



Original Research Article

Carbapenem sparing options for the treatment of ESBL and AmpC producing Enterobacteriaceae in hemodynamically stable patients – an in vitro study

S.Amreliwala, S.Durgad and A.Poojary*

Department of Pathology and Microbiology, Breach Candy Hospital Trust, Mumbai, India

*Corresponding author

ABSTRACT

Infections due to multidrug resistant (MDR) Enterobacteriaceae pose a major therapeutic challenge. ESBL and AmpC enzymes are two of the most common enzymes encountered. The dry antibiotic pipeline mandates antibiotics like Carbapenems to be reserved for critically ill, hemodynamically unstable patients. This in- vitro prospective study looked into options that would be carbapenem sparing, for treatment of MDR gram negative Enterobacteriaceae in hemodynamically stable patients. Multidrug resistant (MDR) Enterobacteriaceae isolated from various clinical samples except blood culture were studied. ESBLs were identified by the Clinical Laboratory Standards Institute (CLSI) criteria. AmpC were identified using the boronic acid inhibition method. Kirby Bauer disc diffusion methods was used to determine the antibiotic susceptibility to test the following bactericidal antimicrobial agents: Cefepime (FEP), Cefoperazone /Sulbactam (CFS), Piperacillin /Tazobactam (TZP), Cefepime/Tazobactam (CPT), Amikacin (AK), Gentamicin (GM) and Levofloxacin (LVX). Out of 400 MDR isolates studied, 210 (52.5%) were ESBL producers, 89 (22.25%) were AmpC producers, and 101 (25.25%) produced both types of enzymes. Among the MDR Enterobacteriaceae, the susceptibility observed was as follows: CPT 88%, AK 76%, CFS 66%, TZP 55%, GM 51%, FEP 49%, and LVX 17%. CPT, AK & CFS can empirically serve as carbapenem sparing agents in the treatment of infections other than blood stream infections, in hemodynamically stable patients.

Keywords

MDR-GNB,
BL-BLIs,
Carbapenem
sparing,
Hemo-
dynamically
stable

Introduction

In India, multi drug resistant (MDR) Enterobacteriaceae continue to be an important cause of infections both in the community and in healthcare settings . [1,2] Resistance is commonly encountered to Betalactam antibiotics, mediated by a diverse group of enzymes called as Beta-lactamases.

Betalactamses have been classified according to their genetic make up into 4 classes namely: Class A, B, C & D based on the Ambler's Molecular classification [3]. ESBLs belong to group A, comprising of > 150 enzymes and are widely produced by Enterobacteriaceae. [4,5,6]

These enzymes can hydrolyze penicillins, cephalosporins of the 1st, 2nd & 3rd generation like ceftazidime and cefotaxime, & also monobactams. They are usually encoded by plasmids which carry resistance genes to other group of antibiotics like the quinolones and aminoglycosides.[4] ESBLs are inhibited by beta-lactamase inhibitors like tazobactam, sulbactam & clavulanic acid.[4] AmpC β -lactamases, on the other hand, belong to class C of Ambler's molecular classification and hydrolyze penicillins, cephalosporins and monobactams except the fourth generation cephalosporins.[7] Amp C are not inhibited by beta lactamase inhibitors. AmpCs are encoded on chromosomes as well as plasmids. [8]

While carbapenems are active against both ESBL and AmpC producers, they are best reserved for critically ill patients. The dry antibiotic pipeline particularly against gram negative bacteria has forced us to look into opportunities for improving usage of the existing antimicrobial agents, particularly in patients who are hemodynamically stable. [9] The aim of our study was to analyze the antibiotic susceptibility pattern of MDR Enterobacteriaceae producing ESBL, AmpC or both to bactericidal antimicrobial agents like Beta lactam /Beta lactamase (BL-BLI) inhibitor combinations, Fluoroquinolones and aminoglycosides.

