

Original Research

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Antimicrobial Susceptibility Testing of Cefepime Enmetazobactam Against Multidrug Resistant Pathogens

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ABSTRACT: Multidrug-resistant (MDR) bacteria are becoming more common, which is a serious threat to public health and makes treating a variety of illnesses more difficult. Pathogens that show resistance to several kinds of antibiotics, such as carbapenems, extended-spectrum β -lactams, and other widely used medicines, are among the most alarming. The purpose of this study is to assess the antimicrobial susceptibility of a novel β -lactam/ β -lactamase inhibitor combination, cefepime-enmetazobactam, against a group of MDR pathogens. The minimum inhibitory concentrations (MICs) of cefepime-enmetazobactam against a variety of clinical isolates of MDR pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and other clinically significant resistant organisms, were found using conventional broth microdilution techniques. The findings show that cefepime-enmetazobactam has strong in vitro action against a variety of MDR bacteria, including those that produce carbapenemases and extended-spectrum β -lactamases (ESBLs). It was discovered to be very efficient against *A. baumannii* and *K. pneumoniae*, which are frequently linked to serious hospital-acquired infections. According to these results, cefepime-enmetazobactam is a potentially effective treatment for infections brought on by MDR microorganisms. To properly evaluate its safety, effectiveness, and possible role in controlling resistant infections in hospital settings, more research—including clinical trials—is required.

INTRODUCTION:

One of the most important issues facing contemporary medicine is the rise of multidrug-resistant (MDR) microorganisms. These organisms cause a variety of challenging illnesses in both community and healthcare settings because they are resistant to several types of antibiotics. MDR infections frequently result in longer hospital stays, higher fatality rates, and significant medical expenses. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter* species are among the most worrisome MDR pathogens since they have been linked to serious infections like pneumonia, urinary tract infections, and bloodstream infections [1][2]. These pathogens possess a variety of resistance mechanisms, including the production of extended-spectrum β -lactamases (ESBLs) and carbapenemases, which degrade commonly used β -lactam antibiotics, rendering them ineffective [3]. In response to the growing problem of antimicrobial resistance (AMR), new therapeutic agents are urgently needed. One such agent is the combination of cefepime, a fourth-generation cephalosporin, with enmetazobactam, a novel β -lactamase inhibitor. Cefepime exhibits broad-spectrum activity against Gram-negative and Gram-positive bacteria, but its efficacy is compromised by β -lactamase-producing organisms. Enmetazobactam, designed to inhibit a wide range of β -lactamases, including ESBLs and carbapenemases, enhances the activity of cefepime against resistant strains [4]. The cefepime-enmetazobactam combination has shown promising results in preclinical and clinical studies, suggesting that it may offer a viable treatment option for infections caused by MDR pathogens [5]. Antibiotic

