PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF ESBL AND AMPC β-LACTAMASES PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE FROM VARIOUS CLINICAL SAMPLES: AN EMERGING THREAT

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ABSTRACT
Resistance to broad spectrum β-lactams mediated by Extended Spectrum β-lactamases (ESBL) and AmpC β-lactamases enzymes is a growing threat worldwide.

AIM
The aim of the study was to detect the prevalence and antimicrobial susceptibility of ESBL and AmpC β-lactamase producing Escherichia coli and Klebsiella pneumoniae isolated from various clinical samples.

MATERIALS AND METHODS
A total of 288 isolates comprising of 180 Escherichia coli and 108 Klebsiella pneumoniae isolated from various clinical samples were included. ESBL was detected by Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and Double Disk Synergy Test (DDST). AmpC detection was done by AmpC disk test.

RESULTS
Out of 180 Escherichia coli and 108 Klebsiella pneumoniae isolates 91 (50.5%) and 63 (58.3%) were confirmed to be ESBL producers by PCDDT and D1 and 81 (45%) and 57 (52.7%) by DDST respectively. AmpC was detected in 35 (19.4%) of Escherichia coli and 33 (30.5%) of Klebsiella pneumoniae isolates. Co-production of ESBL and AmpC was detected in 6 (3.3%) Escherichia coli and 11 (10.2%) of Klebsiella pneumoniae isolates. Majority of ESBL producers were from blood in both organisms. Multidrug resistance (MDR) was seen in 79.1% of ESBL Escherichia coli and 63.5% of ESBL Klebsiella pneumoniae isolates. MDR was seen in 28 (96.5%) of AmpC producing Escherichia coli and all AmpC producing Klebsiella pneumoniae isolates.

CONCLUSION
It is essential to report ESBL and AmpC beta lactamase production along with routine susceptibility, which will aid the clinicians in prescribing antibiotics. Strict adherence to the hospital antibiotic policy and good infection control practices would go a long way in curtailing the menace of drug resistance.

KEYWORDS
AmpC β-lactamase, Escherichia coli, Extended-spectrum β-lactamase, Klebsiella pneumoniae, Multidrug resistance.


INTRODUCTION
The rapid global dissemination of Enterobacteriaceae harbouring plasmid borne extended-spectrum β-lactamases (ESBL) and plasmid mediated AmpC β-lactamases represents a significant clinical threat. The predominant mechanism for resistance to β-lactam antibiotics in gram negative bacteria is by synthesis of β-lactamases. Among the β-lactamases, the production of ESBLs and AmpC β-lactamases are the most common. ESBLs are plasmid-mediated β-lactamases that are capable of efficiently hydrolyzing penicillin, narrow and broad-spectrum cephalosporins and monobactams (aztreonam), but they do not hydrolyse cephapenem or carboxypenem (imipenem, meropenem). β-lactamases, inhibitors such as clavulanic acid, sulbactam and tazobactam generally inhibit ESBL producing strains. They have evolved from genes of TEM-1, TEM-2 or SHV-1 by mutation that alter the amino acid configuration around the active site of these β-lactamases rendering them susceptible to hydrolysis by these enzymes. There are also new families of ESBLs including the CTX-M and OXA-type enzymes as well as novel unrelated β-lactamases. ESBL producing isolates are most commonly found in Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli). AmpC β-lactamases are primarily chromosomal and plasmid-mediated and are resistant to β-lactamase inhibitors such as clavulanic acid, but can hydrolyse cephapenem. Plasmid mediated AmpC β-lactamases (PMABLs) have evolved by the movement of chromosomal genes on to plasmids and are found in E. coli, K. pneumoniae, Salmonella spp., Proteus
**mirabilis, Citrobacter freundii, Enterobacter aerogenes** which confer resistance similar to their chromosomal counterparts. Carbapenems are one of the antibiotics of last resort for many bacterial infections, such as *E. coli* and *K. pneumoniae* producing AmpC and ESBL. These organisms are responsible for a variety of infections like urinary tract infections, septicaemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscesses and device related infections and are typically associated with multidrug resistance. Treatment failures after instituting β-lactam antibiotic therapy for infections caused by ESBL producing gram negative bacilli have been reported. It has been demonstrated that ESBL and AmpC production by infecting organisms adversely affects the clinical outcome.