Materials and Methods

This was a prospective study in which, 400 MDR Enterobacteriaceae producing ESBL, AmpC or both, isolated consecutively from non repetitive clinical specimens, except blood cultures were included over two years of the study period (June 2011 to May 2013). The anatomical sites of isolation were as follows: urine(310), wound swab (45) ,intra abdominal sites(25) ,sputum(15)

and vaginal swab(5) . The isolates comprised of E.coli(328), K.pneumoniae(48), M.morganii(10) E.cloacae (8) E.aerogenes (3) Serratia marcesans (1) C.freundii(1) and P.mirabilis (1) . The identification was performed using conventional biochemical methods.

Antimicrobial susceptibility testing

(AST): AST and interpretation was performed using the Kirby Bauer disc diffusion method on Mueller Hinton agar plates with 0.5 Mcfarland density suspension as per CLSI guidelines. [10]. The antibiotic discs(μ g) used were follows: cefepime (30 μ g) , cefoperazone sulbactam(75/30 μ g), piperacillin tazobactam(100/10 μ g), cefepime tazobactam(30/10 μ g), amikacin(30 μ g), gentamicin(30 μ g) and levofloxacin(5 μ g) (Becton Dickinson Pvt India Ltd.) . Reference strains included in the study were as follows: ESBL positive Klebsiella pneumoniae ATCC 700603, ESBL negative Escherichia coli ATCC 25922 .

ESBL detection: All isolates that were resistant to Cefotaxime and Ceftazidime were confirmed for ESBL detection. The tests were performed as per the Clinical Laboratory Standard Institute recommendations as follows: [10].

Initial screen test for ESBL: The test inoculum (0.5 McFarland's turbidity) was spread onto Mueller-Hinton agar (MHA) using a sterile cotton swab. A disc of cefotaxime (30 μ g) and ceftazidime (30 μ g) were kept placed on the agar surface in addition to the other antimicrobial agents. The plate was incubated at 37°C overnight. The zone diameter of ≤ 27 mm & ≤ 22 mm for Cefotaxime & Ceftazidime respectively indicated that the isolate may produce ESBL. This was further confirmed by the phenotypic confirmatory test.

Phenotypic disc confirmatory test (PDCT): The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA using a sterile cotton swab; a cefotaxime (30 µg) disc containing of the antibiotic and a cefotaxime-clavulanic acid disc were placed 16 to 20 mm apart. The plate was incubated at 37°C overnight. A zone enhancement of ≥ 5 mm with the inhibitor containing disc compared to cefotaxime alone confirmed the presence of ESBLs. (Fig 1)

AmpC Detection: Since the CLSI has not recommended a testing methodology for AmpC enzymes, the phenotypic AmpC detection method used was as follows:

All cefoxitin resistant isolates were tested by an Inhibitor based method using boronic to identify AmpC producers. To prepare the combination disk, 120 mg phenyl boronic acid was added to 3ml Dimethyl sulfoxide (DMSO). DMSO - boronic acid solution was diluted with an equal volume of sterile distilled water (D/W), and 20µl of this solution was added to cefotaxime disks. Discs of cefotaxime (30 µg) alone and that in combination with boronic acid were then placed on a lawn culture of the isolate on MHA at a distance of 15 to 20 mm apart. The plate was incubated at 37°C overnight. Increase in zone diameter of >5 mm with the inhibitor containing disc compared to cefotaxime alone were categorized as AmpC producers. [11] Fig 2

Result and Discussion

In our study, 57% of the MDR Enterobacteriaceae were from outpatient specimens, 38% from wards and 5% from the intensive care units. (Fig 3) Urine was the most common specimen (77%) to grow MDR GNBs, followed by wound swabs (9%), Intra-abdominal specimens (6%),

Sputum (3%) & vaginal swab (1%). (Fig 4) E.coli (81.7%) & Klebsiella pneumoniae (12%) were the common organisms found in the study. The remaining 2.5 % was comprised of Morganella morganii, Enterobacter species (3.5%), Serratia marcescens (0.25%), Citrobacter freundii (0.25%) and Proteus mirabilis (0.25%) (Fig 5) Approximately, 52.5% of the isolates included in the study were ESBL producers, 25.25% were ESBL + AmpC co-producers & 22.25% were only AmpC producers (Table 1).