resistance is a complex, multifactorial process by which bacteria evolve mechanisms to survive exposure to antibiotics that would normally inhibit their growth or kill them. Both intrinsic (occurring naturally) and acquired (through mutation or horizontal gene transfer) pathways can lead to resistance. Antibiotic resistance poses a serious danger to world health since it can result in treatment failures, longer hospital admissions, and higher mortality [6]. Enzymatic antibiotic modification or degradation is one of the main mechanisms of antibiotic resistance. The synthesis of enzymes that render antibiotics inactive is one of the most prevalent processes, particularly in Gram-negative bacteria. For instance, β -lactamases render carbapenems, cephalosporins, and penicillins useless by hydrolyzing their β -lactam ring [7]. Reduced permeability makes some bacteria reduce their ability of antibiotic uptake by altering the structure or expression of porin channels in the outer membrane. This is particularly significant in *Pseudomonas aeruginosa*, where decreased porin expression limits β -lactam entry [8]. Biofilms protect bacterial communities from antibiotics and host immune responses. Because of their restricted penetration, modified microenvironment, and inactive persister cells, biofilm cells can be up to 1,000 times more resistant to drugs than planktonic cells [9]. Mobile genetic elements including plasmids, transposons, and integrons, which speed up the spread of resistance genes throughout bacterial populations, frequently mediate these resistance mechanisms. Certain Gram-negative bacteria create enzymes called extended-spectrum β -lactamases (ESBLs), which provide resistance to a variety of β -lactam medicines, such as penicillins, aztreonam, and third-generation cephalosporins like cefotaxime and ceftazidime. But β -lactamase inhibitors like clavulanic acid, sulbactam, tazobactam, and more recent drugs like enmetazobactam usually suppress them [10]. The hydrolysis of the β -lactam ring, a crucial structural element of β -lactam antibiotics, is the main way that ESBLs cause antibiotic resistance. The antibiotic molecule loses its ability to bind to its target, the penicillin-binding proteins (PBPs), which are necessary for the production of bacterial cell walls, after it has been hydrolyzed [11]. Most frequently, plasmid-mediated genes that enable horizontal transfer between bacterial populations encode ESBLs. The most prevalent families of ESBLs include TEM-type such as TEM-1, TEM-3 which was first identified in *Escherichia coli*, these enzymes are derived from earlier penicillinases and evolved to hydrolyze a wider range of β -lactams. SHV-type such as SHV-2, SHV-5 are commonly found in *Klebsiella pneumoniae*, these variants can hydrolyze third-generation cephalosporins. CTX-M-type such as CTX-M-15 which is now the most globally widespread ESBLs, these enzymes preferentially hydrolyze cefotaxime and are associated with pandemic clones of *E. coli* and *Klebsiella* spp. [12]. The genetic mobility of ESBL genes is a major concern, as they are often located on plasmids that also carry genes for resistance to other antibiotic classes, such as aminoglycosides and fluoroquinolones. This contributes to the development of multidrug-resistant (MDR) phenotypes [13]. ESBL production can be difficult to detect in clinical laboratories because phenotypic expression can vary. Therefore, specific tests such as the double-disk synergy test (DDST), combination disk test (CDT), and molecular methods (e.g., PCR) are often used to confirm ESBL production. Antimicrobial susceptibility testing is critical for determining the effectiveness of new antibiotics against MDR pathogens. In this study, we aim to evaluate the in vitro antimicrobial activity of cefepime-enmetazobactam against a panel of MDR pathogens. By disk diffusion method, we have assessed the zone of inhibition of cefepime-enmetazobactam for various resistant strains, providing valuable insight into its potential clinical application in treating infections caused by these challenging pathogens.

MATERIALS AND METHODS:

STUDY DESIGN AND SETTING:

This six-month study was carried out at Saveetha Medical College and Hospital in Chennai between January and June of 2025. Every sample that came into the microbiology lab was processed for sensitivity and culture. Strict confidentiality was applied to all patient data.

CULTURE PROCESSING:

All of the samples—aside from the urine samples—were processed by streaking them on Macconkey, chocolate, and blood agar. Cystine-Lactose-Electrolyte-Deficient (CLED) agar was streaked with urine samples. Plates which has growth of lactose-fermenting and non-fermenting colonies were processed using standard biochemicals, and cation-adjusted Mueller Hinton agar was used for antimicrobial susceptibility testing.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

Those organisms which were found multi drug resistant were tested with cefepime enmetazobactam by the disc diffusion method using cation adjusted mueller hinton agar.

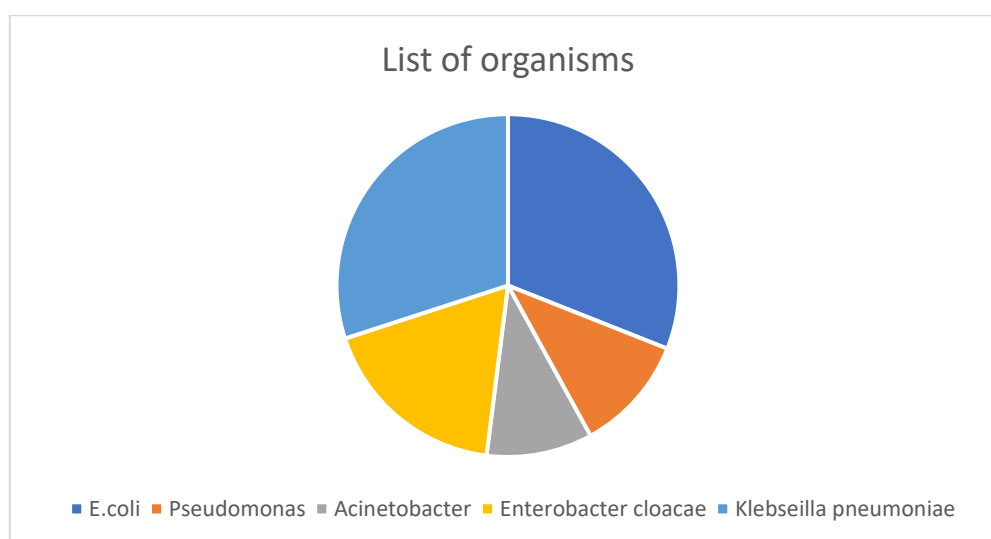
RESULTS:

