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TO EVALUATE THE *IN-VITRO* ANTIMICROBIAL ACTIVITY OF CEFEPIME ENMETAZOBACTAM AND PLAZOMICIN AGAINST CLINICAL ISOLATES OF MULTI DRUG RESISTANT KLEBSIELLA *PNEUMONIAE*

Microbiology

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ABSTRACT

BACKGROUND: Infections due to multidrug-resistant (MDR) pathogens cause significant morbidity and mortality. **METHODS:** The study aimed to assess the in-vitro antimicrobial activity of Cefepime enmetazobactam (FPE) and Plazomicin (PLZ) against 50 MDR Klebsiella pneumoniae isolates- 27 carbapenem-resistant (CRE) and 23 non-CRE strains. **RESULTS:** Among CRE isolates, limited susceptibility was observed for PLZ (18.5%) and FPE (7.4%). Non-CRE isolates of K.pneumoniae showed high susceptibility to PLZ (78.2%) and FPE (100%). Thus, reduced effectiveness of these antibiotics was noted in CRE isolates compared to the high susceptibility seen in non-CRE strains, with FPE showing promising activity especially in non-CRE K. pneumoniae infections. **CONCLUSION:** Added research with greater number of clinical isolates is essential to validate and the promising roles of FPE and PLZ in the management of CRE infections.

KEYWORDS

Cefepime enmetazobactam, Plazomicin, Antibiotic susceptibility test

INTRODUCTION

Carbapenem-resistant *Klebsiella (K.) pneumoniae* (CRKP) has become a formidable challenge to global health systems, and its importance is highlighted by the World Health Organization's (WHO) designation of CRKP as a "critical priority pathogen" [1]. This status reflects its association with high mortality rates, frequent outbreaks, and the scarcity of effective treatment options. CRKP is a leading cause of difficult-to-treat hospital-acquired infections, raising the stakes for patient safety, escalating healthcare costs, and straining infection control protocols worldwide, including in low-and middle-income countries such as India, where resistance rates are particularly alarming [1].

K. pneumoniae is a Gram-negative, encapsulated, non-motile, rod-shaped bacterium normally inhabiting the gastrointestinal tract in humans and is found in soil and water. While often harmless in healthy individuals, it is an opportunistic pathogen—capable of causing life-threatening illnesses such as pneumonia, genito-urinary tract infections, bloodstream infections, and sepsis, especially in hospitalized or immunocompromised patients [2]. Plazomicin, a novel aminoglycoside, represents a major advancement in the treatment of multi-drug resistant Gram-negative infections. Engineered to evade many aminoglycoside-modifying enzymes that inactivate older drugs like gentamicin and amikacin, plazomicin maintains high efficacy against a range of multi-drug resistant (MDR) *K. pneumoniae* strains, including those producing extended-spectrum β-lactamases (ESBLs) and carbapenemases. *In-vitro* studies reveal that plazomicin demonstrates potent bactericidal activity even against isolates resistant to most other aminoglycosides and β-lactams. Its activity encompasses both ESBL-positive and many carbapenemase-positive isolates, making it a critical option in settings with high rates of resistance [3].

Cefepime/enmetazobactam is a next-generation pairing antibiotic that unites cefepime, a broad-spectrum fourth-generation cephalosporin and enmetazobactam, a novel β-lactamase inhibitor. This combination was specifically developed to address growing resistance driven by ESBLs and certain carbapenemases—particularly OXA-48 types. Enmetazobactam extends the spectrum of cefepime by targeting β-lactamases, restoring efficacy against ESBL-producers and, importantly, many OXA-48 carbapenemase-producing *K. pneumoniae* strains. Studies demonstrate that cefepime/enmetazobactam significantly reduces bacterial burden in experimental (murine) models of hospital-acquired pneumonia caused by OXA-48 *K. pneumoniae*, achieving meaningful reductions even where cefepime or meropenem alone failed [4].

METHODOLOGY

Study design and site

The present study was conducted at Max Super Speciality Hospital, Dwarka, with the aim to evaluate the effectiveness of novel antibiotics Plazomicin and Cefepime/enmetazobactam against multidrug-resistant *K. pneumoniae* strains. It was a laboratory based *in-vitro* analytical study.