Distinguishing between the AmpC and the ESBL producing organisms has epidemiological significance and it may have a therapeutic importance as well. Moreover, these strains are no longer confined to the hospital environment, but of late are being isolated from the community at increasing frequencies. Therefore, it is necessary to know their prevalence so as to enable the clinician to select appropriate antibiotic regimen at the earliest. The routine susceptibility tests performed by clinical laboratories fail to detect these strains making treatment options difficult. With this background, the current study was conducted to determine the prevalence of ESBL and AmpC β-lactamases in *E. coli* and *K. pneumoniae*, which were isolated from various clinical samples from both in-patients and out-patients who attended a tertiary care hospital in North-West India.

**MATERIAL AND METHODS**

A total of 288 consecutive, non-repetitive isolates comprising of 108 *K. pneumoniae* and 180 *E. coli* were recovered from different clinical samples between January 2014 and May 2014 (Table 1). The isolates were identified by standard biochemical methods.

**Antimicrobial Susceptibility Testing**

Antibiotic susceptibility of the isolates was done by Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines, using commercially available discs (HiMedia, Mumbai, India). Cefepime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), amikacin (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), meropenem (10 µg), norfloxacin (10 µg), nitrofurantoin (300 µg) and cefoperazone/sulbactam (75/10 µg).

**Screening for ESBLs and AmpC β-lactamases**

As per CLSI recommendation, isolates showing resistance (zone ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone) by disc diffusion method were considered potential ESBL producers and further preceded for confirmation. Isolates showing resistance to cefotixin (inhibition zone < 18 mm) by disc diffusion method were considered potential AmpC producers and further tested for presence of AmpC β-lactamase enzyme by AmpC disk test.

**Detection of ESBLs and AmpC β-lactamases**

The Phenotypic Confirmatory Disc Diffusion Test (PCDDT) All strains that were potential ESBL producers were subjected to confirmation using the PCDDT as recommended by CLSI. A disc of cefotaxime (30 µg) and ceftazidime (30 µg) alone and a disc of cefotaxime/clavulanic acid (30 µg/10 µg) and ceftazidime/clavulanic acid (30 µg/10 µg) were placed independently 30 mm apart center to center on a lawn culture of 0.5 McFarland turbidity of the test isolate on Mueller-Hinton Agar (MHA) plate and incubated for 18-24 hours at 35°C. A ≥5 mm increase in zone diameter for either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production (Figure 1).
Detection of AmpC β-lactamases
Out of 145 screen positive isolates, 68/145 (46.89%) were confirmed as AmpC β-lactamase producers by AmpC disk test. AmpC β-lactamase production was seen in 35/180 (19.4%) of *E. coli* and 33/108 (30.5%) of *K. pneumoniae* isolates (Table 2).

Co-production of ESBL and AmpC β-lactamases
Among the 154 ESBL positive isolates, 17 also tested positive for AmpC β-lactamase. Co-production of ESBL and AmpC was observed in 17/288 (5.9%) isolates. It was higher in *K. pneumoniae* (10.2%) than in *E. coli* (3.3%).

Antimicrobial Sensitivity Pattern
A wide spectrum of antimicrobial resistance pattern to various antimicrobial agents were detected in ESBL positive *E. coli* and *K. pneumoniae* (Figure 4, 5). Both *E. coli* and *K. pneumoniae* strains showed a high degree of resistance to 4th generation cephalosporin cepime accounting for 91.7% and 94% respectively. Least resistance was seen with meropenem in *E. coli* isolates accounting for 1.2%. However, among *K. pneumoniae* isolates the resistance was 14.3%. Among the urinary *E. coli* isolates, a high resistance of 89.2% was seen with norfloxacin.

A high multi-drug resistance (MDR) of 79.1% and 65.2% respectively was observed among ESBL producing strains of *E. coli* and *K. pneumoniae*. MDR was significantly higher in ESBL *E. coli* strains than non-ESBL strains. \( P=0.036 \) multi-drug resistance was seen in 28/29 (96.5%) of AmpC producing *E. coli* and 22/22 (100%) of *K. pneumoniae* isolates.