We have isolated around 123 multidrug resistant organisms which includes various different isolates like *E.coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. The results showing list of various organisms are tabulated below.

TABLE 1: Depicts the list of various organisms

S.no	organism	No.of isolates susceptible
1	<i>E.coli</i>	31
2	<i>Pseudomonas aeruginosa</i>	11
3	<i>Acinetobacter baumannii</i>	10
4	<i>Enterobacter cloacae</i>	18
5	<i>Klebsiella pneumoniae</i>	30

FIGURE 1: Depicts the list of various organisms



Various isolates showed different zone size with the highest zone size of around 38mm. The list of organisms and their varying zone diameter is tabulated below.

TABLE 2: Depicts the zone diameter of various isolates

S.no	Zone diameter	No.of isolates	<i>E.coli</i>	No.of <i>Pseudomonas aeruginosa</i> isolates	No.of <i>Acinetobacter baumannii</i> isolates	No.of <i>Enterobacter cloacae</i> isolates	No.of <i>Klebsiella pneumoniae</i> isolates
1	24mm	4	4	4	1	2	4
2	25mm	6	1	3	5	6	
3	26mm	2	0	1	1	2	
4	27mm	2	0	1	1	0	

5	28mm	2	1	1	3	2
6	29mm	0	0	0	0	0
7	30mm	4	1	1	4	6
8	31mm	2	0	0	0	0
9	32mm	2	0	0	0	1
10	33mm	0	1	1	0	0
11	34mm	2	0	0	0	1
12	35mm	2	1	1	2	4
13	36mm	2	0	0	0	0
14	37mm	0	0	0	0	0
15	38mm	1	1	0	0	2

