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EVALUATION OF A COMBINATION OF THIRD GENERATION CEPHALOSPORIN, ESBL INHIBITOR AND MBL INHIBITOR: AN IN VITRO STUDY TO ASSESS THE EFFICACY FOR TREATMENT OF CARBAPENEMASE PRODUCING GRAM NEGATIVE BACILLI

Microbiology

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ABSTRACT

Gram negative bacteria belonging to Enterobacteriaceae and non-fermenting gram negative bacilli are the major causes of infections in hospitalized patients. In the past two decades, there has been remarkable increase in the resistance to beta-lactam antibiotics in these organisms. Beta-lactam ases enzymes are one of the major causes.[1, 2]. A therapeutic preparation of third generation cephalosporin combined with extended spectrum beta-lactamase (ESBL) inhibitor sulbactam and metallo -beta-lactamase (MBL) inhibitor EDTA is available for intravenous use[3,4]. The present study was aimed at testing the sensitivity of combination of these antimicrobials for the gram negative bacilli of Enterobacteriaceae and non-fermenting GNBs showing in-vitro resistance to third generation cephalosporins. This ceftriaxone combination appears to be a suitable option for management of infections by GNBs occurring in CCUs.

KEYWORDS

Gram negative bacilli (GNB), Carbapenemase, Combination Of Third Generation Cephalosporin, ESBL Inhibitor And MBL Inhibitor

Gram negative bacteria belonging to Enterobacteriaceae and non-fermenting gram negative bacilli are the major causes of infections in hospitalized patients. In the past two decades, there has been remarkable increase in the resistance to beta-lactam antibiotics in these organisms. Beta-lactamases enzymes are one of the major causes for this upsurge in resistance in these organisms. The prevalence of ESBLs reported to be as high as 62% to 100% in most tertiary care hospitals in India accounting for the resistance to third generation cephalosporins. [1]. Beta-lactam- betalactamse inhibitor combinations like piperacillin –tazobatam and carbapenems are often the alternatives for such strains. Besides ESBLs, carbapenem hydrolyzing enzymes like plasmid coded MBLs are also becoming widespread in Enterobacteriaceae and non-fermenting bacteria in India which further compromise the treatment options for infections by these organisms[2].A therapeutic preparation of third generation cephalosporin combined with ESBL inhibitor sulbactam and metallo -beta-lactamase inhibitor EDTA is available for intravenous use[3,4]. The present study was aimed at testing the sensitivity of combination of these antimicrobials for the gram negative bacilli of Enterobacteriaceae and non-fermenting GNBs showing in-vitro resistance to third generation cephalosporins.

Material & Methods:

A total of 260 isolates of Gram negative bacilli (GNB) resistant to third generation cephalosporins like ceftriaxone or cefotaxime or ceftazidime were studied. The isolates were recovered from the patients admitted in Critical Care Units(CCUs) and medical and surgical wards of a tertiary care hospital. The organisms were recovered from clinical specimens like blood, sterile body fluids, pus, sputum and tracheal-bronchial secretions. GNB were identified using standard biochemical reactions[5,6] and subjected to antibiotic sensitivity testing by Modified Kirby Bauer Disc diffusion test (CLSI 2015)[7]. All the isolates were tested for the susceptibility to carbapenems, imipenem and meropenem, a fourth generation cephalosporin cefepime, aminoglycosides including amikacin and gentamicin and a fluoroquinolone, ciprofolxacin. An antibiotic disc impregnated with ceftriaxone, disodium edetate, an inhibitor of metallo-betalactamase (MBL) and Sulbactam, an ESBL inhibitor (HI Media) was used against all the isolates to test the susceptibility to this combination of drugs. The results were interpreted according to break point zone diameters provided by Hi Media Laboratories Pvt. Ltd. A total of 193 isolates from the study recovered from the patients admitted in CCUs were also subjected to phenotypic tests for detection of Extended spectrum [7] and AmpC beta-lactamases[8] . These isolates were also screened for the presence of carbapenemases by Modified Hodge Test [7]. The isolates showing positive MHT were subjected to phenotypic tests for identification of Metallo-beta-lactamses using Imipenem and Imipenem –EDTA discs[9] and Klebsiella pneumoniae carbapenemases (KPC). For identification of KPC, boronic acid discs containing 400 mcg boronic acid/ml as KPC inhibitor were used [10].

