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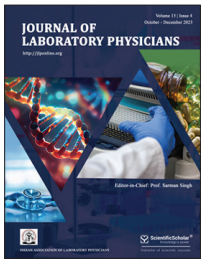

Original Article

A comparative *in vitro* sensitivity study of "cefepime-tazobactam" and other antibiotics against Gram-negative isolates from intensive care unit

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ABSTRACT

Objectives: The major health problem today includes the emergence of multidrug-resistant (MDR) bacteria, especially extended-spectrum βlactamase, carbapenemases, and Amp C-producing Gram-negative bacilli (GNB). Our study is aimed to recognize the *in vitro* susceptibility pattern of cefepime/tazobactam compared to other antibiotics used against GNB in an intensive care unit (ICU) setting.

Materials and Methods: We conducted a prospective observational research comprising all GNB isolated from clinical samples of patients admitted to the ICU throughout the study period from January 2021 to December 2021. All of the isolates were analyzed using " Matrix Assisted Laser Desorption/ ionization - time of flight - Mass spectrometry assay (MALDI-TOF-MS)" for identification and the Kirby-Bauer disc diffusion technique to test for susceptibility. Cefepime-tazobactam was tested by E-test (Hi-Media, Mumbai) method. The minimum inhibitory concentrations (MIC) of cefepime (as in Clinical Laboratory Standards Institute, 2021) has been utilized to elucidate the sensitivity of cefepime-tazobactam, as no criteria for cefepime-tazobactam is available.

Statistical Analysis: All statistical analysis was performed using the software program IBM Statistical Package for Social Sciences (SPSS) Statistics version 20.0, with $P < 0.05$ considered statistically significant.

Results: We included a total of 480 GNB isolated from blood, pus, body fluids, endotracheal aspirates (ETA), and sputum samples. The most common microorganism tested for susceptibility to cefepime-tazobactam was *Klebsiella pneumoniae* (182/480, 37.92%) followed by *Acinetobacter baumannii* (135/480, 28.12%), and *Pseudomonas aeruginosa* (94/480, 19.58%). *K. pneumoniae* and *Enterobacter aerogenes* were most resistant to all the antibiotics tested against them. *K. pneumoniae* was most resistant to meropenem (41/182, 22.53%), followed by imipenem (42/182, 23.08%) and cefoperazone-sulbactam (49/182, 26.92%) and was predominantly found susceptible to cefepime-tazobactam (122/182, 67.04%).

Conclusions: Cefoperazone-tazobactam is a new "β-lactam/β-lactamase combination" found effective in the *in vitro* analysis of drug-resistant isolates of GNB.

Keywords: Carbapenemases, Cefepime, Cefoperazone-sulbactam, Cefepime-tazobactam, MALDI-TOF-MS

INTRODUCTION

The indiscriminate use of third and fourth -generation cephalosporin and carbapenems in the past few decades has led to widespread antibiotic resistance among Gram (−ve) bacteria; Thereby restricting the use of several classes of antibiotics and using the antibiotic of last resort, Colistin.[1,2] Recently, some microorganisms have developed resistance to colistin leading to pan-drug resistant infections with an imperative demand for newer antibiotics.[3-5] Beta-lactam resistance has been encountered as the most common form of resistance encountered among the microorganisms. The use of Beta-

lactam/ Beta-lactamase inhibitor is a better method to counter the resistance to Beta-lactams.[6,7]

Among Indian hospitals, there is high resistance to extended-spectrum βlactamases (ESBLs), and a similar trend is being observed in carbapenems due to irrational use in most critical care units.[8,9] Thus, an ever-increasing menace of Gram-negative antibiotic resistance is being encountered in Indian healthcare, which can be attributed to the indiscriminate usage of antibiotics, over-the-counter availability of all antibiotic agents, and the absence of national antibiotic policy to counter the skyrocketing antimicrobial resistance.[10]

The discovery of new antibiotics is the need of the hour to counter the ever-increasing antibiotic resistance. A fourth-generation cephalosporin with a β-lactamase inhibitor combination provides coverage to OXA and AmpC enzymes over third -generation cephalosporins.[7] Among newer BL/BLI combinations, cefepime/tazobactam is a new drug approved for use in Indian hospitals by the Drug Controller General of India.[11] No clinical data is available in the literature for this drug combination, and very limited studies have been published on this drug combination to date. The aim of the research was to elucidate the *in vitro* sensitivity of "Cefepime-tazobactam" and some other antibiotics against Gram (−ve) isolates from the intensive care unit (ICU).

