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Original Article

A comparative *in vitro* study of Cephalosporin/Beta-lactamase inhibitor combinations against Gram negative bacilli

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Abstract

The present study aims at comparing the in-vitro susceptibility of six commercially available cephalosporin – BLI combinations with cephalosporins alone against hospital isolates of Gram negative bacilli. Gram negative bacilli, numbering 500, isolated from various clinical samples, were included in the study. The isolates were also screened for ESBL production by the methods recommended by CLSI. Susceptibility pattern of six Cephalosporins/ Betalactamase inhibitor (BLI) combinations were compared with their partner cephalosporins. Overall, 29.6% of Gram negative bacilli were susceptible to the five Cephalosporins (IIIrd & IVth gen); the highest activity being shown by cefepime. Susceptibility was much higher (more than double) to the Cephalosporin combinations containing Tazobactam (TZB) & sulbactam (SLB) (62.7%). However such enhanced susceptibility was completely lacking with combinations containing clavulanate (29.1%). Gram negative bacilli, as a group, exhibited very high resistance to the new cephalosporins (IIIrd & IVth gen). When these agents were tested as fixed-dose combinations with TZB & SLB, the overall susceptibility was enhanced by more than 100%. Such an enhancement was absent with clavulanate combinations. Cefepime/TZB revealed the highest activity against ESBL producing GNB. Further studies are needed in the clinical settings as they can play an important role as good alternatives to carbapenems.

INTRODUCTION

Indiscriminate use of third & fourth generation cephalosporins during the last decade has led to the development of widespread resistance to these agents among Gram negative bacilli (GNB) (1). Since betalactamase production is the major mechanism of resistance to the betalactam agents, their use in combination with BLI's provides a logical & effective measure to counter this problem. Four combinations of various Penicillins with Beta-lactamase inhibitors (BLI) were introduced during 1982-90, all of which have been established as very valuable agents in the management of serious infections. The situation is, however, different with cephalosporins. While Cefoperazone-Sulbactam is one fixed-dose combination approved for use in many European countries, such combinations have not yet been approved for use in the U.S or U.K or other developed countries (2). At the same time, many cephalosporin - BLI combinations are readily available in the Indian market, but very few in-vitro studies are available regarding their superiority over their partner cefalosporins (2). Also, studies have shown that the same betalactam combined with different BLIs have differential efficacy against Enterobactericiae (3).

The present study aims at comparing the in-vitro susceptibility of six commercially available cephalosporin – BLI combinations with cephalosporins alone against hospital isolates of Gram negative bacilli.

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Materials and Methods

Antibiotic sensitivity discs :

The following discs from HiMedia, Mumbai, were used for sensitivity testing **:**

Cephalosporin discs (plain) :

1. Cefepime (30 mcg)
2. Cefoperazone (75 mcg)
3. Cefotaxime (30 mcg)
4. Cefixime (5 mcg)
5. Ceftriaxone (30 mcg)

Cephalosporin – BLI combination discs :

1. Cefepime – Tazobactam (80/10)
2. Ceftriaxone – Sulbactam (30/15)
3. Cefotaxime – Clavulanic acid (30/10)
4. Ceftriaxone – Tazobactam (30/10)
5. Cefoperazone – Sulbactam (75/30)
6. Cefixime – Clavulanic Acid (5/10)

Methodology

Biological samples obtained from patients admitted to the MOSC Medical college hospital, Kolenchery, Kochi, Kerala were used for the study. Isolation and identification of bacterial pathogens were performed according to standard microbiological techniques.[4] All isolates of Gram negative bacilli were included in the study.

Susceptibility testing was performed on Mueller-Hinton Agar plates (Hi Media, Mumbai) by the disc diffusion method as per CLSI (formerly NCCLS) guidelines. The diameters of the zones of inhibition of growth were expressed as 'sensitive' or 'resistant' based on CLSI guidelines (5). Moderately sensitive strains were considered resistant.

Testing for ESBL production :

Each isolate of Gram negative bacilli was considered a potential ESBL producer, if the zones of inhibition in the disc diffusion tests were as follows :

cefotaxime < 27 mm, ceftriaxone < 25 mm, ceftazidime < 22 mm & cefpodoxime < 30 mm.

For disc diffusion testing, a > 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism. Escherichia.coli ATCC 25922 (ESBL negative) & Klebsiella pneumoniae ATCC 700603 (ESBL positive) strains were used as control (5). The performance & interpretation of the tests were done as per CLSI guidelines.

