Original Article

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Comparative Evaluation of the *in-vitro* Activity of Six β-lactam/β-lactamase Inhibitor Combinations against Gram Negative Bacilli

Microbiology Section

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ABSTRACT

Background: The extensive use of the β-lactam antibiotics in hospitals and in the community has created major resistance problems which has led to increased morbidity, mortality and healthcare costs. The use of the β-lactamase inhibitors in combination with the β-lactam antibiotics is currently the most successful strategy used for circumventing the resistance mechanisms.

Objective: To evaluate the in-vitro activity of six commercially available β-lactam/β-lactamase inhibitor combinations against Gram Negative Bacilli (GNB).

Materials and Methods: A total of 384 non duplicate, consecutive, gram negative bacilli (278 Enterobacteriaceae and 106 non fermenters) isolated from various clinical samples were subjected to antimicrobial sensitivity testing by the Kirby-Bauer method. The following β-lactam/β-lactamase inhibitor combinations were tested: amoxycillin-clavulanic acid, ampicillin-sulbactam, cefoperazone-sulbactam, piperacillin-tazobactam, cefepime-tazobactam and ticarcillin-clavulanic acid.

Results: Against the Enterobacteriacae, the sensitivity of Cefepime- tazobactam was 90. 64%, followed by Cefoperazone-sulbactam (84.89%) and Piperacillin - tazobactam (53.95 %). The sensitivity of the non fermenters was the highest for Cefepime-tazobactam (49.04%) and was least for Ampicillin-sulbactam and Amoxycillin-clavulanic acid (4.71% each). Cefepime-tazobactam was sensitive for all the extended spectrum β-lactamase (ESBL) isolates.

Conclusion: Among the six β-lactam/β-lactamase inhibitor combinations tested, Cefepime-tazobactam exhibited the best in-vitro activity against the gram negative bacilli isolated at our centre.

Key Words: β-lactam/β-lactamase inhibitor combinations, Gram negative bacilli

INTRODUCTION

Antimicrobial resistance constitutes one of the major threats which are related to the pathogenic microorganisms. Gram-negative pathogens such as *Enterobacteriaceae* (especially those which produce the extended-spectrum β-lactamases), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* have acquired an important role in the nosocomial infections, which is of particular concern, because of the associated broad spectrum of the antibiotic resistance [1].

The β-lactam antibiotics have been the cornerstone of our antibiotic armamentarium since the beginning of the antibiotic era. These act by disrupting the bacterial cell-wall synthesis and they are characterized by the presence of a β-lactam ring, which is crucial for the antimicrobial activity [2]. Unfortunately, bacteria have evolved sophisticated resistance mechanisms to combat the lethal effects of the β-lactam antibiotics. In the gram-negative pathogens, β-lactamase production is the main mechanism which leads to an acquired β-lactam resistance [3]. β -lactamases are responsible for the resistance to penicillins, the extended-spectrum cephalosporins, the monobactams, and the carbapenems [4].

One successful method of circumventing the threat of the plasmid-encoded β-lactamases is to use a combination of inhibitors of these enzymes with a penicillin [5]. The β-lactamase inhibitors alone have little or no antimicrobial activity. However, when they are co-administered with a β-lactam antibiotic, an inhibitor acts to bind and inactivate the beta-lactamases, thereby protecting the "partner" antibiotic from hydrolysis and potentiating the activity of the partner antibiotic, perhaps by binding directly to the bacterial penicillin binding proteins. As a result, a synergistic activity is observed against a variety of bacteria [6].

Three β-lactamase inhibitors are in the clinical use: clavulanic acid, sulbactam, and tazobactam. These agents are used in the empirical treatment of respiratory, intra-abdominal, and skin and soft tissue infections. There is also evidence to suggest that they are efficacious in treating the patients with neutropaenic fever and nosocomial infections, especially in combination with other agents [7].

The current study was undertaken to compare the *in -vitro* activity of 6 commercially available β-lactam/β-lactamases inhibitor combinations against gram negative bacteria, in order to evaluate their effectiveness in our set up.