MATERIALS AND METHODS

Duration and place of study

This prospective work has been done in the "Bacteriology Section" of the microbiology department at our university hospital from January 2021 to December 2021.

Sample collection

During the study period, all non-duplicate Gram-negative bacilli (GNB) isolates obtained from all clinical samples including blood, pus, body fluids, endotracheal aspirates (ETA), and sputum were taken from the ICU patients and send to "Bacteriology Section" of Laboratory of Microbiology department.

Inclusion criteria

As multiple samples were sent from a single patient admitted to the ICU, we included only the first sample growing GNB from a single patient. Thus, non-repeat samples were included in this study [Figure 1].

Exclusion criteria

All samples coming to the laboratory after 2 h from sample collection, growing contaminants, and commensals were excluded from the study.

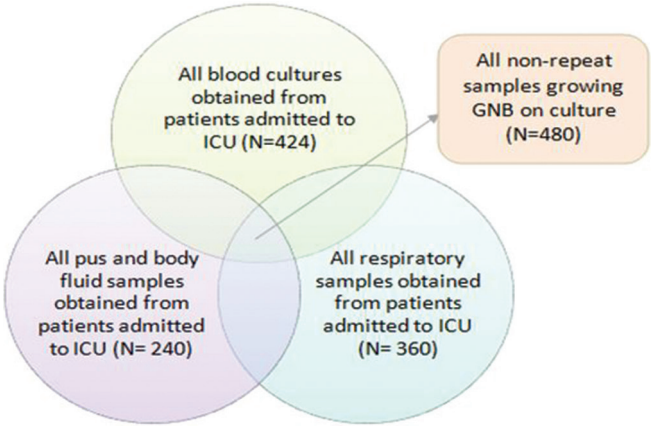

Figure 1: Inclusion of the pathogenic GNB isolated from various samples obtained from patients admitted to the ICU (n = 1024). GNB: Gram negative bacteria, ICU: Intensive care unit, N: Number of samples.

Characterization of samples

The production for the Screening of ESBL and Metallo-β-lactamase (MBL) was performed on all the GNB isolates respecting Clinical Laboratory Standards Institute (CLSI) guidelines.[12] ESBL production possibly was demonstrated by isolates zone size "≤22 for ceftazidime (30 μg), ≤27 with cefotaxime (30 μg), and ≤25 with ceftriaxone (30 μg"). Disc potentiation test has been performed using "cefotaxime (30 μg) and ceftazidime (30 μg) antibiotic disk with or without clavulanic acid (10 μg)" and by "double disk susceptibility test" has been used to confirm the production of ESBL.[12] Yong *et al.*[13] used imipenem (10 μg) only and imipenem (10 μg) along with ethylenediaminetetraacetic acid (750 μg) disk for phenotypic MBL detection in clinical isolates.

Antimicrobial susceptibility testing

As recommended by the CLSI guidelines (2019),[12] Kirby-Bauer disc diffusion test was employed to carry out an antibiotic susceptibility test. The disk of "piperacillin-tazobactam" (100/10 μg), cefepime (30 μg), "cefoperazone-sulbactam" (75/30 μg), 10 μg of meropenem, ertapenem and imipenem, and E-strips of cefepime-tazobactam were taken from HiMedia (Mumbai, India). The zone diameters were interpreted as susceptible, intermediate, or resistant respecting CLSI guidelines. As any criteria for interpretation of zone diameter of cefepime-tazobactam and cefoperazone-sulbactam were not available, to evaluate the susceptibility of these two medication combinations, cefepime and cefoperazone zone size as per CLSI 2016 were utilized.[14]

For cefepime-tazobactam (30/10 μg) to be susceptible, a ≥25 for *Enterobacteriaceae* and ≥18 zone size for non-fermenters was considered. Quality control of every disc was done according to "CLSI 2016"[14] approved disk diffusion QC ranged against "*Pseudomonas aeruginosa* ATCC 27853,

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Klebsiella pneumoniae* ATCC 700603."

