Study of simultaneous production of ESBL and AmpC beta lactamases among Enterobacteriaceae in Chennai

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ABSTRACT

Introduction

Resistance to antimicrobials among Enterobacteriaceae, especially to commonly used beta-lactam antibiotics by production of Extended Spectrum Beta Lactamases (ESBL) and AmpC Beta Lactamases (AmpCBL) has become a global healthcare challenge. The magnitude of the problem varies with geographical areas and with time, and such data helps in formulating infection control strategies. Simultaneous production of ESBL and AmpCBL may lead to confusing antibiogram in routine Kirby-Bauer disc diffusion method requiring further testing.

Methods

The present study was done to estimate ESBL and AmpC beta-lactamases among Enterobacteriaceae isolated from various clinical specimens. Screening for ESBL and AmpC were done based on resistance to indicator cephalosporins in Kirby-Bauer disc diffusion method. Double disk synergy technique using cephotaxime, ceftazidime and their combination with clavulanic acid was used for confirming ESBL production as per CLSI guidelines. Boronic acid along with ceftazidime and ceftazidime-clavulanic acid were used to detect AmpC production in suspected isolates.

Results

Out of 100 isolates 78 were from urine specimens. E.coli (65%) was the commonest isolate followed by Klebsiella spp. (31%). Multidrug resistance was seen in 46% of the isolates. A total of 32% isolates were producing ESBL alone, 6% AmpCBL alone and 8% isolates produced both ESBL and AmpCBL. Among E.coli isolated in this study 29% produced ESBL alone, 8% produced AmpCBL alone and 9% produced both. Among the beta-lactamase producing E.coli 93% were resistant to ciprofloxacin, 83% to co-trimoxazole, 53% to gentamicin and 7% to imipenem. Only 13% and 17% of beta-lactamase producing E.coli were resistant to nitrofurantoin and amikacin respectively.

Conclusion

Simultaneous production of ESBL and AmpCBL is seen in 6% of Enterobacteriaceae. Co-resistance to non-beta-lactam antibiotics is high among Enterobacteriaceae producing beta lactamases. Since E.coli was the commonest urinary isolate and most of them were sensitive to nitrofurantoin, amikacin and imipenem, nitrofurantoin and amikacin can be used for empirical therapy and imipenem as reserved drug for serious infections in our hospital.

Key words: ESBL, AmpC beta lactamase, Escherichia coli, Enterobacteriaceae
cephalosporins, which can lead to the prescription of the inappropriate therapy for the infected patients.  

The prevalence of resistance and the pattern among Enterobacteriaceae keeps changing with geographical areas and different periods of time. It is also influenced by antibiotic usage/prescription practices and infection control practices in that area. The present study was conducted with the aim of estimating the resistance among enterobacteriaceae and providing locally applicable data to guide empirical therapy. Special emphasis was given in detecting ESBL and AmpC enzyme mediated resistance and co-resistance patterns of such isolates. A particular type of disc placement was used for the purpose.

**Methods:**

The study was conducted after approval from Institutional Ethics committee and after taking informed consent from the participants. Cross sectional study design was used. The study was conducted at a Tertiary Care Hospital attached to Medical College in South India during August and September 2013. A total of 100 consecutive, non-repetitive Gram negative bacterial isolates of Enterobacteriaceae family isolated from different clinical specimens (urine, pus, sputum, etc) submitted to microbiology laboratory for routine diagnostic purposes were included in the study.

All the isolates were identified using standard microbiological techniques. Antibiotic sensitivity testing was done by using Kirby Bauer’s disk diffusion method as described by the Clinical Laboratory Standards Institute (CLSI) guidelines 2011. Isolates resistant to Cefotaxime and/or Ceftazidime were considered probable ESBL producers. Isolates resistant to Cefoxitin were considered as probable AmpC BL producers.

All probable ESBL and AmpC producing strains were further subjected to antibiotic sensitivity testing using Cefotaxime (CTX), CTX+Clavulanic Acid (CEC), Ceftazidime (CAZ), CAZ+Clavulanic Acid (CAC), CAZ+Boronic Acid (CAZ+BA), CAC+BA and Imipenem (IPM) disks placed as in Fig 1. A stock (60 g/l of Dimethyl Sulfoxide) solution of 3-Aminophenylboronic acid (Sigma Aldrich, India) was prepared and 5 µl was added to disks of CAZ and CAC (300µg/disk) to detect AmpC enzymes.  

Based on the zone sizes obtained following interpretations were done:

1. A ≥5 mm increase in the zone diameter with CTX and/or CAZ with CA than CTX and/or CAZ alone confirms ESBL production
2. A ≥5 mm increase in zone diameter with CAZ+BA than CAZ alone indicates AmpC production
3. A ≥5 mm increase in zone diameter with CAC+BA than CAC and CAZ+BA indicates combined ESBL and AmpC production (Fig No. 2.1)
4. Flattening of zone of CTX or CAZ towards the Imipenem disk indicates production of inducible-AmpC production (Fig No.2.2)

Quality control was done by using Klebsiella pneumoniae ATCC 700603 and E. coli ATCC 25922 standard strains. All the data obtained was tabulated using Microsoft excel and appropriate calculations such as percentages were done for analysis.

