible to cefepime. ESBL is inhibited by clavulanic acid whereas AmpC β-lactamase is inhibited by boronic acid. ESBL and AmpC β-lactamase producers are responsible for majority of the cephalosporin resistance. [3] Plasmid-mediated AmpC β-lactamases are uninducible, whereas chromosomal AmpC β-lactamases are inducible. ESBLs along with plasmid-mediated AmpC β-lactamases are associated with broad multidrug resistance (as usually genes for other antibiotic resistance mechanisms also residing on the same plasmid).

Many clinical laboratories currently test *E. coli* and Klebsiella spp. for ESBL production but do not try to detect AmpC β-lactamases. Many infectious disease personnel remain unaware of the clinical importance of AmpC β-lactamases. Plasmid-mediated AmpC β-lactamases lead to false in vitro susceptibility to cephalosporins. The prevalence of bacteria producing ESBLs varies from 20-71% in India and 8-45% worldwide. In case of AmpC β-lactamase, prevalence of the AmpC production varies from 10.67% and 15.1% in other parts of the world whereas in India, prevalence of AmpC ranges from 3.3-47.3%. [4]

We conducted a study to assess the rate of ESBL and AmpC β-lactamases in *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. The objective of the study was to detect prevalence of ESBL, plasmid and chromosome mediated AmpC β-lactamases.

## MATERIALS AND METHODS

### Sample size

The study was carried out during three months study period in a tertiary care hospital. The sample comprised of 130 consecutive clinical isolates from blood cultures and exudates, including *E. coli* (n=60), *Klebsiella* spp. (n=30) and *Enterobacter* spp. (n=40). The isolates were identified by standard biochemical methods. [5]

### Antimicrobial susceptibility testing

The Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. The antibiotics disks (Hi-Media, Mumbai, India) tested were tested for different cephalosporins and interpreted as per Clinical Laboratory Standards Institute (CLSI- 2009) guidelines. [6] *Escherichia coli* ATCC 25922 strain was used for quality control.

### Phenotypic Testing for ESBLs

Isolates were tested for ESBL via the combination disk method using ceftazidime (30 µg) and a disc of ceftazidime-plus-clavulanate (30 µg plus 10 µg). A ≥5 mm increase in diameter of the inhibition zone of the ceftazidime-plus-clavulanate disc, when compared to the ceftazidime disc alone, was interpreted as phenotypic evidence of ESBL production. [7]

### Phenotypic Testing for AmpC β-lactamases

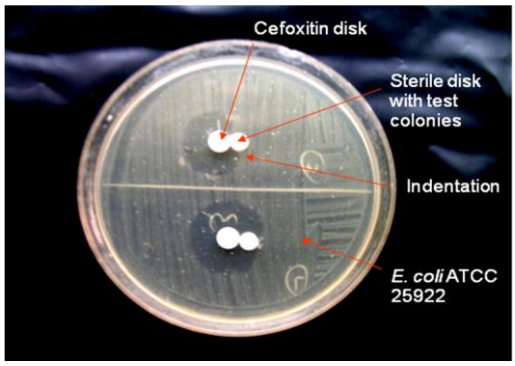
Strains with a cefoxitin inhibition zone of <18mm and resistant to all the 3$^{rd}$ generation cephalosporins tested were considered screen positive for AmpC β-lactamase production. [8] Confirmation of AmpC β-lactamases was carried out by directly using the whole bacterial isolates, a technical variation of the conventional three dimensional extract test. [9]

ISSN: 0975-8585

**Plasmid mediated AmpC β-lactamase detection by AmpC disk test**

The test was carried out according to the procedure previously described. [10] Briefly, a 0.5 McFarland suspension of *E. coli* ATCC 25922 was inoculated on the surface of Mueller-Hinton agar (MHA) plate. A cefoxitin disc (30 µg) was placed on the inoculated surface of the agar. Sterile disks (6 mm) were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism is placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk. A negative test showed an undistorted zone (**Figure 1**).