MATERIALS AND METHODS

This study was conducted in the Microbiology lab of a private hospital in Jaipur, Rajasthan, India. 384 non-duplicate, consecutive, gram negative bacilli which were isolated from various clinical specimens and were received in the laboratory over a period of 5 months (between May-September 2010), were included in the study. A total of 278 *Enterobacteriaceae* and 106 non fermenters were assessed. The routine bacterial identification and the antibiotic sensitivity testing for all the isolates in the Microbiology lab, was performed on an automated system: Microscan autoScan-4(Siemens, West Sacramento, California, USA). For the purpose of this study, the antibiotic sensitivity testing of the six commercially

available β-lactam/ β-lactamase inhibitor combinations against 384 gram negative bacterial isolates (which were identified routinely by the automated system) was performed by the Kirby Bauer method [8] For determining the sensitivity of the inhibitor combinations, the following antibiotic discs were used - Cefoperazone -sulbactam (75/30mcg), Ampicillin -sulbactam (10/10mcg), Amoxicillin -clavulanic acid (20/10mcg), Ticarcillin-clavulanic acid (75/10mcg), Piperacillin-tazobactam (100/10mcg) and Cefepime - tazobactam (30/10mcg). Except for Cefoperazone-sulbactam which was obtained from BD (Becton Dickinson), all the other antibiotic discs and the media which were used for the study were obtained from Himedia, India. The diameter of the zones of inhibition of growth was recorded and interpreted as sensitive, intermediate resistant or resistant, based on the Clinical Laboratory Standards Institute (CLSI) guidelines. For Cefoperazone-sulbactam and Cefepime-tazobactam, the zone interpretation criteria were used as was stated for Cefoperazone and Cefepime respectively, in the CLSI-2010 guidelines [9]. The organisms with intermediate levels of resistance to the antibiotics were included in the percentage of resistant organisms for the final analysis. *Escherichia coli* ATCC 25923 and *Pseudomonas aeruginosa* ATCC were used as the controls.

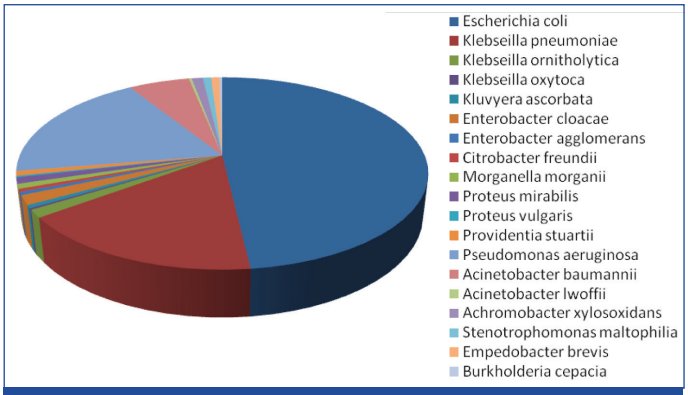
RESULTS

Of the 384 gram negative bacteria which were isolated during the study period, 278 (72.40%) were *Enterobacteriaceae* and 106(27.60%) were non fermenting, gram negative bacilli. These gram negative bacterial isolates were obtained from the patients who presented to the Outpatient Departments -OPDs (34.90%) or were admitted to the Intensive Care Units-ICUs (25.26%) and wards (39.84%) of our hospital. Urine specimens accounted for a majority (33.60%) of the clinical specimens which yielded gram negative bacteria, followed by respiratory samples (21.87%), stool (16.40%), pus (13.54%), blood specimens (11.46%) , fluids and swabs (1.56% each).

[Table/Fig-1] shows the frequency distribution of the gram negative bacilli which were obtained from various clinical samples. *Escherichia coli* was the most frequent gram negative bacteria which was isolated, which constituted 48.18% of all the GNB isolates. *Pseudomonas aeruginosa* and *Klebseilla pneumoniae* were the other common isolates which accounted for 18.75% and 16.66% of the total GNBs respectively.

[Table /Fig-2] has compared the *in- vitro* activity of the six β-lactam/ β-lactamse combinations against the GNBs. Among all the GNBs which belonged to *Enterobacteriacae,* the highest sensitivity was observed for Cefepime-tazobactam (90. 64%), followed by Cefoperazone-sulbactam (84.89%) and Piperacillin – tazobactam (53.95 %). The sensitivity of the non fermenters was the highest for Cefepime- tazobactam (49.04%) and it was the least for Ampicillin – sulbactam and Amoxycillin-clavulanic acid (4.71% each).

[Table/Fig-3] has compared the activity of various β-lactam/β-lactamase inhibitor combinations against the ESBL producing *E. coli* and *Klebseilla pneumoniae*. Cefepime-tazobactam showed100% sensitivity in all the ESBL positive isolates .However, Cefoperazone-sulbactam showed 90% sensitivity among the ESBL positive *Klebseilla pneumoniae* and 94.73% sensitive among the ESBL positive *E. coli*. The poorest sensitivity among all the β -lactam/β-lactamase inhibitor combinations against the ESBL positive isolates was observed for Ampicillin-sulbactam.