Results

Cefalosporins alone :

A total of 500 isolates of GNB obtained from biological samples were included in the study. E.coli constituted > 50% of the organisms; K.pneumoniae, Ps. Aeruginosa and A.boumanii together accounted for another 40% (Fig. 1). On an average, 30% of gm negative bacilli were susceptible to the five cefalosporins. Cefepime exhibited the highest activity (34.2%), followed by cefotaxime and ceftriaxone (29.6%) (Fig. 2).

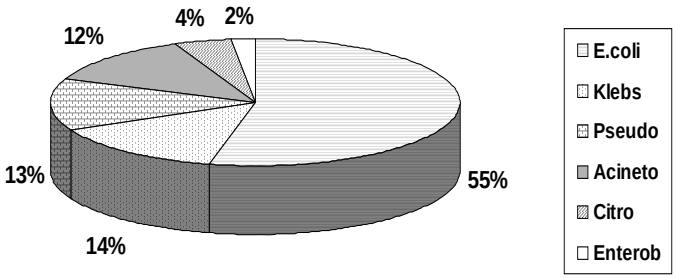

Fig. 1 : Percentage of Gm – ve isolates from clinical samples.

Combinations with TZB and SLB :

Fig. 3 shows the comparative activities of the three BLI combinations with cefalosporins. Substantially enhanced activity against gm negative bacilli was

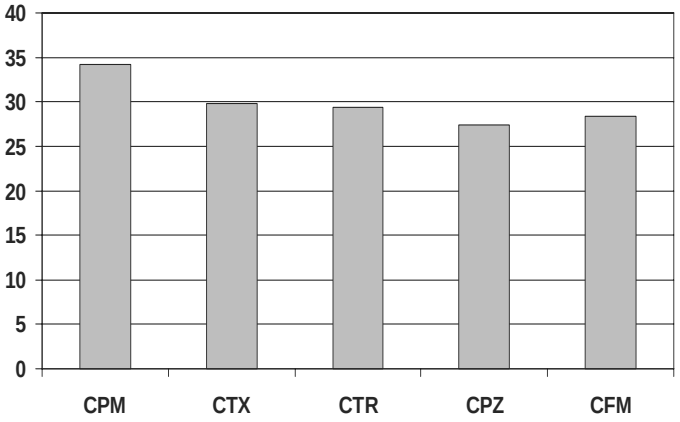

Fig. 2 : Comparative overall suceptibility (%) of Cefalosporins against gm –ve bacilli

CPM – cefepime, CTX – cefotaxime, CTR – ceftriaxone, CPZ – cefoperazone, CFM – cefixime.

demonstrated by sulbactam (SLB) and tazobactam (TZB) combinations, TZB being superior to those with SLB (66.8% and 58.6% respectively) (Fig. 4). Enhanced activity with the combinations was expressed mostly against E.coli, K.pneumoniae, A.boumanii and Ps.aeruginosa, and this was statistically significant. Among the two BLI combinations involving ceftriaxone too, the one with TZB was superior to SLB combination (63.4% to 58%) (Table I). Ps.aeruginosa exhibited a difference in being more susceptible to SLB combinations rather than the combination with TZB, but this was not statistically significant.

Cephalosporin – Clavulanate combinations :

In contrast to the other two, the cephalosporin combinations involving clavulanate (CLV) did not show any significant increase in activity against GNB (Fig. 3).

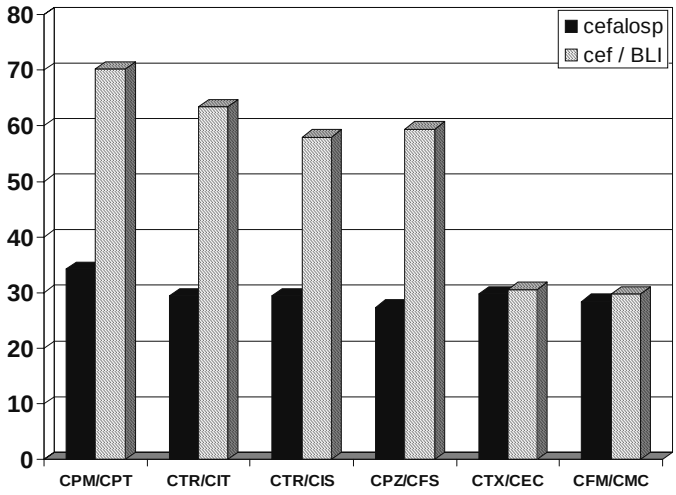

Fig. 3 : Susceptibility pattern (%) of cefalosporins vs BLI combns.