RESULTS

We included 480 total GNB isolated from blood, pus, body fluids, ETA, and sputum samples in our study cohort. The maximum number of GNB isolates tested for susceptibility to cefepime-tazobactam was obtained from endotracheal aspirate samples (120/480, 25%), followed by those isolated from bloodstream infections (115/480, 23.95%) and sputum (112/480, 23.33%), as shown in Figure 2.

The most common microorganism tested for susceptibility to cefepime-tazobactam was *K. pneumoniae* (182/480, 37.92%) followed by *Acinetobacter baumannii* (135/480, 28.12%) and *P. aeruginosa* (94/480, 19.58%). Figure 3 describes the distribution of the microorganisms from the various samples included in the study. The most common microorganism was *K. pneumoniae*, which was mostly isolated from endotracheal aspirate (75/182, 41.21%) followed by bloodstream infections (69/182, 37.91%). *A. baumannii* was isolated most commonly from ETA (68/135, 50.37%) followed by sputum samples (42/135, 31.11%), while *P. aeruginosa* was isolated mostly from endotracheal aspirate samples (43/94, 45.74%).

The microorganisms were further divided into two groups, namely, non-inducible *Enterobacteriaceae* group (which includes *E. coli* and *K. pneumoniae*) and the inducible *Enterobacteriaceae* group (which includes *Enterobacter aerogenes* and *Serratia marcescens*) and those belonging to non-inducible *Enterobacteriaceae* group (n = 217, 45.21%) were predominantly tested for susceptibility to cefepime-tazobactam in comparison to inducible *Enterobacteriaceae* group (n = 36, 7.5%). We also analyzed the results of antibiotic susceptibility testing with cefepime-tazobactam and other first-line drugs against the isolates included in the study [Table 1]. The drug in question was also tested for susceptibility against non-fermenters (n = 229, 47.71%).

Table 1 also represents the antibiotic susceptibility of all Gram (−-ve) isolates to cefepime, cefoperazone-sulbactam, cefepime-tazobactam, meropenem, and imipenem. *Serratia marcescens* was the most susceptible isolate to all the antibiotics and was 75.0%–100.0% (12/16, 75.0% to 16/16, 100.0%) susceptible to all the five antibiotics. *K. pneumoniae* and *E. aerogenes* were most resistant to all the antibiotics tested against them. *K. pneumoniae* was most resistant to meropenem (41/182, 22.53%), followed by imipenem (42/182, 23.08%) and cefoperazone-sulbactam (49/182, 26.92%), and was predominantly found susceptible to cefepime-tazobactam (122/182, 67.04%). *E. aerogenes* was least susceptible by cefoperazone-sulbactam (2/18, 11.11%), followed by both carbapenems (3/18, 16.67%) each and cefepime (5/18, 27.47%) and primarily found susceptible to cefepime-tazobactam (11/18, 61.11%). Overall, susceptibility to all the antibiotics in descending order was as follows: cefepime-tazobactam (322/480, 67.08%), cefepime (137/480, 28.54%), cefoperazone-sulbactam (127/480, 26.46%), imipenem (120/480, 25.0%), and meropenem (116/480, 24.17%).

Table 2 describes the comparative activity of cefepime-tazobactam and cefepime against the most common three microorganisms isolated from the samples involved in the present study. *K. pneumoniae* has been identified as the most susceptible (50/182, 27.47%) and most resistant (60/182, 32.96%) microbe to both the cefepime and cefepime-tazobactam. Seventy-two (72/182, 39.56%) isolates of *K. pneumoniae* were found susceptible to cefepime-tazobactam but were resistant to

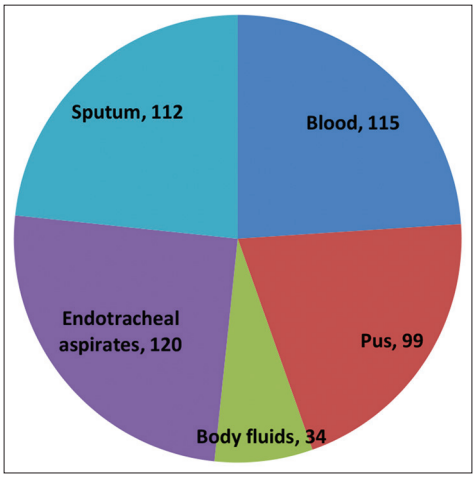

Figure 2: The distribution of all the samples included in the study cohort (n = 480). n: Number of samples.