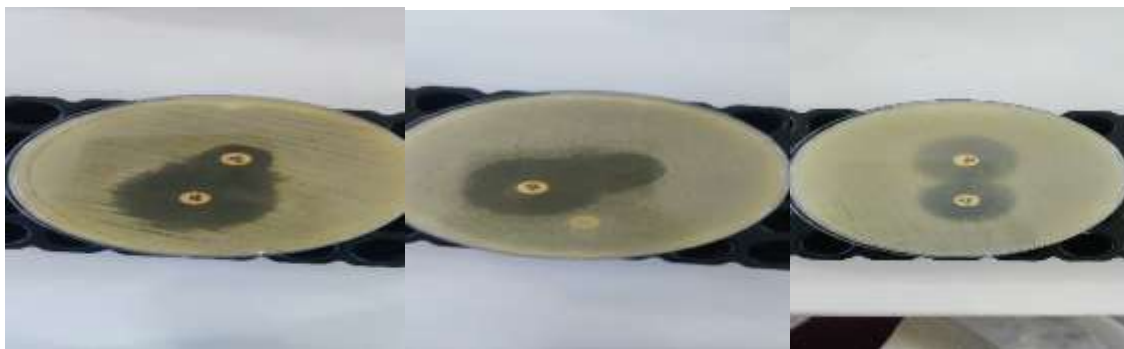


FIGURE 2: Depicts the antimicrobial susceptibility testing for *E.coli*

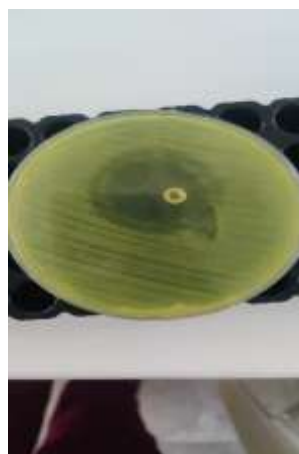


FIGURE3: Depicts the antimicrobial susceptibility testing for *P.aeruginosa*



FIGURE4: Depicts the antimicrobial susceptibility testing for *A.baumannii*



FIGURE 5: Depicts the antimicrobial susceptibility testing for *E.cloacae*

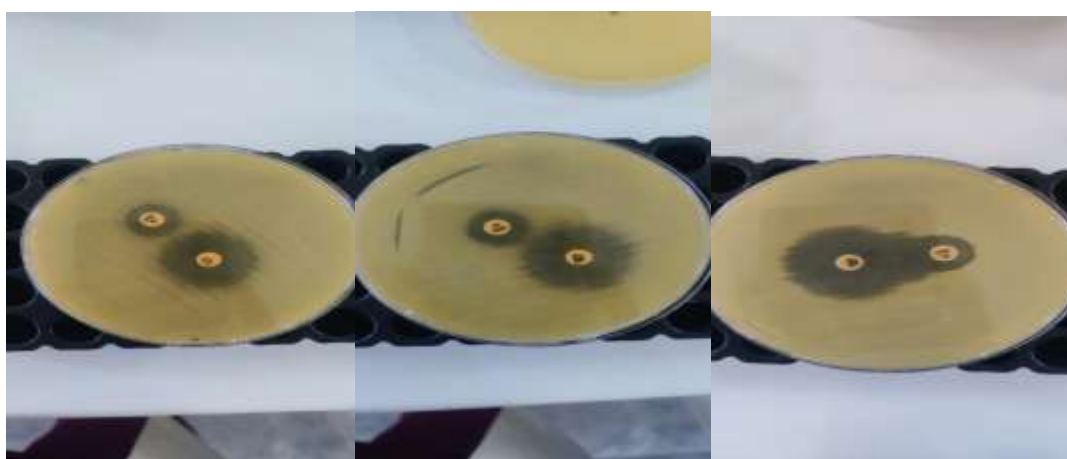


FIGURE 6: Depicts the antimicrobial susceptibility testing for *K.pneumoniae*

Among the 123 isolates, few isolates were resistant to the drug.the list of isolates resistant to cefepime-enmetazobactam is tabulated below.

TABLE 3: Depicts the list of organisms resistant to cefepime-enmetazobactam

S.no	organism	No.of isolates resistant
1	<i>E.coli</i>	6
2	<i>Pseudomonas aeruginosa</i>	2

3	<i>Acinetobacter baumannii</i>	3
4	<i>Enterobacter cloacae</i>	4
5	<i>Klebsiella pneumoniae</i>	8