Cefipime/Tazobactam had the best overall susceptibility against all the MDR isolates (88%), followed by Amikacin 76%, Cefoperzone/Sulbactam 66% and Piperacillin/ Tazobactam 53%. Levofloxacin was susceptible in only 17% of the MDR isolates suggesting Fluoroquinolones to be unreliable in the treatment of infections caused by MDR GNBs. (Fig6) ESBL producers showed sensitivity to CPT(84.6%), AK(69.2%) and CFS(59.75%) in that order. AmpC producers showed sensitivity to CPT(88.5%) and AK(88.5%) followed by FEP(86.5%) in that order. The co-producers showed sensitivity to CPT(93%), CFS(85%) and AK(77.4%) in that order. The overall susceptibility of the MDR Enterobacteriaceae strains were as follows: CPT(88%), AK(76%) and CFS(66%) (Table 2).

Antimicrobial resistance increases the morbidity, mortality and costs of treating infectious diseases. (12) What is alarming is the increasing isolation of these bacteria from outpatient specimens. While 57% of the MDR isolates in our study were from outpatient specimens, data from both India and globally also suggest an increasing trend in MDR GNB isolation from the community [2,9,13,14].

ESBLs were first described in 1983, and have the ability to hydrolyse oxyimino-cephalosporins, and monobactams, but not cephamycins or carbapenems. [4,15] There are more than 500 different ESBLs described all of which are mutations of the classical broad-spectrum beta lactamase enzymes that were initially named TEM and SHV (TEM-1, TEM-2, SHV-1). CTX-M 15 is the most common variety found in India [16]. In our study, about 52.5% of the MDR isolates were pure ESBL producers. While in India, the ESBL prevalence ranges from 60 to 70%, in our study 52.5% of our MDR isolates were pure ESBL producers. [17,18] Out of the 229 outpatient clinical specimens, 50.2% were ESBL producers. This conforms with other studies from India suggesting that the ESBL carriage in the community can be anywhere between 20% to 53%. [14, 19,20,21] *E.coli* and *K.pneumoniae* are the two most common isolates ESBL producing uropathogens [21] In our study too, 82% of ESBL producers were *E.coli* followed by 17% of *K.pneumoniae*. ESBL producers showed highest susceptibility to CPT(84.6%), AK(69.2%) and CFS(59.75%). Cefepime, gentamicin, levofloxacin and piperacillin tazobactam may not be as reliable choices for treating infections due to ESBL producing bacteria as they show <50% susceptibility. Other studies have also pointed out resistance to quinolones and gentamicin among ESBL producers [22]

AmpC beta-lactamases differ from ESBL's in that they are cephalosporinases and are resistant to beta-lactamase inhibitors. They hydrolyze the cephamycins, but not the 4th generation cephalosporins (eg. cefepime) [7] Genes for AmpC β -lactamases can be found both on the chromosomes as well as on plasmids of Enterobacteriaceae. Plasmid mediated AmpC have arisen by transfer of chromosomal genes on to plasmids and are inducible. [23]

They can be found in *E. coli*, *Klebsiella pneumoniae*, *Salmonella* species, *Citrobacter freundii*, *Enterobacter aerogenes* and *Proteus mirabilis*. The plasmid mediated AmpC beta lactamases are more dangerous because they can jump across species to confer drug resistance among different species of Enterobacteriaceae. [24]

In our study, pure AmpC production was seen in 22.25% of the MDR Enterobacteriaceae isolates. Studies from different parts of India suggest that the prevalence of AmpC among MDR Enterobacteriaceae can be anywhere between 5% to 25%. [12,22,25,26] Amikacin (88.5%), Cefipime/Tazobactam(88.5%), Cefipime (86.5%) and Gentamicin (81.25%) showed best susceptibility towards AmpC producers in this study. Piperacillin /Tazobactam (41.67%), Cefoperazone /Sulbactam (50.7%), Levofloxacin (32.3%) showed \leq 50% susceptibility for AmpC producers. Similar patterns of susceptibility are seen in other Indian studies [22,25]