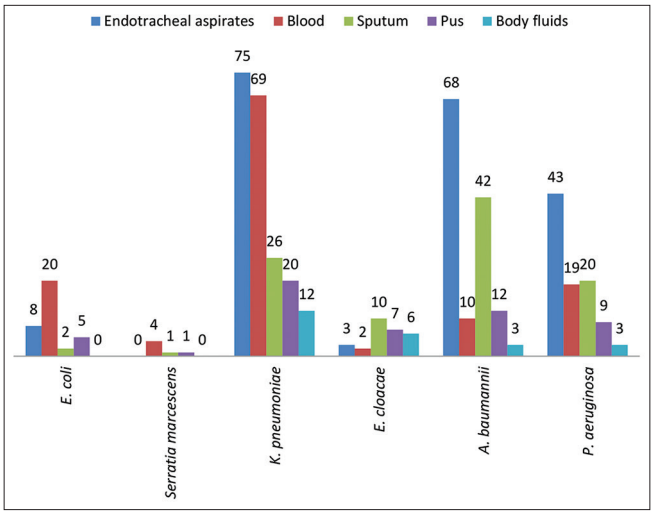

Figure 3: The distribution of pathogenic microorganisms isolated from various samples included in this study (n = 480). n: Number of samples. *E.coli: Escherichia coli, K.pneumoniae, Klebsiella pneumonia, E.cloacae: Enterobacter cloacae, A.baumannii: Acinetobacter baumannii, P. aeruginosa, Pseudomonas aeruginosa.*

Table 1: Antibiotic susceptibility patterns of Gram-negative bacilli isolates included in our study (*n*=480).

Antibiotics	Cefepime (%)	Cefepime-tazobactam (%)	Cefoperazone-sulbactam (%)	Imipenem (%)	Meropenem (%)
Non-inducible *Enterobacteriaceae* (*n*=217)					
Escherichia coli (*n*=35)	11/35 (31.43)	26/35 (74.28)	10/35 (28.57)	8/35 (22.86)	7/35 (20.0)
Klebsiella pneumoniae (*n*=182)	50/182 (27.47)	122/182 (67.04)	49/182 (26.92)	42/182 (23.08)	41/182 (22.53)
Inducible *Enterobacteriaceae* (*n*=34)					
Enterobacter cloacae (*n*=18)	5/18 (27.78)	11/18 (61.11)	2/18 (11.11)	3/18 (16.67)	3/18 (16.67)
Serratia marcescens (*n*=16)	12/16 (75.0)	16/16 (100.0)	12/16 (75.0)	15/16 (93.75)	14/16 (87.5)
Non-fermenters (*n*=229)					
Acinetobacter baumannii (*n*=135)	34/135 (25.18)	89/135 (65.92)	32/135 (22.22)	31/135 (22.96)	30/135 (22.22)
Pseudomonas aeruginosa (*n*=94)	25/94 (26.59)	58/94 (61.70)	22/94 (23.40)	21/94 (22.34)	21/94 (22.34)
All isolates (*n*=480)	137/480 (28.54)	322/480 (67.08)	127/480 (26.46)	120/480 (25.00)	116/480 (24.17)

n: Number of samples.

Table 2: Comparative activity of cefepime-tazobactam and cefepime against the three most common microorganisms isolated from the samples included in our study (*n*=411).