**Results:**

One hundred consecutive enterobacteriaceae isolated from urine (78), sputum (8), swabs (8) and Pus (6) specimens were included in the study. The isolates included E.coli (65%), Klebsiella Spp (31%), Proteus Spp (03%) and Citrobacter freundii (1%).

Sensitivity patterns of all the bacterial isolates is tabulated in Table 1. The co-resistance patterns to different antibacterials in various Enterobacteriaceae isolated are summarised in table 2. Since urine specimens constituted the majority among all the specimens included in the study, urinary isolates are analysed separately as in table 3. Majority (88%) of the urinary isolates were sensitive to nitrofurantoin.

**Discussion:**

Extended spectrum β-lactamases (ESBLs) belong to Group 2be of Bush’s functional classification and are derived from the point mutation in original plasmid-mediated TEM-1 and SHV-1 β-lactamases. ESBLs are capable of hydrolyzing penicillins, cephalosporins of the first, second, third, and fourth generations and the monobactam antibiotic aztreonam. Cephamycins (e.g., cefoxitin) or carbapenems (e.g., imipenem, meropenem, and ertapenem) are not affected by these enzymes. They are inhibited by clavulanic acid (CA) and this property is used in detecting them in the laboratory. ESBLs are most commonly produced by Escherichia coli and Klebsiella species but can also be produced by other Enterobacteriaceae.

Since ESBL-producing organisms are frequently susceptible to a β-lactam/β-lactamase inhibitor combination (e.g., amoxicillin / clavulanic acid) in vitro, it may be assumed that the same may be true in vivo. But it is essential to keep in mind that if an organism has an AmpC β-lactamase then the β-lactam/β-lactamase inhibitor combination may fail to work.

AmpC β-lactamases are group C enzymes belonging to Class I of Bush-Jacoby’s...
They confer resistance to penicillins, cephalosporins (except advanced spectrum cephalosporins like cefepime, cefpirome, and cefclidin), and monobactams. They can be differentiated from extended-spectrum β-lactamases (ESBLs) by their ability to hydrolyze cephamycins and not inhibited by clavulanic acid (CA). AmpC enzymes may be plasmid mediated or chromosomal. Organisms such as Enterobacter, Citrobacter, Shigella, Morganella, Serratia, and Escherichia coli possess AmpC enzymes on their chromosomes. Chromosomal expression is usually inducible variety, but it can be expressed constitutively when there is a promoter mutation (derepressed strains). Generally, they are susceptible to advanced spectrum cephalosporins (ASCs, i.e., cefepime and cefpirome).

The CLSI-recommended phenotypic confirmatory test (PCT) would fail to detect ESBLs in the presence of AmpCBL, as

<table>
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<tr>
<th>Table 1: ESBL and AmpC production in enterobacteriaceae</th>
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<tr>
<td>Sensitivity Patterns</td>
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<tr>
<td>Sensitive (Not producing ESBL or AmpC)</td>
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<tr>
<td>ESBL alone</td>
</tr>
<tr>
<td>AmpC alone</td>
</tr>
<tr>
<td>ESBL+AmpC</td>
</tr>
<tr>
<td>Total ESBL producers</td>
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<tr>
<td>Total AmpC producers</td>
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<tr>
<th>Table 2: ESBL and/or AmpCBL producing E.coli and Klebsiella spp. and their co-resistance patterns to other antibiotics</th>
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<td>Resistant to</td>
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<tr>
<td>CIP</td>
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<td>AK</td>
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The latter enzyme is resistant to CA. CA may induce high-level expression of chromosomal AmpCBL, masking the synergy arising from the inhibition of an ESBL. The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. The AmpCBL producing organisms can act as a hidden reservoir for the ESBLs. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs and it may result in a fatal and inappropriate antimicrobial therapy.

The BA disk test in combination with the CLSI phenotypic confirmatory test described by Rudresh SM et al was very simple, highly sensitive and specific for the identification of ESBL and/or AmpC among Enterobacteriaceae. The test showed a 100% positive and negative predictive value for ESBL and/or AmpCBL detection. The BA disk test requires the addition of two more disks to the CLSI described PCT, making it more simple and easy to adapt on a routine basis by a clinical microbiology laboratory. The test results were reproducible when carried out in duplicate. The CPM–CA disk test detected all the ESBLs correctly but another test for AmpC detection like the M3D test has to be incorporated into the susceptibility testing.