[Table/Fig-1]: Frequency distribution of gram negative bacteria isolated from various clinical specimens

[Table/Fig-4] depicts the sensitivity of all the six β- lactam/β-lactamase inhibitor combinations against the Carbapenem Resistant (CR) *Enterobacteriaceae* GNB. 60.97% CR *Enterobacteriaceae* GNB isolates were sensitive to Cefepime –tazobactam and 48.78% were sensitive to Cefoperazone-sulbactam.

DISCUSSION

The present study has demonstrated Cefepime- tazobactam as the most effective β-lactam/β-lactamase inhibitor combination with an *in- vitro* sensitivity of 90.64 % for the *Enterobacteriaceae* GNB and of 49.06 % for the non fermenting GNB. Most of the previous Indian studies have evaluated the *in -vitro* activity of the following three β-lactam and β-lactamase inhibitor combinations: Piperacillin-tazobactam, cefoperazone sulbactam and ticarcillin-clavulanic acid and they have shown variable results against the gram negative bacteria. Few studies have reported Piperacillin -tazobactam as the best combination agent [10,11]. Anuradha *et al*., have also reported Piperacillin-tazobactam as the most active combination of these three agents against *Enterobacteriaceae* and *Pseudomonas species,* but they found Ticarcillin-clavulanic acid to be highly effective against *Acinetobacter* species and *Stenotrophomonas maltophilia* [12]. However, a study from Chandigarh reported both Cefoperazone- sulbactam and Piperacillin-tazobactam as effective antibiotic combinations against gram negative isolates, but Ticarcillin-clavulanate was observed to be poorly effective [13]. A study on the gram negative bacteria which were isolated from Medical Oncology patients, reported the activity of Cefoperazone-sulbactam to be comparable to that of piperacillin-tazobactam [14].

Very few studies are available on the sensitivity profile of the cefepime-tazobactam combination. In a recent study from south India, the sensitivity of cefepime- tazobactam was reported to be 80.4% against 1003 gram negative bacteria which were tested [15]. It has been reported to be 100% sensitive against the *Escherichia coli* which was isolated from adult patients with community acquired UTIs [16]. Further, it has also been demonstrated that in comparison to cefepime and ceftazidime, the cefepime -tazobactam combination exhibited good activity against gram +ve and gram-ve organisms [17].

This study demonstrated that Amoxycillin/clavulanic acid and Ampicillin/sulbactam had poor activities against the gram negative bacteria in our set up. In concordance to our findings, Mulla *et al.,* have also reported that combinations like ampicillin – sulbactam and amoxicillin – clavulanic acid are not much effective against the Enterobacteriaceae [18].

Organism	No. of isolates	Amox/clav No. S (%S)	Amp/sul No. S (%S)	Cefo/sul No. S (%S)	Pip/taz No .S (%S)	Cfp/taz No. S (%S)	Tic/clav No .S (%S)
Escherichia coli	185	56(30.27%)	45(24.32%)	172(92.97 %)	107(57.84%)	176(95.14%)	80 (42.70%)
Klebseilla pneumoniae	64	17(26.56%)	13(20.31%)	40(62.50%)	26(40.63%)	53 (82.81%)	19(29.68%)
Klebseilla ornitholytica	5	0(0%)	0(0%)	4(80%)	4(80%)	4(80%)	0(0%)
Klebseilla oxytoca	1	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)
Kluvyera ascorbata	2	1(50%)	1(50%)	2(100%)	1(50%)	2(100%)	1(50%)
Enterobacter cloacae	6	0(0%)	0(0%)	5(83.33%)	4(66.66%)	6(100%)	3(50%)
Enterobacter agglomerans	2	0(0%)	0(0%)	1(50%)	0(0%)	1(50%)	0(0%)
Citrobacter freundii	2	1(50%)	0(0%)	2(100%)	1(50%)	2(100%)	1(50%)
Morganella morganii	3	0(0%)	0(0%)	3(100%)	2(66.66%)	3(100%)	2(66.66%)
Proteus mirabilis	4	1(25%)	1(25%)	3(75%)	1(25%)	3(75%)	1(25%)
Proteus vulgaris	1	0(0%)	0(0%)	1(100%)	1(100%)	1(100%)	1(100%)
Providentia stuartii	3	0(0%)	0(0%)	2(66.66%)	2(66.66%)	2(66.66%)	0(0%)
Total Enterobacteriaceae	278	77(27.69%)	61(21.94 %)	236(84.89 %)	150 (53.95%)	252 (90.64%)	108(38.84%)
Pseudomonas aeruginosa	72	3(4.17%)	3(4.17%)	26(36.11%)	17(23.61%)	25(34.72%)	14(19.44%)
Acinetobacter baumannii	22	1(4.54%)	1(4.54%)	12(54.54%)	2(9.09%)	18(81.82%)	1(4.54%)
Acinetobacter lwoffii	1	0(0%)	1(100%)	1(100%)	0(0%)	1(100%)	1(100%)
Achromobacter xylosoxidans	4	0(0%)	0(0%)	3(75%)	2(50%)	2(50%)	2(50%)
Stenotrophomonas maltophilia	3	1(33.33%)	0(0%)	3(100%)	0(0%)	3(100%)	2(66.66%)
Empedobacter brevis	3	0(0%)	0(0%)	1(33.33%)	1(33.33%)	2(66.66%)	0(0%)
Burkholderia cepacia	1	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)
Total Non fermenters	106	5(4.71%)	5(4.71%)	47(44.33%)	22(20.75%)	52 (49.06%)	20(18.86%)

