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In-Vitro Sensitivities of Combinations of Cefipime, Ceftazidime, Cefpirome Along With Beta Lactamase Inhibitors Sulbactam against ESBL Producing Enterobacteriaceae

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Abstract: ESBL (extended spectrum beta lactamase) producing enterobactericeae are resistant to betalactam group of antibiotics causing a great threat to their treatment options .The present study was undertaken to determine in-vitro sensitivity of some betalactam/ betalactamase combinations like cefipime, cefpirome, ceftazidime, ceftriaxone with sulbactam along with Tobracef (Tobramycin+Ceftazidime) against these group of enterobacteriaceae in order to choose an alternative treatment options. A total of 505 clinical samples were tested. 168 gram negative bacilli were isolated from those samples. Those isolates were screened for ESBL production by methods recommended of CLSI. Maximum ESBL production was seen amongst E.coli & Klebsiella species. Susceptibility pattern of different Cephalosporin with beta lactamase inhibitor combinations were tested amongst these ESBL strains. Showed E.coli & Klebsiella showed highest sensitivity to Supime disc (cefipime + sulbactam), Pseudomonas to Supime (cefipime + sulbactam), Zydotum (Ceftazidime + sulbactum), Acinetobacter to Pirotum (Cefpirome + Sulbactam), Citrobacter to vancoplus (Ceftriaxone + vancomycin), Enterobacter to salbactomax (Ceftriaxone + salbactum). This betaLactam/betalactamase combination therapy may be tried against ESBL producing enterobacteriaceae where sole choice of therapy is with Imepenem.
Keywords: Gram Negative bacilli, Extended spectrum beta lactamase, beta – lactamase inhibitors, Enterobacteriaceae.

INTRODUCTION

With extensive use of 3rd & 4th generation cephalosporin antibiotic resistance has now become a major problem all over the world , particularly in hospital set up[1]. Multiple drug resistance in Gram negative bacteria is mediated through R -Plasmids results in wide spread resistance not only to beta lactam antibiotics but also to amino glycosides and fluoroquinolones.

Thus effective treatment in hospital setup is compromised due to high prevalence of ESBL (Extended spectrum Beta lactamase) producing Enterobacteriaceae. They are able to hydrolyse oxyiminocephalosporins (3rd generation cephalosporin) and monobactums (Aztreonum) but cannot hydrolyse Cephamycins (Cefoxitins) & carbapenems (Imepenems) [2]. But there is no substantial clinical data to support the use of cephamycins in treating infections with ESBL producing organisms. Although carbapenems are effective but continuous use may substitute one problem for another because this therapy can select out for other resistant organisms like Acinetobacter[2]. Hence beta lactamase mediated resistance is overcome by several beta lactamase inhibitor combinations which bind irreversibly to beta lactamase and render them ineffective & thus sparing beta lactam antibiotics & ensuring their effectiveness. Currently many new beta lactam antibiotics along with beta lactamase inhibitor combinations have been marketed to combat these ESBL problems [3].

The present study carried out to evaluate the in- vitro activities of three commercially available beta –lactam antibiotics like cefipime, cefpiromes (4th generation cephalosporins) and Ceftazidime & ceftriaxone (3rd generation cephalosporins) with beta lactamase inhibitors Sulbactam combinations against ESBL producing enterobacteriaceae in a tertiary care hospital. Along with these in vitro sensitivity of Tobracef (Tobramycin + Ceftazidime) (CFT) & Sulbactamax (ceftriaxone + sulbactam) (SBX) has been determined.