Sample size

A total of 50 non-duplicate MDR *K. pneumoniae* clinical isolates and 5 *E. Coli* Carbapenem-resistant Enterobacterales (CRE) isolates were included.

Selection criteria

Inclusion criteria: Clinical isolates of *K. pneumoniae*, confirmed by VITEK, were included in the study.
Exclusion criteria: Duplicate isolates from the same patient and isolates with mixed growth of multiple organisms were excluded.

Microbiological diagnosis

Detection of carbapenemase

The Carbapenemase RESIST-5 O.K.N.V.I. card, a rapid immunochromatographic lateral flow assay detecting the five major clinically relevant carbapenemases—OXA-48, KPC, NDM, VIM, and IMP—was used in our study for accurate and timely identification of carbapenemase-producing Enterobacterales.

Lawn Culture and Disc Diffusion Method

Each isolate was suspended in normal saline and adjusted to a McFarland turbidity standard of 0.5. A lawn culture was prepared by uniformly swabbing the suspension onto cation adjusted Mueller-Hinton Agar (MHA) plates. After the agar surface was allowed to dry, antibiotic discs were placed aseptically on the agar. The standard disc concentration for plazomicin recommended by CLSI is 30µg [5]. For cefepime-enmetazobactam, the standard disc for susceptibility testing contains 30µg of cefepime and 20µg of enmetazobactam [6,7]. The MHA plates were then incubated aerobically at 37°C overnight (18–24 hours). Zone diameters around disc of plazomicin and Cefepime enmetazobactam was measured and interpreted according to CLSI and EUCAST guidelines, respectively [5,7].

Data analysis

Descriptive analysis was performed on the collected data, which included zone of inhibition diameters. All categorical data was entered into Microsoft excel and analyzed using SPSSv25. All data was expressed in percentage for susceptibility and resistance. Interpretation was done based on the zone of inhibition and the data was recorded as an excel sheet. Further statistical analysis was carried out on the data.

RESULT

A total of 50 *K. pneumoniae* isolates were analyzed for antibiotic susceptibility, comprising 27 carbapenem-resistant (CRE) and 23 non-

CRE strains (Table 1). The CRE isolates exhibited resistance to most commonly used β-lactam antibiotics. Among these resistant isolates, limited susceptibility was observed for plazomicin (18.5%) and cefepime-enmetazobactam (7.4%). In contrast, the non-CRE *K. pneumoniae* isolates retained susceptibility to plazomicin (78.2%) and cefepime-enmetazobactam (100%).

This highlights the substantially reduced effectiveness of these antibiotics in CRE isolates compared to the high susceptibility seen in non-CRE strains, with cefepime-enmetazobactam showing promising activity especially in non-CRE *K. pneumoniae* infections. Among Piperacillin/Tazobactam resistant enterobacterales, CRE *K. pneumoniae* (n=7) and CRE *E. coli* (n=5) were cefepime enmetazobactam sensitive. (Table 1 and 2)

The study also included carbapenem-resistant OXA-48 positive isolates of *K. pneumoniae* (n=5) and *E. coli* (n=5) (Table 2). Among these OXA-48 positive *K. pneumoniae* isolates , susceptibility was 20% to amikacin, 60% to gentamicin, and 40% to fosfomycin. None were susceptibile to tigecycline. Intermediate susceptibility to colistin was seen in 83.3% of isolates, while all were susceptible to to cefepime-enmetazobactam. No susceptibility was observed to amoxicillin/clavulanate, cefepime, cefoperazone/sulbactam, ceftriaxone, ciprofloxacin, carbapenems, or piperacillin-tazobactam. OXA-48 positive CRE *E. coli* isolates showed susceptibility to several antimicrobials, with 100% susceptibility to amikacin, fosfomycin, tigecycline, and cefepime-enmetazobactam. Gentamicin susceptibility was 80%, and colistin showed intermediate susceptibility at 100%. Other β-lactam and carbapenem agents showed no susceptibility in this group as well. (Table 2)