[Table/Fig-2]: Comparison of in- vitro activity of the six β-lactam /β-lactamse combinations against the *Enterobacteriaceae* and Non fermenter GNB
Abbreviations: Amoxy/clav-Amoxycillin-clavulanic acid, Amp/sul-Ampicillin-sulbactam, Cefo/sul-Cefoperazone-sulbactam, Pip/taz-Piperacillin- tazobactam, Cfp/taz-Cefepime-Tazobactam , Ticar/clav-Ticarcillin/clavulanic acid

Organism	Total	ESBL isolates (%)	Amox/clav	Amp/sul	Cefo/sul	Pip/taz	Cfp/taz	Ticar/clav
Escherichia coli	185	57(30.81%)	9(15.78%)	5(8.77%)	54(94.73%)	31(54.38%)	57(100%)	13 (54.38%)
Klebseilla pneumoniae	64	10(15.62%)	1(10%)	1(10%)	9(90%)	6(60%)	10(100%)	2(20%)
Total	249	67(26.90%)	10(14.92%)	6(8.95%)	63(94.02%)	37(55.22%)	67(100%)	15(22.38%)

[Table/Fig-3]: Comparative in vitro activity of six β-lactam/β-lactamase inhibitor combinations for ESBL organisms
Abbreviations: Amoxy/clav-Amoxycillin-clavulanic acid, Amp/sul-Ampicillin-sulbactam, Cefo/sul-Cefoperazone-sulbactam, Pip/taz-Piperacillin- tazobactam, Cfp/taz-Cefepime-Tazobactam ,Ticar/clav-Ticarcillin/clavulanic acid, ESBL-extended spectrum beta lactamase

S. No.	Organism	Total isolates	No. of C R isolates	%CR	Amoxy/ clav No. S(%S)	Amp/ Sul No. S (%S)	Cefo/sul No. S (%S)	Pip/taz No. S (%S)	Cfp/taz No. S (%S)	Ticar /clav No. S (%S)
1	Escherichia coli	185	11	12.94	0(0%)	1(9.09%)	7(63.63%)	1(9.09%)	7(63.63%)	1 9.09%)
2	Klebseilla pneumoniae	64	20	31.25	0(0%)	0(0%)	7(35%)	1(5%)	11(13.75%)	0(0%)
3	Klebseilla ornitholytica	5	1	20	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)
4	Klebseilla oxytoca	1	0	0	ND	ND	ND	ND	ND	ND
5	Kluvyera ascorbata	2	0	0	ND	ND	ND	ND	ND	ND
6	Enterobacter cloacae	6	1	16.66	0(0%)	0(0%)	1(100%)	1(100%)	1(100%)	1(100%)
7	Enterobacter agglomerans	2	2	100	0(0%)	0(0%)	1(50%)	0(0%)	1(50%)	0(0%)
8	Citrobacter freundii	2	1	50	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)
9	Morganella morganii	3	0	0	ND	ND	ND	ND	ND	ND
10	Proteus mirabilis	4	4	100	1(25%)	1(25%)	3(75%)	1(25%)	3(75%)	1(25%)
11	Proteus vulgaris	1	0	0	ND	ND	ND	ND	ND	ND
12	Providentia stuartii	3	1	33.33	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Total	278	41	14.75%	1(2.43%)	2(4.87%)	20(48.78%)	4(9.75%)	25(60.97%)	3(7.31%)

[Table/Fig-4]: Sensitivity pattern of Carbapenem Resistant (CR) Enterobacteriaceae against the β-lactam/β-lactamase inhibitor combinations
Abbreviations: Amoxy/clav-Amoxycillin-clavulanic acid, Amp/sul-Ampicillin-sulbactam, Cefo/sul-Cefoperazone-sulbactam, Pip/taz-Piperacillin- tazobactam, Cfp/taz-Cefepime-Tazobactam , Ticar/clav-Ticarcillin/clavulanic acid,CR-carbapenem resistant, ND - Not done.