Results

In the total of 260 isolates of Gram Negative Bacilli resistant to third generation cephalosporins, *Acinetobacter baumannii* was the predominant organism(28.8%), followed by *Klebsiella pneumoniae* (28.1%), *Pseudomonas aeruginosa* (10%)and *E. Coli* (8.8%) (Table1).

Table1: Distribution of isolates showing resistance to third generation cephalosporins

Name of the isolate	Number	Percentage
Klebsiella pneumoniae	73	28.1
E. coli	23	8.8
K. oxytoca	02	0.8
C. koseri	12	4.6
C. freundii	01	0.4
E. aerogens	16	6.2
P. vulgaris	5	1.9
P. mirabilis	03	1.2
A. baumannii	75	28.8
Acinetobacter spp	19	7.3
P. aeruginosa	31	11.9
Total	260	100

A total of 193 isolates were isolated from patients admitted in ICUs, 61 from patients admitted in surgical wards and 06 from medical wards. Of the 193 isolates from CCU patients, 46 were ESBL producers of

which 30 isolates belonged to Enterobacteriaceae and 16 were Non-fermenters. The distribution of isolates(103) producing various beta-lactamses is shown in Table2.

Table 2: Beta-lactamases distribution of CCU isolates (n=193)

Beta-lactamase	Number	Enterobacteriaceae	Non-fermenters
ESBL	46	30	16
AmpC	17	3	14
KPC	16	14	2
MBL	24	7	17

Out of total 260 isolates studied carbapenem resistance was observed in 159(61.2%) and 138(53.1%) isolates with meropenem and imipenem respectively. Resistance and an intermediate susceptibility was seen in 72(27.7%) and 54(20.8%) isolates respectively to antimicrobial combination containing ceftriaxone, sulbactam and disodium edetate(Table3).

Table3: Showing resistance to carbapenems and third generation cephalosporin with beta-lactamase inhibitors

Name of the isolate	Number	Ceftriaxone + Sulbactam + EDTA			Imipenem			Meropenem		
		S	I	R	S	I	R	S	I	R
Klebsiella pneumoniae	73	64	9	0	28	8	37	19	18	36
E. coli	23	23	0	0	14	4	5	13	3	7
K. oxytoca	02	2	0	0	2	0	0	0	0	2
C. koseri	12	1	4	7	3	1	8	0	2	10
C. freundii	01	1	0	0	1	0	0	1	0	0
E. aerogens	16	4	7	5	0	2	14	0	1	15
P.vulgaris	05	4	0	1	2	0	3	2	0	3
P. mirabilis	03	3	0	0	3	0	0	3	0	0
A. baumannii	75	18	25	32	16	7	52	17	3	55
Acinetobacter spp	19	8	4	7	6	1	12	4	1	14
P.aeruginosa	31	6	5	20	23	1	7	14	0	17
Total	260	134	54	72	98	24	138	73	28	159

Table 4: Showing susceptibilities to aminoglycosides and fluoroquinolone

Name of the isolate	Number	Gentamicin		Amikacin		Ciprofloxacin	
		S	I +R	S	I + R	S	R
Klebsiella pneumonaie	73	21	52	26	47	22	51
E. coli	23	16	7	18	5	10	13
K. oxytoca	02	1	1	1	1	0	2
C. koseri	12	10	2	10	2	0	12
C. freundii	01	0	1	0	1	0	1
E. aerogens	16	0	16	6	10	0	16
P.vulgaris	05	2	3	2	3	0	5
P.mirabilis	03	3	0	3	0	3	0
A. baumannii	75	4	71	4	71	25	50
Acinetobacter spp	19	1	18	1	18	5	14
P.aeruginosa	31	13	18	13	18	11	20
Total	260	71	189	84	176	76	184

Discussion

Antimicrobial resistance among enterobacteriaceae and non- fermenting gram negative bacilli like *Acinetobacter baumanii* and *Pseudomonas aeruginosa* is of increasing concern. Beta-lactam resistance in these organisms occurs through various mechanisms including production of beta-lactam hydrolysing enzymes, beta-lactamases. In the present study a total of 260 isolates of GNBs showing resistance to third generation cephalosporins were recovered during the study period of which 193 were from patients admitted in CCUs. Enterobacteriaceae organisms accounted for 135 isolates whereas 125 isolates were Non fermenting Gram negative bacilli.