CPM – cefepime, CPT – cefepime / TZB, CTR – ceftriaxone, CIT – ceftriaxone / TZB,

CIS – ceftriaxone / SLB, CPZ – cefoperazone, CFS – cefoperazone / SLB, CTX – cefotaxime,

CEC – cefotaxime / CLV, CFM – cefixime, CMC – cefixime / CLV

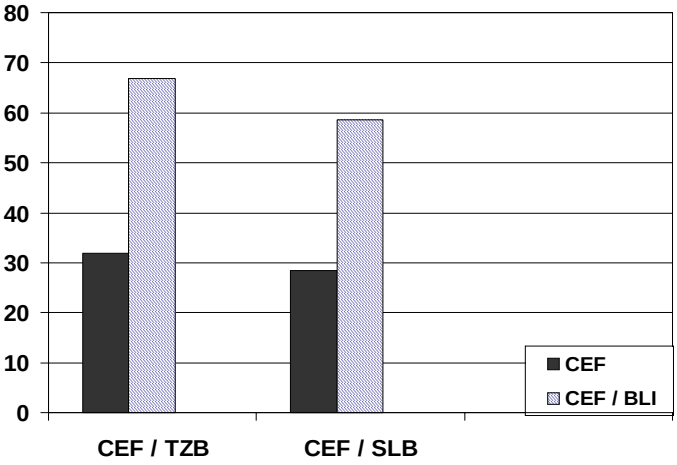

Fig. 4 : Comparative overall susceptibility of Tazobactam and Sulbactam comibanations.

CEF/TZB – cefalaosporin/TZB, CEF/SLB – cephalosporin/SLB

TABLE I : Comparative susceptibility (%) of Cephalosporins and Cephalosporin/ BLI Combinations.

Organism	*CPM*	*CPT*	*CTR*	*CIT*	*CIS*	*CPZ*	*CFS*	*CTX*	*CEC*	*CFM*	*CMC*
All bacilli	34.2	70.2	29.4	63.4	58.0	27.4	59.4	29.8	30.6	28.4	29.8
E.coli	38.6	86.9*	34.1	80.9*	72.3	30.3	72.7*	35.2	35.2	31.1	33.0
Kleb.pneu	27.1	67.1*	25.7	62.9*	48.6	25.7	51.4*	24.3	24.3	25.7	25.7
Ps. Aeru.	44.8	55.2	37.3	43.3	49.3	37.3	61.2	35.8	44.8	38.8	44.8
Acineto.	9.7	25.8	3.2	16.1	19.4	3.2	19.4	4.8	1.6	4.8	3.2
Citro.	27.3	50.0	22.4	40.9	36.4	22.4	27.3	27.3	27.3	27.3	27.3
Entero.	50.0	60.0	40.0	70.0	70.0	40.0	60.0	30.0	30.0	40.0	30.0

CPM – C cefepime, CPT – C cefepime/TZB, CTR – C ceftriaxone, CIT – C ceftriaxone/TZB, CIS – C ceftriaxone/SLB, CPZ – C cefoperazone, CFS – C cefoperazone/SLB, CTX – C cefotaxime, CEC – C cefotaxime/CLV, CFM – C cefixime, CMC – C cefixime/ CLV.

*P<0.05 compared to cefalosporins alone.

TABLE II : *In vitro* activity of Cephalosporin - BLI combinations against ESBL positive organisms.

Organism	*Total no*	*ESBL positive*	*No (%) of organisms susceptible*						*No (%) of organisms resistant to all combinations*
			CPT	*CIT*	*CIS*	*CFS*	*CEC*	*CMC*	
All Gm–ve bacilli	500	339 (68)	248 (73)	203 (59)	175 (51)	172 (50)	02 (0.005)	–	91 (26)
E.coli	267	171 (64)	134 (78)	120 (70)	99 (58)	102 (60)	2 (01)	–	37 (21)
Kleb.pneu	70	51 (73)	30 (59)	27 (53)	17 (33)	20 (39)	–	–	21 (41)
Ps. Aeru.	67	34 (48)	10 (29)	8 (24)	9 (26)	10 (29)	–	–	24 (71)
Acineto.	62	58 (94)	13 (22)	8 (14)	9 (16)	10 (17)	–	–	45 (83)
Citro.	22	16 (73)	5 (31)	2 (13)	2 (13)	2 (13)	–	–	11 (69)
Entero.	10	07 (70)	2 (29)	2 (29)	2 (29)	1 (14)	–	–	5 (71)