<table>
<thead>
<tr>
<th>Name of Sample</th>
<th>Total No.</th>
<th>ESBL Positive (Pure) No. (%)</th>
<th>AmpC Positive (Pure) No. (%)</th>
<th>AmpC &amp; ESBL Co-producers No. (%)</th>
<th>Total No.</th>
<th>ESBL Positive (Pure) No. (%)</th>
<th>AmpC Positive (Pure) No. (%)</th>
<th>AmpC &amp; ESBL Co-producers No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>134</td>
<td>65 (48.5%)</td>
<td>22 (16.4%)</td>
<td>4 (2.9%)</td>
<td>14</td>
<td>07 (50%)</td>
<td>02 (14.3%)</td>
<td>03 (21.4%)</td>
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<tr>
<td>Pus</td>
<td>13</td>
<td>04 (30.7%)</td>
<td>04 (30.7%)</td>
<td>00</td>
<td>31</td>
<td>10 (32.2%)</td>
<td>09 (29.0%)</td>
<td>03 (9.6%)</td>
</tr>
<tr>
<td>Blood</td>
<td>10</td>
<td>08 (80%)</td>
<td>01 (10%)</td>
<td>00</td>
<td>28</td>
<td>23 (82.1%)</td>
<td>02 (7.1%)</td>
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<tr>
<td>Sputum</td>
<td>09</td>
<td>04 (44.4%)</td>
<td>00</td>
<td>01 (11.1%)</td>
<td>05</td>
<td>02 (40%)</td>
<td>02 (40%)</td>
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<tr>
<td>Tracheal swab</td>
<td>02</td>
<td>00</td>
<td>01 (50%)</td>
<td>00</td>
<td>11</td>
<td>04 (36.3%)</td>
<td>04 (36.3%)</td>
<td>01 (9.1%)</td>
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<tr>
<td>Suction tip</td>
<td>02</td>
<td>01 (50%)</td>
<td>00</td>
<td>00</td>
<td>08</td>
<td>01 (12.5%)</td>
<td>03 (37.5%)</td>
<td>01 (12.5%)</td>
</tr>
<tr>
<td>Vaginal Swab</td>
<td>04</td>
<td>01 (25%)</td>
<td>00</td>
<td>00</td>
<td>01</td>
<td>01 (12.5%)</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>CSF</td>
<td>02</td>
<td>01 (50%)</td>
<td>00</td>
<td>00</td>
<td>03</td>
<td>01 (33.3%)</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Others</td>
<td>04</td>
<td>01 (25%)</td>
<td>01 (25%)</td>
<td>01 (25%)</td>
<td>07</td>
<td>04 (57.1%)</td>
<td>00</td>
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</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>85 (47.2%)</td>
<td>29 (16.1%)</td>
<td>06 (3.3%)</td>
<td>108</td>
<td>52 (48.1%)</td>
<td>22 (20.3%)</td>
<td>11 (10.1%)</td>
</tr>
</tbody>
</table>

**Table 1: Distribution of ESBL and AmpC β-lactamases in Different Clinical Samples**

<table>
<thead>
<tr>
<th>Name of Microorganism</th>
<th>Total No. of Isolates</th>
<th>No. of Isolates Resistant to 3GCs in Screening Test</th>
<th>No. of Isolates Positive by PCDDT*</th>
<th>No. of Isolates Resistant to Cefoxitin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>180</td>
<td>148</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>108</td>
<td>102</td>
<td>63</td>
<td>57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>288</td>
<td>250</td>
<td>154</td>
<td>138</td>
</tr>
</tbody>
</table>

**Table 2: Results of Screening and Confirmatory Tests for ESBL and AmpC Production**

*PCDDT = Phenotypic Confirmatory Disc Diffusion Test; †DDST = Double Disc Synergy Test
ESBL production confirmed by an increase in zone of ≥5 mm for Ceftazidime (CAZ) and Ceftazidime/Clavulanic Acid (CAC) and Cefotaxime (CTX) and Cefotaxime/Clavulanic Acid (CEC).

AmpC Disk Test: Blunting towards Cefoxitin Disc Indicating Positive Test (A), Flattening (Borderline Positive) (B) and Absence of Blunting Indicating Negative Test (C).

Antimicrobial Resistance Patterns of Clinical Isolates of β-lactamase and non β-lactamase Producing Escherichia coli

Antimicrobial Resistance Patterns of Clinical Isolates of β-lactamase and non β-lactamase Producing Klebsiella pneumoniae.
DISCUSSION
With the spread of ESBL and AmpC producing strains all over the world, it is necessary to know the prevalence of these strains in hospitals. The overall prevalence of ESBL in the present study was 15.4/288 (53.5%). ESBL was detected in 58.3% of K. pneumoniae and 50.5% of E. coli strains.