MATERIALS & METHODS

The study was carried out in Bacteriology laboratory of a tertiary care Medical centre over a period of Jan 2013 – Jan 2014. Samples like urine, sputum, pus, tracheal aspirate, Broncho alveolar lavage fluid, blood culture received from all departments were processed for isolation and identification of bacterial pathogens according to standard Microbiological techniques. All gram negative bacteria isolated from these samples were tested for ESBL productions by using cefotaximes (30 ug), Ceftazidime (30 µg), (Hi – Media Mumbai), Ceftazidime + Clavulinic acid (Cac) (Hi media) disc. Escherichia coli

(ATCC 25922) (Beta lactamase negative) and Klebsiella Pneumoniae (ATCC 700603) (ESBL positive) strains were used as control organisms. The performance and interpretation of these test was done as per Clinical laboratory standards Institute(CLSI) guidelines[4]. Anti-Microbial sensitivity testing was done in Muller- Hinton Agar plates (Hi – Media, Mumbai) by disk diffusion methods according to CLSI guidelines. The following disks were tested Supime(CPS) (Cefipime + Sulbactum) (30: 15 µg), Zydotum (CAS)(Ceftazidime + Sulbactum) (30:15 µg), Pirotum (CRS)(30: 15 µg (Cefpirome + Sulbactum), Sulbactomax (SBX)(Ceftriaxone+ Sulbactum) (30: 15 µg) ,Vancoplus (CVA)(Vancomycin + Ceftriaxone) ,Tobracef(CFT) (Tobramycin + ceftazidime) obtained from Venus remedies, India. The diameter of the zones of inhibition was recorded and interpreted as Sensitive, Resistant and Intermediate based on CLSI guidelines [10].

RESULTS

A total of 505 samples were processed, amongst which 168 strains isolated were Gram Negative bacteria (33.6%) Of these 82 strains were ESBL producers (49%).E.coli & Klebsiella were amongst highest ESBL producers. (Table 1: showing organisms producing ESBL supported by pie diagram) E.coli has highest sensitivity to Supime (Cefipime + sulbactum)(CPS) (76.59%) (Table 2: showing sensitivity of E.coli along with bar diagram), Klebsiella showed highest sensitivity to (CPS) Supime (Cefipime + Sulbactum) (30: 15 µg)(57.44%)(Table 3: showing sensitivity of Klebsiella along with bar Diagram), Pseudomonas sensitive to Supime (Cefipime+sulbactum (CPS75%), Zydotum (Ceftazidime +sulbactum)(CAS)(50%), (Salbactomax)(SBX) (Ceftriaxone+Sulbactum) (50%) & Vancoplus (CVA) (Vancomycin + ceftriaxone) (50%) but 25% resistance to Tobracef (CFT)(Tobramycin +ceftazidime)(Table 4 showing sensitivity of Pseudomonas along with a bar diagram). Citrobacter 100% sensitive to Vancoplus (CVA) (Vancomycin + ceftriaxone) & (Pirotum) (CRS) (Cefpirome + Sulbactum) & 100% resistance to CAS (Zydotum) (Ceftazidime + sulbactum) & CFT (Tobracef) (Tobramycin+vancomycin)(Table 5 showing sensitivity of citrobacter along with bar diagram). Enterobacter 100% sensitive to (Salbactomax)SBX (Ceftriaxone + Sulbactum) but 100% resistance to CFT(Tobramycin +ceftazidime), CVA (Ceftriaxone + Vancomycin) & CAS(Ceftazidime + Sulbactum)(Table 6 showing sensitivity of Enterobacter along with bar diagram). Acienetobacter 50% sensitive to CRS (Cefpirome + Sulbactum) but 50% resistant to CAS, CVA &CFT. (Table7 showing sensitivity of Acinetobacter along with bar diagram)

DISCUSSION

A total of 505 different clinical samples were processed of which 168 isolates were Gram Negative bacilli (33.6%) amongst which 82 strains were ESBL producers (49%) This incidence was quite low as compared to earlier studies by Mahanti *et al*. (68%) [1] & Susan *et al*. (67%) [10] ESBL production was maximum in case of E.coli (58.02%) & Klebsiella (33.33%).

In fact each and every organism exhibiting resistance to Cephalosporins were invariably ESBL producers. Combining Third generation cephalosporins with Beta lactamase inhibitors like Supime (CPS) (Cefipime + Sulbactum) enhanced the sensitivity of ESBL producing E.coli & Klebsiella.