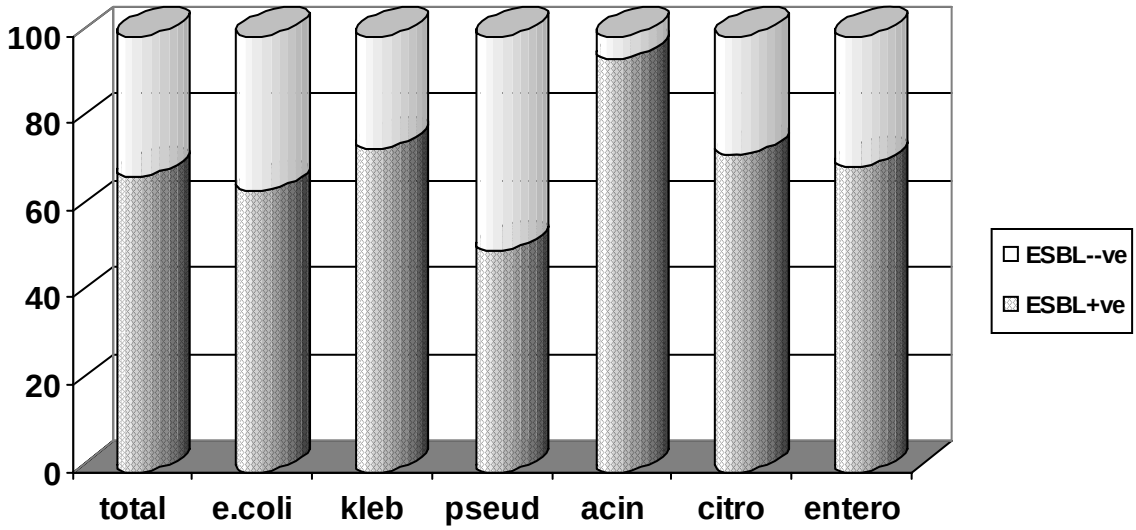

Fig. 5 : Percentage of ESBL positive organisms in clinical samples.

ESBL production among GNB :

On the whole, 67.8% of gm negative bacilli were found to be ESBL producers – whereas 94% of Acinetobacter strains were ESBL positive, the property was seen in only 50.7% of pseudomonas; for others it was in the range of 64 to 73% (Fig. 5).

Susceptibility of ESBL positive organisms to cefalosporins :

None of the five cefalosporins studied exhibited any activity against organisms elaborating ESBL enzymes when used alone; this was uniformly the case against all GNB. In contrast, combinations of cefalosporins with TZB as well as SLB exhibited very high activity against ESBL producers (66% and 50.5% respectively) (Table II); the difference between TZB and SLB combinations was statistically significant. Susceptibility to Cefepime – TZB and ceftriaxone – TZB were 73% and 59& respectively. In the case of clavulanate combinations, only a negligible two out of 339 ESBL positive strains were susceptible (Table II, Fig. 6).

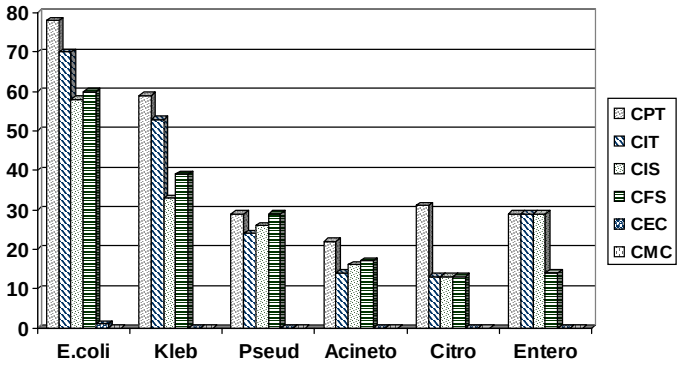

Fig. 6 : *In vitro* activity of Cephalosporin – BLI combinations against ESBL positive organisms.

CPT – cefepime / TZB, CIT – ceftriaxone / TZB, CIS – ceftriaxone / SLB, CMC – cefixime / CLV, CEC – cefotaxime / CLV, CFS – cefoperazone / SLB

Discussion

In the present study, only around 30% of the GNB were susceptible to third and fourth generation cefalosporins. Cefepime was clearly superior to the other four cephalosporins. Higher activity of cefepime was directed mainly against E.coli, Pseudomonas & Acinetobacter. Cefepime being the only fourth generation agent, is resistant to hydrolysis by a majority of the betalactamase subtypes including chromosomally encoded Amp C enzymes produced by Gram negative bacilli (with the exception of ESBLs) (7). In contrast, Amp C enzymes are capable of hydrolyzing third generation cephalosporins, resulting in a decreased susceptibility of the pathogen. Individual Gram negative bacilli are known to be capable of elaborating more than one subtype of betalactamases.