Approximately 25% of the isolates in our study were co-producers of ESBL & AmpC betalactamases. Such co-producing complex phenotypes are being increasingly reported worldwide [27,28]. Other studies from India have also shown that ESBL production among AmpC producing MDR GNB can range from 25% to 92% [25,29]. For co-producers, our study showed the following susceptibilities: Cefipime /Tazobactam (93%), Cefoperazone /Sulbactam (85.4%), Amikacin (77.4%), and Piperacillin/Tazobactam (72.17%) which could be carbapenem sparing options.

The carbapenems are considered to be antibiotics of last resort to combat serious infections caused by multidrug resistant bacteria, especially in ICUs and high risk

wards housing critically ill patients. A dry antibiotic pipeline particularly for Gram negative bacilli mandates that we choose carbapenem sparing regimens in less serious infections. [30]

This study suggests that Betalactam plus betalactamase combinations like Cefipime /Tazobactum, Cefoperazone /Sulbactum, Piperacillin /Tazobactum and

aminoglycosides like Amikacin may be useful carbapenem sparing antibiotics in the Indian scenario where infections due to gram negative multidrug resistant organisms pose a major problem both in the hospital as well as in the community. Clinical studies need to be conducted to understand the extent to which the above carbapenem sparing therapies will be useful.

Table.1 Distribution of ESBL, AMPC and ESBL +AMPC co-producers

Organisms (Nos.)	ESBL (%)	AMPC(%)	ESBL +AMPC(%)
Ecoli (328)	172 (82)	68 (77)	88(87)
Kpn (48)	36(17)	2(2)	10(10)
M.morganii (10)	-	9(10)	1(1)
E.cloacae (8)	2(1)	5(6)	1(1)
E.aerogenes (3)	-	2(2)	1(1)
Serratia (1)	-	1(1)	-
C.freundii (1)	-	1(1)	-
P.mirabilis (1)	-	1(1)	-
Total	210 (52.5%)	89(22.25%)	101(25.25%)

Table.2 Susceptibility pattern for ESBL, AmpC & Co-producers

Antimicrobial agents	% Susceptibility		
	ESBL	AmpC	ESBL+AmpC
Cefepime	33	86.5	49.5
Cefepime + Tazobactum	84.6	88.5	93
Piperacillin + Tazobactum	48	41.67	72.17
Cefoperazone + Sulbactum	59.75	50.7	85.4
Levofloxacin	11.75	32.3	14.8
Amikacin	69.2	88.5	77.4
Gentamicin	39.3	81.25	48.7