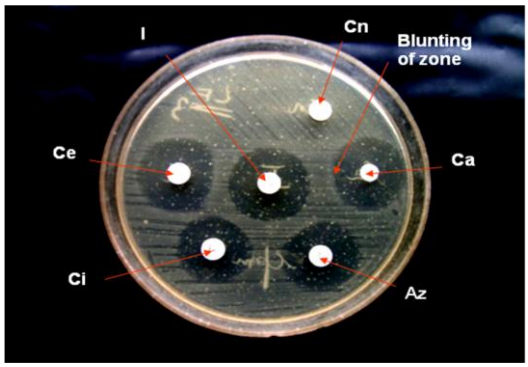
**Figure 1: Plasmid mediated AmpC β-lactamase detection by AmpC disk test**




**Chromosome mediated (Inducible) AmpC β-lactamase detection by Disk Antagonism Test (DAT).**

Disks of inducing agent cefoxitin (Cn) and cephalosporins (cefepime- Cpm, ceftazidime- Ca, ceftriaxone- Ci and cefotaxime- Ce) were placed on the surface of the test bacterial inoculation (0.5 McFarland suspension) on MHA plate. The plates were examined after overnight incubation at 37$^{o}$C. [11] Imipenem was also used as an inducing agent and was compared with cefoxitin, in the disk antagonism test
(**Figure 2**).

**Figure 2: Chromosome mediated (Inducible) AmpC β-lactamase detection by Disk Antagonism Test (DAT)**




**I - Imipenem, Cn- cefoxitin, Ca- ceftazidime, Az- Aztreonam, Ci- ceftriaxone, Ce-Cefotaxime.**

**RESULTS**

Of 130 isolates, 79 were ESBL-producers, 66 were plasmid-mediated (derepressed) AmpC β -lactamase producers and 08 were inducible (chromosome mediated) AmpC β -lactamase producers. Among the *Escherichia coli* and *Klebsiella* spp. none were positive for inducible AmpC β -lactamase producers. Moreover, 67% and 65% of *Escherichia coli* were positive for ESBLs and plasmid-mediated AmpC β -lactamases, respectively. Similarly, 57% and 50% of *Klebsiella* spp. were positive for ESBLs and plasmid-mediated AmpC β-lactamases, respectively. Among *Enterobacter* spp., 55%, 30% and 20% were positive for ESBLs, plasmid-

mediated AmpC β-lactamases and inducible AmpC β-lactamases, respectively (**Table 1, Figure 1 & 2**). No resistance to imipenem was recorded.

**Table 1: Extended-spectrum β - lactamases (ESBLs) and AmpC β-lactamases produced in *Escherichia coli*, *Klebsiella* spp and *Enterobacter* spp.**

| Organism | No. tested | ESBL | ESBL + derepressed | De-repressed | Inducible AmpC | Inducible + Derepressed | Nil |
|---|---|---|---|---|---|---|---|
| *E. coli* | 60 | 40 (67%) | 31 (52%) | 39 (65%) | - | - | 12 (20%) |
| *Klebsiella* spp. | 30 | 17 (57%) | 12 (40%) | 15 (50%) | - | - | 10 (33%) |
| *Enteroba-cter* spp. | 40 | 22 (55%) | 10 (25%) | 12 (30%) | 08 (20%) | 06 (15%) | 08 (20%) |
| Total | 130 | 79 (61%) | 53 (41%) | 66 (51%) | 08 (6%) | 06 (5%) | 30 (23%) |

**Derepressed- plasmid-mediated AmpC β-lactamase; inducible- chromosome mediated AmpC β –lactamase.**

## DISCUSSION AND CONCLUSION

Infections due to resistant gram-negative organisms have largely been regarded as a healthcare-associated phenomenon. The infections due to ESBL producing organisms are difficult to treat due to their resistance to wide spectrum of antibiotics including the third generation cephalosporins. *Escherichia coli* that produces extended-spectrum β-lactamase (ESBL) has become widespread in hospitals. [12] The rate of ESBL production in bacteria differs greatly all over the world, and it has been changing rapidly. There are marked geographical differences in the proportions of ESBL production among clinical isolates of *Klebsiella pneumoniae* and *E. coli*. The prevalence of ESBLs was reported to be over 10% in east Europe, 3.5% in a Canadian study and 20-48.8% in Asia. [13] In recent years, a significant increase in ESBL producing *Enterobacteriaceae* has been reported in India and neighboring countries mostly identified using phenotypic methods. [14] A high rate of ESBL production has been reported for South America (>40% of *K. pneumoniae* and 5 to 10% of *E. coli* isolates) and Asia (20 to 30% of *K. pneumoniae* and 15 to 20% of *E. coli*). The very high rates were reported from a multicenter survey study in India (>55% and >60%, respectively). Considerably lower rates of ESBL phenotypes have been reported for Europe (10 to 15% and 5 to 10%, respectively) and North America (5 to 10% and <5%, respectively). [15] In a study from Chennai, a total of 67.4% of isolates were positive for ESBLs. [16] In *Enterobacter species* ESBL prevalence varies in different reports from 33% to 50%. [17] In our study, 67%, 57% and 55% of *Escherichia coli, Klebsiella* spp. *and Enterobacter* spp. respectively were found to be ESBL producers. Similar to some reports, *E. coli* had highest prevalence rate of ESBL production. [13]