What is more revealing is that more than 65% of the gram negative pathogens isolated from biological samples exhibited resistance to the all the five cephalosporins (third & fourth generation) included in the study.

Such high degree of resistance could be mainly due to their indiscriminate use, this being a mechanism of defence against these agents. Beta-lactamase production is the main mechanism leading to this form of acquired bacterial resistance (2).

Extended Spectrum Beta-Lactamases (ESBL) are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of beta-lactams including third generation cefalosporins (8). The beta-lactamase inhibitors are capable of inhibiting a variety of beta-lactamases including ESBL enzymes. A highly effective approach for tackling beta-lactamase induced resistance is the use of beta-lactam/BLI combinations (9). The well-established usefulness of BLI combinations with penicillins is suffice to validate this point.

The results of this study are consistent with this approach. In the present study, 68% of gm-ve bacilli showed positive results for ESBL production, the incidence is quite similar (68.78%) to an earlier study by Mohanty et al. (9). ESBL production was maximum for Acinetobacter (94%) (Fig. 5). In fact, each and every organism exhibiting resistance to the cefalosporins was invariably an ESBL producer.

While only < 30% of the organisms were susceptible to these antibiotics used alone, combining them with BLIs (TZB or SLB) substantially enhanced their activity to more than 60% (Table I, Fig. 3). Enhanced susceptibility was exhibited by all gm-ve bacilli; maximum being E.coli and Klebsiella. TZB combinations were superior to those with SLB in this regard (66.8% to 58%). Many studies have documented the superiority of TZB over SLB and CLAV when combined with piperacillin (9, 10). In one study, TZB showed significantly greater activity than SLB against TEM-1 and SHV-1 enzymes, the most prevalent plasmid mediated enzymes produced by gm –ve bacilli (11). The greater activity exhibited by the two TZB combinations over SLB combinations in the current study is in concurrence with the above finding, though the partner antibiotic was different. Among the many variants of the betalactamase superfamily, certain TEM variants (inhibitor resistant TEMs) identified in many Gram negative bacilli including E.coli & Klebsiella are not inhibited by SLB or Clavulanate, but remain susceptible to TZB; so is the case with OXA enzymes (12). Thus the better inhibitory effect of TZB vs clavunate and sulbactam is enough to inhibit a majority of beta-lactamases in a complex betalactamase background (13).

Among the TZB combinations, those involving cefepime exhibited significantly higher activity (73% to 59%). This is in accordance with another study done by Smita Sood (14). As stated earlier, this stems from the ability of Cefepime, a fourth generation drug, to withstand hydrolysis by a larger proportion of betalactamases including Amp C enzymes, in contrast to ceftriaxone (15). It is noteworthy that the available B.L.I.s primarily inhibit Class A serine based enzymes (including ESBLs), but have no effect on Class C enzymes (13).

Surprisingly, clavulanate, another beta lactamase inhibitor, failed to demonstrate any increase in activity of its partner cephalosporins – only Pseudomonas shows some enhanced sensitivity, but this was not statistically significant. This difference

with clavulanate combinations is on expected lines, since this is the only inhibitor capable of inducing production of AmpC beta lactamase enzymes by gm negative bacilli – this evidently could negate the advantage of inhibition of other enzymes including ESBLs (16, 17). This possibility needs confirmation by studies using techniques for detecting AmpC production – this institution lacks this facility. Poor activity for clavulanate combinations compared to TZB and SLB combinations was also observed in a previous studies involving BLI/Penicillin combinations (18, 19).

Conclusions

A majority (>60%) of the GNB isolated from clinical specimens exhibited resistance to third and fourth generation cefalosporins. Beta-lactamase inhibitors need not always enhance the activity of cefalosporins, when used in combination. Whereas CLV had virtually no effect, TZB and SLB substantially enhanced the activity of cefalosporins. Cefepime/TZB revealed the highest activity against ESBL producing GNB. Further studies are needed in the clinical settings as they can play an important role as good alternatives to carbapenems. The emergence of widespread resistance to third and fourth generation cefalosporins has resulted in poor patient outcomes, increased total health care costs, and increased use of carbapenems. Carbapenems are currently considered the agents of last resort to combat gram negative infections in intensive care units and high risk wards. Cefepime is a fourth generation drug most stable against beta-lactamases like AmpC and OXA. Thus cefepime/TZB covers all three major mechanisms of resistance (ESBL, AmpC & OXA); so it can virtually act as a carbapenem.

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