Microorganisms	Susceptible to both cefepime and cefepime-tazobactam (%)	Resistant to both cefepime and cefepime-tazobactam (%)	Sensitive to cefepime-tazobactam and resistant to cefepime (%)	Resistant to cefepime-tazobactam and sensitive to cefepime (%)
Klebsiella pneumoniae (*n*=182)	50 (27.47)	60 (32.96)	72 (39.57)	0 (0.0)
Acinetobacter baumannii (*n*=135)	34 (25.18)	46 (34.07)	55 (40.74)	0 (0.0)
Pseudomonas aeruginosa (*n*=94)	25 (26.59)	36 (38.29)	33 (35.11)	0 (0.0)

n: Number of samples.

cefepime, while none of the isolates were found susceptible to cefepime and resistant to cefepime-tazobactam. *A. baumannii* (55/135, 40.74%) was found to be most susceptible to cefepime-tazobactam but was resistant to cefepime.

DISCUSSION

There is serious global concern regarding the rise of antibiotic resistance in recent times,with few to no newer antibiotics in the pipeline. With the advent of increasing Carbapenem resistance, there is an increasing interest in new combinations like "cefepime-tazobactam." Cefepime is a fourth generation, synthetic cephalosporin with broad-spectrum action.[15] The antibiotic exhibits better action against the Gram (−ve) bacteria in comparison to the Gram-positive bacteria. Tazobactam is a beta-lactamase that inhibits the activity of the beta-lactamase enzymes including "group 1-cephalosporinases, group 2br-TEM beta-lactamases, and group 3-metallo-beta-lactamases." "Cefepime-tazobactam" is expected to overcome ESBL, AmpC, and OXA enzyme production.[16,17]

There is a lack of studies comparing the efficacy of drugs most commonly used in the ICU, which include a variety of cephalosporins, carbapenems, and BL/BLI combinations. This study reports a better susceptibility of the Gram (−ve) bacteria to cefepime-tazobactam in comparison to carbapenems, and other cephalosporins that are profusely used in the ICU setup, which agrees with research done by Sood *et al.*[17] and Mushtaq *et al.*[18] The common microorganisms isolated from various samples such as *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* showed better susceptibility to cefepime-tazobactam in comparison to cefepime alone. Therefore, similar findings were stated by Susan *et al.*,[6] and other authors'[17-19].

In the wake of increasing carbapenem resistance of ~ 25% susceptibility of the commonly isolated microorganisms reported in our study, the susceptibility of the microorganisms was better to cefepime-tazobactam (67.08%). This is in comparison to the research by Agarwal *et al.*[15] where carbapenems showed comparable performance to cefepime-tazobactam. A similar study performed in patients with hematological malignancies by Benanti *et al.*[20] also showed comparable results with both the antibiotics. However, the patients were shifted back on carbapenems early in the treatment. Other Indian studies by Ghafur *et al.*[21] and Sharma *et al.*[22] reported either equitable or better susceptibility of the microorganisms to both carbapenems and cefepime-tazobactam which are contradictory to our finding of better susceptibility of the organisms to cefepime-tazobactam. A similar rising trend of carbapenem resistance is noticed in research by Vu *et al.*[23] that further compels us to identify new antibiotics to combat the

drug resistance among the isolates. Vu *et al*.[23] supported the use of cefepime-tazobactam in place of carbapenems to prevent the rapid emergence of drug resistance among commonly isolated microorganisms from the ICU settings.

The main limitations of the present analysis are: primary, it is a single-center study and does not represent the rate of drug resistance in a particular geographical region: secondary, due to the unavailability of any zone diameters for the antibiotics susceptibility of cefepime-tazobactam and no specific CLSI guidelines for the same, we had to improvise using the disc diameters of cefepime recommended in CLSI.

CONCLUSIONS

Our study defines a good *in vitro* activity of "cefepime-tazobactam" against non-inducible, inducible, and non-fermenters in comparison to other BL/BLI combinations and carbapenems used routinely in ICUs. It is a potential antimicrobial agent for treating an array of Gram-negative bacteria-associated infections, and it improves the outcome of patients suffering from infections caused by GNB in comparison to other antimicrobial agents.

Ethical approval

The study has been approved by the institutional ethics committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow with the ethical approval number: 2021-48-EMP-EXP, dated 29/11/2021.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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