Several clinical laboratories now test *Escherichia coli* and Klebsiella spp. for production of ESBLs but do not try to detect AmpC β-lactamases. These enzymes are associated with multiple antibiotic resistances. It is important to know the occurrence of ESBL and AmpC producing strains to guide empirical therapy for various infections. AmpC β-lactamases can be plasmid or chromosomal mediated. The chromosome mediated AmpC β-lactamases are produced by *Citrobacter freundii*, *Enterobacter cloacae*, *Morganella morganii*, *Hafnia alvei*, and *Serratia marcescens*. Plasmid mediated AmpC β-lactamases genetically related to chromosomally encoded AmpC enzymes were identified in late 1980s. [18] The plasmid borne gene could easily spread between *Klebsiella* spp., *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* spp. The spread of plasmid mediated AmpC β-lactamases is a great worry worlwide. AmpC β-lactamases are not inhibited by clavulanic acid and can inactivate cephamycins in addition to cephalosporins incativated by ESBLs. AmpC β-lactamase producers are of clinical and epidemiological importance and are responsible for higher morbidity and mortality. [19] AmpC β-lactamase producers lead to misinterpretation of phenotypic detection tests and in

turn treatment failures. Further, there are no Clinical and Laboratory Standards Institute (CLSI) guidelines for detection of AmpC β-lactamase producing pathogens. [20]

The precise prevalence of AmpC β-lactamases is not known, which is mainly because of the nonexistent simple and reliable detection methods or guidelines. There is varied difference in the proportions of AmpC β-lactamase production among different centres in India. Inducible (chromosome mediated) AmpC β-lactamase are uncommon and to the best of our knowledge it has not been reported in *K. pneumoniae* and *E. coli.* In a study from Madhya Pradesh, 15.97% were AmpC β-lactamases and of which 4.86% isolates were positive for both ESBL and AmpC β-lactamases. [21] In another study from India, ESBL was detected in 63% isolates of *E. coli* and 73% of *Klebsiella spp*. The occurrence of AmpC β-lactamases was found to be 9%. [22] In a study from New Delhi 6.97% *E.coli* and 6.18% *K. pneumoniae* were positive for AmpC β-lactamase production. [10] In a report from Kolkota, 47.8% *E. coli* and 13% *K. pneumoniae* were reported to be AmpC β-lactamase producers. [23] In a study from Chennai, 24.1% of *Klebsiella* spp. and 37.5% of *E. coli* were AmpC β-lactamase producers. [24] In Andhra Pradesh, 3.4 per cent of *E. coli*, 4.8 per cent of *K. pneumoniae* were found to AmpC β-lactamase producers. [25] In our study, among the *Escherichia coli* and *Klebsiella* spp. none were positive for inducible AmpC β -lactamase producers. Among *Escherichia coli* 67% and 65% of isolates were positive for ESBLs and plasmid-mediated AmpC β-lactamases, respectively. Similarly, 57% and 50% of *Klebsiella* spp. were positive for ESBLs and plasmid-mediated AmpC β-lactamases, respectively. Among *Enterobacter* spp., 55%, 30% and 20% were positive for ESBLs, plasmid-mediated AmpC β-lactamases and inducible AmpC β-lactamases, respectively. In the Disk Antagonism test, imipenem was used as an inducing agent and was found analogous with cefoxitin.

Further studies must be undertaken to determine the prevalence of these enzymes. Inducible expression of chromosomal AmpC β-lactamases, although not reported to the best of our knowledge in *E. coli* and *K. pneumoniae*, is associated with a significant risk of therapeutic failure with all β-lactam drugs except carbapenems. Hence, an attempt was made not to fail to identify inducible strains by Disk Antagonism test. In addition, detecting a plasmid-mediated AmpC β-lactamases enzyme in a strain with inducible β-lactamases or ESBL coproduction is even more difficult. However, additional investigation of these isolates using polymerase chain reaction for AmpC β-lactamases genes is needed to confirm the phenotypic results. Surveillance is key in controlling the Gram-negative β-lactamase resistance mechanisms we face today and primarily help stop the emergence of a new type of β-lactamases. Clinical laboratories need to have expertise and adequate funding to provide a quick and clinically relevant antibiotic testing service in centers where these resistance mechanisms are encountered.

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