Fig.1 Difference between CTX and CTX/CLA >5mm-an ESBL producer

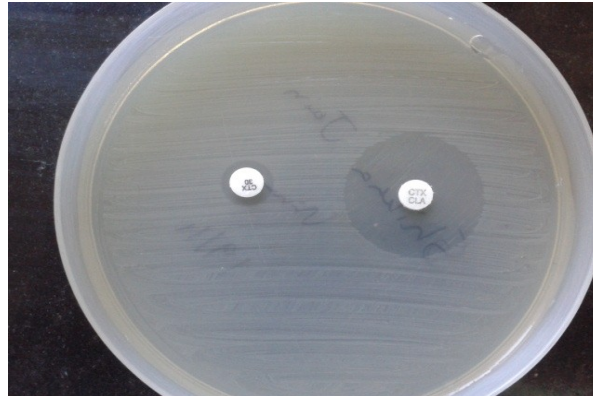


Fig.2 Difference between CTX and CTX/BA >5mm- an AmpC producer

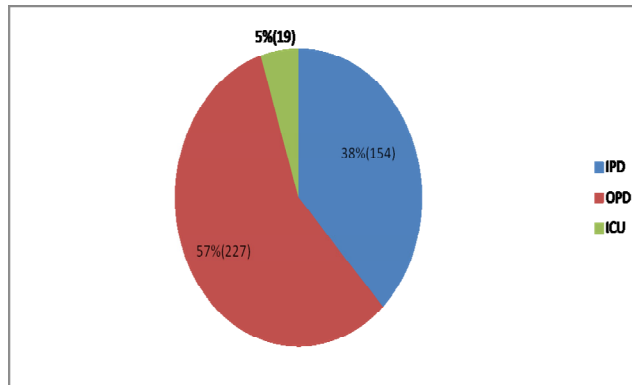


Fig.4 Distribution of the isolates according to the anatomic sites

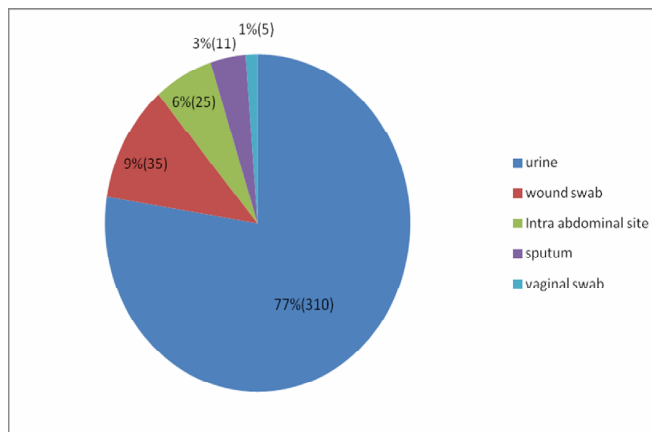


Fig.5 Species of MDR gram negative organisms

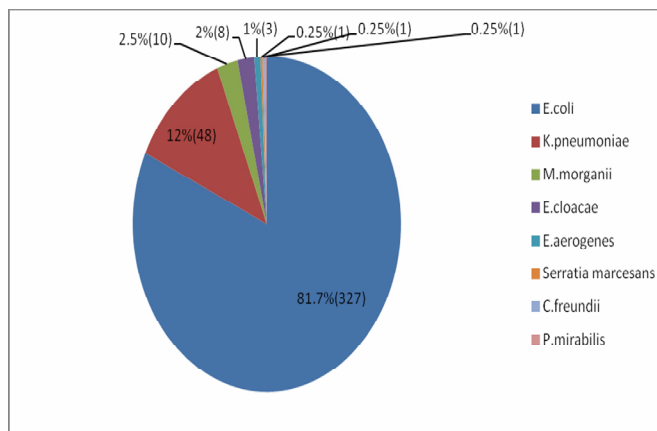
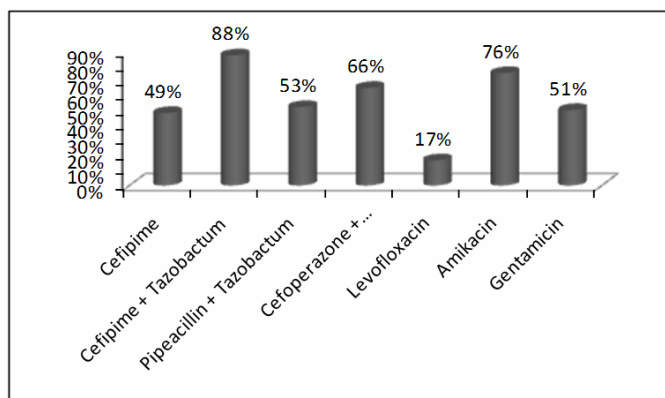


Fig.6 The overall percentage susceptibility of the MDR isolates



FEP – Cefepime, CPT -Cefepime/Tazobactam, TZP –Piperacillin/Tazobactam
 CFS - Cefoperazone/Sulbactam, LVX- levofloxacin, AK- Amikacin, GM-
 Gentamicin

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