Extended spectrum and AmpC beta-lactamses can hydrolyse third generation cephalosporins like ceftriaxone, cefotaxime and ceftazidime. In this study 46(23.8%)and 17(8.8%) isolates from ICUs were ESBL and AmpC producers respectively. As these enzymes don't hydrolyse carbapenems, these are the drugs of choice for treatment of infections caused by the organisms producing ESBL & AmpC enzymes. These broad spectrum beta lactam agents are often used for empiric treatment of very serious infections caused by GNBs. As carbapenems are viewed as agents of last resorts, resistance to them is a significant clinical and public health concern.

Various mechanisms described for carbapenem resistance among gram negative bacilli include, hyperproduction or derepression of AmpC and ESBLs along with loss of outer membrane porins, augmented drug efflux, alteration in penicillin binding proteins and production of carbapenemases belonging to three molecular classes, Ambler class A, B and D. The most important class A carbapenemases is Klebsiella pneumoniae carbapenemase (KPC) mainly accounting for the carbapenem resistance in enterobacteriaceae. Class B carbapenemases require metal ion, usually zinc for beta-lactam hydrolysis and hence are known as metallobetalactamases.

Though initially described MBLs were chromosomal in origin, the plasmid mediated mobile elements described since mid 90s are the major concern for carbapenem resistance encountered in clinical practice. They are most frequently found in the carbapenem resistant *Pseudomonas aeruginosa* and Acinetobacter spp.

The more commonly encountered MBLs include VIM-1 and NDM-1. Though initially confined to non-fermenting gram negative bacilli, VIM-1 is increasingly being reported in Enterobacteriaceae isolates. NDM-1 was first described in 2008 in a Swedish patient admitted in India. Since then this enzyme has become most widely distributed enzyme worldwide including India. In this study as many as 24(12.4%) isolates were MBL producers in ICU isolates. Owing to dependence on zinc ions for hydrolysis, the action of metallobetalactamases is inhibited by chelating agents like EDTA. The present study shows that ESBL producing and MBL producing gram negative bacilli account for the substantial number of infections in CCUs. In the present study out of 260 isolates resistant to third generation cephalosporins, 134 and 54 were sensitive and intermediate sensitive to ceftriaxone combined with ESBL inhibitor sulbactam and MBL inhibitor EDTA. Hence the use of ceftriaxone combined with ESBL inhibitor sulbactam and MBL inhibitor EDTA appears to be a good alternative for the empirical management of such isolates and the use novel antibiotics like carbapenems can be reduced significantly. The sparing use of carbapenem can reduce the emergence of resistance to these life saving drugs against treatment of most serious infections.

The carbapenemases producing gram negative bacilli often exhibit decreased susceptibility to other antimicrobials including fluoroquinolones and aminoglycosides. In the present study 74.07% Enterobacteriaceae (135) and 67.2% NFGNBs (125) showed resistance to fluoroqiunoloes. 85.6% NFGNBs showed resistance to aminoglycosides however in enterobacteriaceae resistance to gentamicin and amikacin was 60.74% and 51.11% respectively. For such isolates, polymyxins and tigecyclines remain as the only options for treatment. The nephrotoxicity of polymyxins limits their use in the management and the role of tigecycline for treatment of blood stream infections is not very encouraging. In view of these observations ceftriaxone combined with ESBL inhibitor sulbactam and MBL inhibitor EDTA appears to be a suitable option for management of infections by GNBs occurring in CCUs.

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