Cefepime-tazobactam, followed by Cefoperazone-sulbactam, was noted as the most sensitive drug combination for the non fermenting GNBs in our study. Cefepime-tazobactam and Cefoperazone-sulbactam have been reported to be most effective for the metallo-β-lactamse producers, Pseudomonas sp. and Acinetobacter *sp.* respectively [19]. The efficacy of Cefoperazone-sulbactam against the non fermenting, gram negative bacilli has also been pointed out in a recent study [20]. A study from Pakistan has reported Cefoperazone-sulbactam to be superior to Piperacillin-tazobactam and Ampicillin-sulbactam in its activity against multi drug resistant *A. baumannii* [21].

The variations in the susceptibility rates of the β-lactam/β- lactamase inhibitor combinations in different studies could possibly be due to the differences in the hospital organisms which were sampled, the test methodologies which were employed, the sites of infection and the study time intervals. The reason for the lower sensitivity of Piperacillin- tazobactam for the GNBs in our study as compared to cefepime- tazobactam and cefoperazone-sulbactam, could be attributed to the fact that Piperacillin-tazobactam was a more commonly utilized empirical agent at our institute. This could have resulted in a selection pressure for the development of resistance to this drug.

In this study , the Cefepime-tazobactam combination showed 100% sensitivity among the ESBL producing *E. coli* and *Klebseilla pneumoniae* isolates, followed by Cefoperazone- sulbactam, which showed 94.73% and 90% sensitivities among the ESBL producing *E.coli* and *Klebseilla pneumoniae* respectively. Cefepime is a semi-synthetic, broad-spectrum cephalosporin which is classified within the fourth generation class. Cefepime has a better activity against the gram-negative bacteria that produce the extended spectrum β-lactamases than the other commercially available oxyiminocephalosporins [22]. Its 3' side chain provides some stability against the Amp C β- lactamases. Tazobactam is the most promising inhibitor, which unlike sulbactam and clavulanic acid, has its own antibacterial activity. The resulting combination, Cefepime-tazobactam, henceforth ensures coverage for all the ESBL producing *Enterobacteriaceae*.

In this study, Piperacillin-tazobactam was observed to show 54.38% sensitivity among the ESBL producing *E.coli* and 60% sensitivity among the ESBL producing *Klebseilla pneumoniae*. A multicentric Indian study has demonstrated the sensitivity of the ESBL producing *E. coli* to Pip- taz as 76% and of the ESBL positive *Klebseilla pneumoniae* as 59% [23].

CLSI recommends that the ESBL producing *Escherichia coli* and *Klebseilla spp*. should be reported as resistant to all the penicillins, cephalosporins and the monobactam antimicrobials and that the β-lactam/β-lactamase inhibitor results should be reported as such [9].

The rapid and the disturbing spread of the extended-spectrum β-lactamases and the AmpC enzymes and the quinolone resistance against the *Enterobacteriaceae* is forcing our reliance on the carbapenems. However, the resistance to these agents is also slowly accumulating via the spread of metallo-, KPC and OXA-48 β-lactamases [24]. The emergence of an alarming resistance to the carbapenems in the gram negative bacteria in India has been highlighted in a few studies [25,26]. Colistin and tigecycline are the drugs with a most likely *in -vitro* activity against the *Enterobacteriaceae* producing carbapenem-hydrolyzing β-lactamases, but the development of resistance to these drugs is of concern [27]. A 14.75% resistance to the carbapenems amongst the *Enterobacteriaceae* GNB was noted in this study. Cefepime-tazobactam and Cefoperazone- sulbactam were found to be sensitive among 60.97% and 48.78% Carbapenem resistant *Enterobacteriaceae* GNB respectively.The activities of these β-lactam/β-lactamse inhibitor combination agents against the carbapenem resistant *Enterobacteriaceae* need to be evaluated further by ascertaining their efficacy in clinical studies.

CONCLUSION

This study showed that among the six β-lactam/β-lactamase inhibitor combinations tested, Cefepime-tazobactam combination was the most effective combination against the gram negative bacilli isolated at our centre. Further *in- vitro* and *in -vivo* studies need to be undertaken to assess the true effectiveness of this combination.

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