The prevalence of ESBL among clinical isolates varies greatly worldwide and in geographical areas and is rapidly changing over time. Reports of ESBL detection among clinical isolates of E. coli range between 20% and 80.6% and those among K. pneumoniae ranges between 20% and 86.7%.\textsuperscript{11,12,13} Variation in the detection rates within and across the states could be due to the differences in the methodology used in these studies. Also it may be due to different patterns of antibiotic use and differences in the selection of organisms for the study. The PCDDT which is recommended by CLSI for phenotypic confirmation of ESBL among E. coli and K. pneumoniae was found to be more sensitive than DDST test. PCDDT detected 154/288 (53.5%) of all the ESBL producers, while DDST detected only 138/288 (47.9%). The DDST lacks sensitivity because of the problem of optimal disc space and the proper storage of clavulanic acid containing discs. Similar observation has been reported by other studies.\textsuperscript{14,15}

Techniques to identify AmpC β-lactamase producing isolates are available, but are still evolving and are not yet optimized for the clinical laboratory.\textsuperscript{16} Due to lack of reliable detection methods, their exact prevalence is unknown. Various studies have reported prevalence of AmpC between 2.2% \textsuperscript{17} and 37.5%.\textsuperscript{18,19} The overall prevalence of AmpC β-lactamases in the present study was 23.6%. Among E. coli it was 19.4%, while it was 30.5% among K. pneumoniae isolates. High level of AmpC production is typically associated with in vitro resistance to 3 GCs and cephamycins leading to clinical treatment failures with broad spectrum cephalosporins.\textsuperscript{19,20}

Co-production of both ESBL and AmpC was observed in (17/288) 5.9% of isolates. It has been stated that AmpC β-lactamases when present along with ESBL can mask the phenotype of the latter.\textsuperscript{4} Thus the coexistence of AmpC and ESBL in the same strain may give false negative results for detection of ESBL. When ESBL production is suspected, but the confirmatory test is negative, the strain should be screened for presence of AmpC β-lactamases.

Due to widespread use of antibiotics, MDR E. coli and K. pneumoniae strains isolated are increasing that poses severe challenges to public health. In the present study, MDR was seen in 79.1% of E. coli and 63.5% of K. pneumoniae isolates of ESBL producing strains. Resistance of ESBL producing isolates to 3GCs among E. coli was found to coexist with resistance to two or more antibiotics such as amikacin (P=0.03), gentamicin (P=0.01), cefepime (P=0.00006), cefotin (P=0.00002) and doxycycline (P=0.02). While in ESBL, K. pneumoniae resistance was seen with doxycycline (P=0.02) and cefoperazone/sulbactam (P=0.04). This coexistence of multidrug resistance has been reported earlier.\textsuperscript{21,22} Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids. The highest drug resistance was observed for cefepime accounting for 90% in E. coli and 93.4% in K. pneumoniae isolates. Similar high resistance has been observed in other studies in India.\textsuperscript{23,24} Resistance to cefepime could be attributed to the high prevalence of CTX-M type ESBLs in these isolates, some of which are capable of hydrolyzing cefepime.\textsuperscript{25} Very high drug resistance of 85.9% was seen for norfloxacin in urinary isolates of E. coli. Imipenem was found to be the most effective drug against ESBL E. coli showing a susceptibility of 98.9 %, whereas 14.3% of ESBL K. pneumoniae isolates were resistant to imipenem, which could be because of carriage of carbapenemase genes.

Multi-drug resistance was observed in 28 (96.5%) of AmpC producing E. coli and 28/28 (100%) of K. pneumoniae isolates. Similar findings have been reported in other studies.\textsuperscript{26,27} This emphasizes the need for detecting AmpC β-lactamase in MDR isolates, so as to avoid therapeutic failures and nosocomial outbreaks.

The increased ESBL and AmpC producing isolates are indicative of the ominous trend of more and more isolates acquiring resistance mechanisms, thus rendering the antimicrobial armament ineffective. The high prevalence of these organisms emphasizes the need for early detection of these β-lactamases, which can help in instituting appropriate antimicrobial therapy and in avoiding the development and dissemination of these multi-drug resistant strains. Every health care institution must develop its own antimicrobial stewardship program, which is based on the local epidemiological data and international guidelines to optimize the antimicrobial use among the hospitalized patients and to improve patient outcomes.\textsuperscript{28} Preventive measures like a continuous surveillance and strict implementation of infection control practices can go a long way in containing the menace of drug resistance in health care settings.

REFERENCES