Table 1: The susceptibility patterns of *Klebsiella pneumoniae* isolates (CRE and non-CRE) against various antibiotics

ANTIBIOTICS	CRE (n=27)	NON CRE (n=23)
AMK	S- 14.8%	S-95.6%
AMX	S-0%	S-65.2%
FEP	S-0%	S-82.6%
CEF/SUL	S-0%	S-91.4%
CRO	S-0%	S-60.8%
CFX	S-0%	S-39.1%
CFXA	S-0%	S-39.1%
CP	S-0%	S-39.1%
COL	S-0%	I-100%
ERT	S-0%	S-100%
FOS	S-44.4%	S-91.3%
GNT	S-18.5%	S-86.9%
IMP	S-0%	S-91.3%
MER	S-0%	S-100%
PITA	S-0%	S-82.6%
TG	S-18.5%	S-86.9%
TRIS	S-25.9%	S-56.5%
PLZ	S-18.5%	S-78.2%
FPE	S-7.4%	S-100%

CRE-Carbapenem-resistant Enterobacterales, S-Sensitive, I-Intermediate, n= number of isolates, AMK-Amikacin, AMX-Amoxicillin, FEP-Cefepime, CEF/SUL-Cefoperazone/Sulbactam, CRO-Ceftriaxone, CFX- Cefuroxime, CFX A- Cefuroxime Axetil, CP- Ciprofloxacin , COL-Colisin, ERT-Ertapenem, FOS- Fosfomycin, GNT- Gentamicin, IMP-Imepenem, MER- Meropenem, PITA-Piperacillin/Tazobactam, TG-Tigecycline, TRIS-Trimethoprim/Sulfamethoxazole, PLZ- Plazomicin, FPE- Cefepime Enmetazobactam.

Table 2- The susceptibility patterns of carbapenem-resistant OXA-48 positive isolates of Klebsiella pneumoniae and Escherichia coli against various antibiotics.

ANTIBIOTIC	*K. pneumoniae* (n=5)	*E. coli* (n=5)
AMK	S-20%	S-100%
AMX	S-0%	S-0%
FEP	S-0%	S-0%
CEF/SUL	S-0%	S-0%
CRO	S-0%	S-0%
CFX	S-0%	S-0%
CFXA	S-0%	S-0%
CP	S-0%	S-0%
COL	I-83.3%	I-100%
ERT	S-0%	S-0%
FOS	S-40%	S-100%
GNT	S-60%	S-80%
IMP	S-0%	S-0%
MER	S-0%	S-0%
PITA	S-0%	S-0%
TG	S-0%	S-100%
TRIS	S-20%	S-60%
FPE	S-100%	S-100%

CRE-Carbapenem-resistant Enterobacterales, S-Sensitive, I-Intermediate, n= number of isolates, AMK-Amikacin, AMX-Amoxicillin, FEP-Cefepime, CEF/SUL-Cefoperazone/Sulbactam, CRO-Ceftriaxone, CFX- Cefuroxime, CFX A- Cefuroxime Axetil, CP- Ciprofloxacin , COL-Colisin, ERT-Ertapenem, FOS- Fosfomycin, GNT- Gentamicin, IMP-Imepenem, MER- Meropenem, PITA-Piperacillin/Tazobactam, TG-Tigecycline, TRIS-Trimethoprim/Sulfamethoxazole, FPE- Cefepime Enmetazobactam.

DISCUSSION

Cefepime/enmetazobactam (FPE) exhibited a marked bactericidal effect in pneumonia caused by OXA-48-producing multidrug-resistant (MDR) *Klebsiella pneumoniae*, outperforming conventional therapies like meropenem. This novel combination presents a promising alternative for treating challenging lung infections caused by such resistant pathogens [8]. These findings align with our study, supporting FPE as a viable treatment option for complicated infections due to carbapenem-resistant Enterobacterales (CRE), including difficult-to-treat *Klebsiella pneumoniae* strains. Thus, FPE may play a critical role in managing severe infections where traditional treatments fail.