In this study, nearly half (46%) of the Enterobacteriaceae isolated were multi-drug resistant. 32% isolates were producing ESBL alone, 6% AmpCBL alone and 8% isolates produced both ESBL and AmpC BL. Thus, 40% isolates produced ESBL and 14% produced AmpC enzymes alone or in addition to other enzymes causing drug resistance. Other similar studies conducted in India have shown that plasmid-borne and chromosomally mediated AmpC and cephalosporinase-producing pathogens are common in resistant E. coli and K. pneumoniae isolates. The reported prevalence of ESBL-producing Gram-negative isolates in various hospitals in India is in the range of 19%–60%. Incidence of ESBL in the present study is comparable to those in studies done by Bandekar et al and Loveena Oberoi et al.

Among E. coli isolated in this study 29% produced ESBL alone, 8% produced AmpC alone and 9% produced both. Among Klebsiellae isolated, 10% produced ESBL alone, 1% AmpC alone and 2% both. Studies done elsewhere in India showed a wide range of figures of AmpC production, from 3.3 to 50%. This wide variation in incidence could be due to various geographical factors, local antibiotic usage pattern, type of hospitals and patient population, etc. In this study 4% isolates were resistant to imipenem. Cabapenem resistance is reported to be 5.3%–59% in various metropolitan tertiary care hospitals in India. The drug of choice remains carbapenems in AmpC producers as the β-lactam and β-lactamase inhibitor combinations fail even if these show sensitivity in vitro.

Generally, isolates producing β-lactamases were found to be more resistant to other antibiotics compared to non-β-lactamase producing strains. The ESBL producing organisms also express the AmpC β-lactamases and they may be co-transferred with the plasmids, thus mediating the fluoroquinolone and the aminoglycoside resistance. Since E. coli was the commonest urinary isolate and most of them were sensitive to nitrofurantoin, amikacin and imipenem, nitrofurantoin and amikacin can be used for empirical therapy and imipenem as reserved drug for serious infections in our hospital.

Table 3: ESBL and AmpC production in uropathogenic enterobacteriaceae

<table>
<thead>
<tr>
<th>Urinary isolates</th>
<th>Sensitive (Non-ESBL, Non-AmpC)</th>
<th>ESBL alone</th>
<th>AmpC alone</th>
<th>ESBL + AmpC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (n=61)</td>
<td>32 (53%)</td>
<td>19 (31%)</td>
<td>04 (6%)</td>
<td>06 (10%)</td>
<td>61</td>
</tr>
<tr>
<td>Klebsiella spp. (n=14)</td>
<td>07 (50%)</td>
<td>05 (36%)</td>
<td>01 (7%)</td>
<td>01 (7%)</td>
<td>14</td>
</tr>
<tr>
<td>Others (n=03)</td>
<td>00</td>
<td>03</td>
<td>00</td>
<td>00</td>
<td>03</td>
</tr>
<tr>
<td>Total</td>
<td>39 (50%)</td>
<td>27 (35%)</td>
<td>05 (6%)</td>
<td>07 (9%)</td>
<td>78</td>
</tr>
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</table>

To improve the clinical outcome of patients in resource-poor settings, treatment guidelines for empirical therapy need to be formulated. These guidelines should ideally be aligned with local susceptibility patterns. Availability of this information will help clinicians select appropriate and effective therapy and reduce the incidence of drug-resistant bacteria. The need for strategies to control antibiotic resistance is greater in resource constraint settings because antibiotic resistance puts further strain on an already fragmented health care system in low and middle-income countries.

It has been greatly emphasised that adequate training should be provided for the undergraduate medical, pharmacy and nursing students regarding the proper prescribing, dispensing and the usage of antibiotics respectively. It
is an important measure which is widely proposed and documented, in order to promote the judicious use of antibiotics. The lack of adequate training during their undergraduate and postgraduate years may be responsible for their inability to undertake these tasks confidently. Hence, teaching about antimicrobial chemotherapy should form a vital part of both the undergraduate and postgraduate medical curricula, considering the frequency with which these agents are prescribed and our continuing and increasing concern regarding antibiotic resistance.

The failure in implementing basic infection control practices has been one of the principle causes of the emergence and the dissemination of resistant organisms. Learning about the antimicrobial prescribing in pharmacology must be connected clearly with the infection control in microbiology. The significance of simple measures like hand hygiene in the control of resistance should be endorsed and its practice should be inculcated at an earlier stage of the medical education.

Though the results of this study are useful for arriving at empirical therapy in the study centre, extension of the study with larger sample size, including confirmatory test for AmpC BL suggested by CLSI and tests for other beta lactamases (e.g., metallo-beta-lactamases) would give a better picture of the situation.

The high prevalence of these organisms in hospitals including sensitive areas like ICUs emphasizes the need for an early detection of the beta-lactamase producing organisms by simple screening methods, which can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains. The need of the hour is that 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, to improve the patient outcomes, to ensure a cost-effective therapy and to reduce the adverse consequences of the antimicrobial use.

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REFERENCES:

Statins are recommended for prevention of progression of cardiovascular disease after percutaneous coronary intervention (PCI). Although high-dose highly efficient statins are recommended, especially in high-risk patients, clinical data are scarce and further investigation in "real-world" settings is needed.

High Intensity Rosuvastatin