Fu Der Wang *et al*. supported the facts that addition of sulbactam lowered the resistance rates significantly for E. cloaca, A. baumani, & ESBL producing K. pneumonia [3]. Sulbactam is a competitive, irreversible inhibitor of a wide variety of beta lactamases produced by common gram positive and gram negative aerobes and anaerobes. The feature that distinguishes sulbactam from other available Beta Lactamase inhibitors is its high level of antimicrobial activity against Bacteroides fragilis and Acinetobacter species organisms against which most cephalosporins display little or no activity [5]. Binding of Sulbactam to PBP2 of these organisms potentiates its intrinsic antibacterial activity.

Acinetobacter infections are frequently associated with long standing ventilators usage. Other risk factors include neurosurgery, ARDS, Large volume of pulmonary aspiration. Given the increasing availability to intensive care facilities and more invasive clinical procedures, the prevalence of Acinetobacter infections is likely to increase [11].

In our study Acinetobacter shows 100% sensitive to Pirotum (Cefpirome + Sulbactam) (CRS) & resistance to other three combinations. F.D Wang *et al.* supported the fact that significant lowering of MIC s for Acinetobacter occurs with combination of cephalosporin + Sulbactam combinations than when used alone[3,7].

Many studies have investigated the in–vitro activity of cefoperazone-sulbactam combination and found it to be superior to that of Cefoperazone alone against clinical isolates of many Gram negative bacilli but particularly against Acinetobacter species, when activity is due to sulbactam alone[6,8].

In accordance with another study by Smitha sood *et al.* cefipime-tazobactum combination showed 100% sensitivity against ESBL producing E.coli & Klebsiella pneumonia isolates, followed by Cefoperazone–sulbactam which showed 94.73% and 90% sensitivity respectively[9]. The poorest sensitivity amongst all the beta–lactum/Beta lactamase inhibitor combinations against ESBL positive isolates was observed for Ampicillin-Sulbactam [11].

The superiority of Tazobactum over Sulbactam was supported by Susan M et al because Tazobactam showed significantly greater activity than Sulbactam against TEM-I & SHV-I enzymes (Inhibitor Resistant beta lactamase), most prevalent plasmid encoded enzymes produced by gram negative bacilli [10].

Cefipime, a fourth generation cephalosporins can withstand hydrolysis by a large proportion of beta lactamases including Amp–C enzymes. This supported the efficacy of this particular beta lactam & beta lactamase combinations (Supime) (cefipime + sulbactam) undoubtedly [12].

Results with ceftazidime+ sulbactam (Ceftazidime)(CAS) & Sulbactamax (SBX) (Ceftriaxone + sulbactum), Vancoplus (CVA) (Vancomycin + Ceftriaxone) Tobracef (Tobramycin+ ceftazidime) also showed significant sensitive strains amongst E.coli, Klebsiella, Pseudomonas.

Table-1: Organisms Producing ESBL

ORGANISMS PRODUCING ESBL	Nos
E.coli	47
Klebsiella	27
Acinetobacter	2
Citrobacter	1
Pseudomonas	4
Enterobacter	1
Total	82

Table-2: Sensitivity of E.coli (total 47 strains)

	Sensitive	Intermediate	Resistant
CPS	37	4	1
CAS	19	14	14
SBX	26	1	3
CVA	9	0	27
CFT	7	6	34
CRS	36	3	0

Table-3: Sensitivity of Klebsiella (Total 27 strains)

	Sensitive	Intermediate	Resistant
CPS	21	0	0
CAS	12	6	6
SBX	16	1	3
CVA	6	0	20
CFT	6	2	18
CRS	1	0	0

Table-4: Sensitivity of Pseudomonas (4 Strains)

	Sensitive	Intermediate	Resistant
CPS	3	0	2
CAS	2	0	2
SBX	2	1	0
CVA	2	0	0
CFT	0	0	1
CRS	1	0	3

Table-5: Sensitivity of Enterobacter :(1 strain)

	Sensitive	Intermediate	Resistant
CAS	0	0	1
SBX	1	0	0
CVA	0	0	1
CFT	0	0	1

Table-6: Sensitivity of Acinetobacter: (2 strains)