In another study, cefepime-enmetazobactam was highlighted as a promising intravenous β-lactam/β-lactamase inhibitor (BL/BLI) combination approved for treating complex urinary and respiratory infections brought on by MDR Gram-negative bacteria. This combination restores cefepime's efficacy against ESBL and OXA-48 producers, presenting a valuable carbapenem-sparing therapeutic option. It had demonstrated strong clinical efficacy and a tolerable safety profile [9]. However, further clinical data are needed to extend its use against a broader range of carbapenemase-producing infections beyond OXA-48 and select KPC variants. These observations align with our study, underscoring the therapeutic potential and importance of cefepime-enmetazobactam in managing resistant infections. Studies favor the use of cefepime/enmetazobactam (FPE) as it demonstrates a spectrum of coverage that is superior to piperacillin-tazobactam (TZP) and comparable to meropenem. These findings align with our study, emphasizing significant role of FPE as an effective carbapenem-sparing option in treatment of infections by ESBL-producing Enterobacterales [10].

In multiple studies, plazomicin showed enhanced *in-vitro* action in comaprsion to gentamicin and amikacin, with high susceptibility rates among non-ESBL and ESBL-producing isolates of *K. pneumoniae* and *E. coli*. One such study included 180 clinical isolates divided equally into non-ESBL-producing, ESBL-producing, and carbapenem-resistant groups, showing strong plazomicin susceptibility in non-ESBL and ESBL producers [11]. Our study supports these findings, with isolates showing limited susceptibility to plazomicin among carbapenem-resistant Enterobacterales (CRE), with only 18.5% CRE strains susceptible, indicating significant resistance in carbapenem-resistant *K. pneumoniae*. However, non-CRE strains exhibited 78.2% susceptibility, suggesting better efficacy in non-carbapenem-resistant infections, aligning with the broader literature on activity of plazomicin.

Another study has demonstrated the efficacy of alternative antibiotics such as fosfomycin, and tigecycline in treating CRE [12]. Despite limited clinical use, tigecycline has shown susceptibility rates (100%) against CRE isolates, consistent with the findings from our evaluation of CRE isolates. This highlights the therapeutic potential of tigecycline as an alternative treatment option for carbapenem-resistant *E. coli* and other Enterobacterales infections, reinforcing the relevance of these agents in managing difficult-to-treat multidrug-resistant infections as observed in our research.

In conclusion, our study highlights cefepime-enmetazobactam (FPE)

and plazomicin (PLZ) as promising treatment options for *Klebsiella pneumoniae* infections, especially in non-carbapenem-resistant strains. FPE also shows strong activity and clinical utility for managing *Escherichia coli* infections, supporting its role as a carbapenem-sparing agent with effectiveness against ESBL-producing strains. Plazomicin, with its strong bactericidal effects and favorable safety profiles, offers an important therapeutic option for multidrug-resistant *K. pneumoniae*. Together, these agents expand available treatment choices for managing difficult-to-treat infections in settings of antimicrobial resistance.

Limitations

A key limitation of the study on cefepime-enmetazobactam (FPE) and plazomicin (PLZ) for carbapenem-resistant Enterobacterales (CRE) infections is the relatively small number of isolates tested. The preclinical and clinical investigations so far have included a limited sample size of isolates, which restricts the generalizability and robustness of the findings in diverse clinical scenarios involving CRE. Thus, ongoing and future research including a greater number of clinical isolates is essential to validate and extend the promising roles of FPE and PLZ in the management of CRE infections.

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The authors declare no conflict of interest

Author contributions:

SM: writing-original draft, methodology, investigation. SJ: methodology, formal analysis, review & editing, data curation. IJ: data curation, formal analysis, review & editing subsequent drafts. JM: Conceptualization, methodology, supervision, review & editing.

All authors had full access to all the data in the study, read and approved the manuscript.

Data sharing statement:

The data that support the findings of this study are available on request from the corresponding author.

Ethics approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee (IEC REF NO: BHR/RS/MSSH/MHPL/DWARKA/MHEC/GIMBS/25-05)

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All authors consented to publication of the manuscript

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