	Sensitive	Intermediate	Resistant
CAS	0	0	1
SBX	0	1	0
CVA	0	0	1
CFT	0	0	1
CRS	1	0	0

Table-7: Sensitivity of CITROBACTER: (1 strain)

	Sensitive	Intermediate	Resistant
CAS	0	0	1
SBX	0	1	0
CVA	1	0	0
CFT	0	0	1
CRS	1	0	0

CONCLUSIONS

A majority of Gram Negative Bacilli that is isolated from clinical specimens are resistant to 3rd & 4th generation cephalosporin. All Beta lactam/Beta Lactamase inhibitors (BL/BLI) combinations need not enhance the activities of cephalosporins, when used in combinations, e.g Clavulanate acid has virtually no effect. Tazobactam and sulbactam however substantially enhanced the activity of cephalosporins.

With the emergence of wide spread resistance to 3rd & 4th generation cephalosporin resulting in poor patient outcomes, increased total health care cost, and increased use of carbapenems. Carbapenems are considered the agents of last resort to combat gram negative infections in intensive care units and high risk wards. Cefipime is a fourth generation cephalosporin stable against beta lactamases like AmpC and OXA. Thus these BL/BLI combinations can virtually act as an alternative to carbapenem.

REFERENCES

1. Mohanty S, Singhal R, Sood S, Dhawan B. Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against gram negative bacteria. Indian Journal of Medical Research. 2005 Nov 1;122(5):425.
2. Labombardi VJ, Rajtman A, Tran K. Use of cefipime for treatment of infections caused by extended spectrum beta lactamase producing Klebsiella pneumoniae and Escherichia coli. Diagn Microbiol Infect Dis 2006; 56:313-15.
3. Wang FD, Lin ML, Lee WS, Liu CY. In vitro activities of β-lactam antibiotics alone and in combination with sulbactam against Gram-negative bacteria. International journal of antimicrobial agents. 2004 Jun 1;23(6):590-5.
4. Combination with sulbactam against Gram-Negative bacteria. Int J Antimicrob Agents 2004;23:590-5.
5. National Committee for Clinical Laboratory standards. Performance standards for antimicrobial disk susceptibility Testing. Twenty fifth informational supplement (M100-S12) 2002. Wayne PA:NCCLS
6. Williams JD. B-Lactamase inhibition and in vitro activity of sulbactam and sulbactam/cefoperazone. Clinical infectious diseases. 1997 Mar 1;24(3):494-7.
7. Jones RN, Wilson HW, Thornsberry C, Barry AL. In vitro antimicrobial activity of cefoperazone-sulbactam combinations against 554 clinical isolates including a review and β-lactamase studies. Diagnostic microbiology and infectious disease. 1985 Nov 1;3(6):489-99.
8. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. Critical care medicine. 1999 May 1;27(5):887-92.
9. Knapp CC, Sierra-Madero J, Washington JA. Comparative in vitro activity of cefoperazone and various combinations of Cefoperazone/Sulbactam. Diagn Microbiol Infect Dis 1990; 13:45-9.
10. Mohanty S, Singhal R, Sood S, Dhawan B. Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against gram negative bacteria. Indian Journal of Medical Research. 2005 Nov 1; 122(5):425.
11. Susan M, Hariharan TS, Sonya J. A comparative in Vitro study of Cephalosporin/Beta lactamase

inhibitor combinations against Gram negative bacilli. Indian J Physiol pharmacol 2013;57:425-31

12. Sader HS, Tosin I, Sejas L, Miranda E. Comparative evaluation of the in vitro activity of three combinations of beta-lactams with beta-lactamase inhibitors: piperacillin/tazobactam, ticarcillin/clavulanic acid and ampicillin/sulbactam. The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases. 2000 Feb;4(1):22-8.
13. Payne DJ, Cramp R, Winstanley DJ, Knowles DJ. Comparative activities of clavulanic acid, sulbactam, and tazobactam against clinically important beta-lactamases. Antimicrobial agents and chemotherapy. 1994 Apr 1;38(4):767-72.