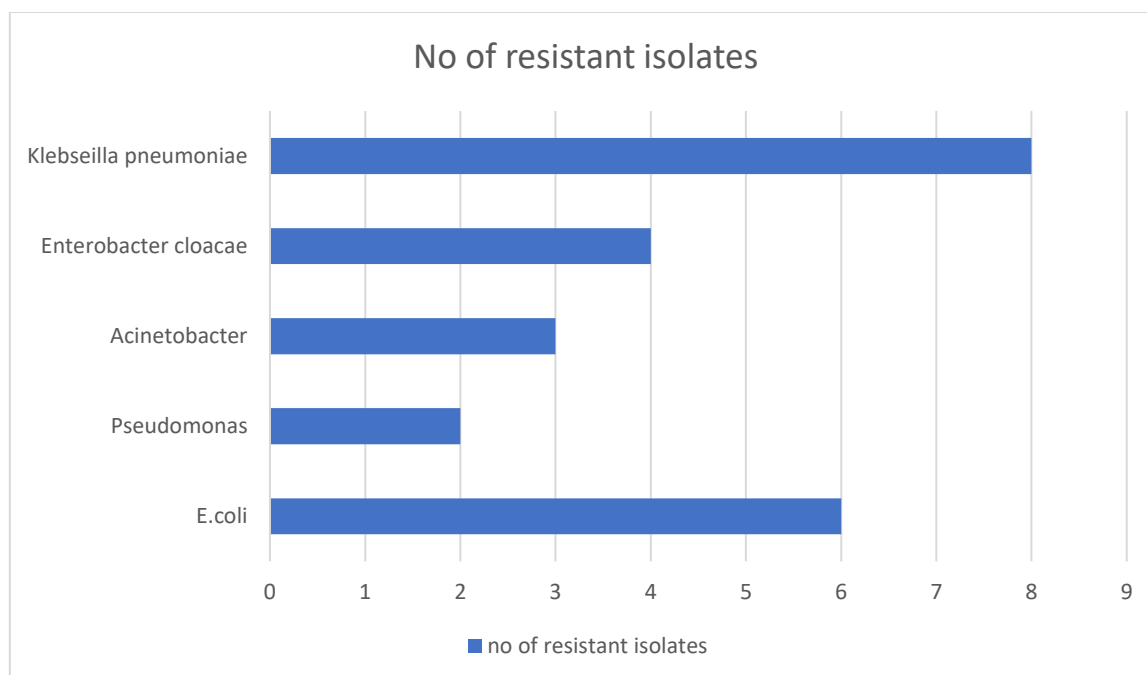


FIGURE 7: Depicts The Resistance Percentage Of Cefepime Enmetazobactam Among Various Organisms

DISCUSSION:

In this study, susceptibility to cefepime-enmetazobactam was 84% among *Escherichia coli* isolates, 83% among *Pseudomonas aeruginosa* isolates, 78% among *Acinetobacter baumannii* isolates, 81% among *Enterobacter cloacae* isolates and 80% among *Klebsiella pneumoniae* isolates. Cefepime/enmetazobactam susceptibility for *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* was 98.8%, 87.1%, and 83.3%, respectively, in a prior study [21]. For all other species susceptibility was 100%. Only 12 (5.9%) isolates exhibited cefepime/enmetazobactam resistance. But in our study the resistance was 16% among *Escherichia coli* isolates, 17% among *Pseudomonas aeruginosa* isolates, 22% among *Acinetobacter baumannii* isolates, 19% among *Enterobacter cloacae* isolates and 20% among *Klebsiella pneumoniae* isolates.

The study's findings show that cefepime-enmetazobactam, a novel β -lactam/ β -lactamase inhibitor combination, has promising in vitro activity against a variety of multidrug-resistant (MDR) Gram-negative pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These findings are consistent with previously published data, suggesting that cefepime-enmetazobactam has significant potential as a therapeutic option in the face of increasing antimicrobial resistance [14][15].

The broad-spectrum β -lactamase inhibition offered by enmetazobactam, a new diazabicyclooctane (DBO) inhibitor, is substantially responsible for the reported efficacy of cefepime-enmetazobactam. Enmetazobactam exhibits better permeability and class inhibition than previous inhibitors like tazobactam or clavulanic acid. Extended-spectrum β -lactamases (ESBLs) and other A β -lactamases are active against some class C enzymes [16]. This pharmacological profile contributes to its enhanced efficacy against ESBL-producing Enterobacterales, a major concern in hospital-acquired infections.

In comparison to cefepime alone and other β -lactam/ β -lactamase inhibitor combinations such as piperacillin-tazobactam or ceftazidime-avibactam, our results demonstrated that cefepime-enmetazobactam proved to be a better drug of choice.

From an antimicrobial stewardship perspective, the use of cefepime-enmetazobactam may help preserve carbapenems by serving as a viable alternative for treating ESBL-related infections. The WHO has designated carbapenem-resistant Enterobacterales (CRE) as essential priority pathogens for research and treatment development, and this could postpone their emergence and spread [17].

However, our study has several limitations. First, it was conducted in vitro, and therefore clinical correlation should be made cautiously. Second, our isolate collection, while representative of MDR organisms, may not fully reflect the diversity of resistance mechanisms globally. Future multicentric studies and post-marketing surveillance data will be essential to further validate cefepime-enmetazobactam's role in real-world clinical settings.

Additionally, cefepime-enmetazobactam's pharmacokinetic/pharmacodynamic (PK/PD) profile supports its usage in clinical settings, especially for complex urinary tract infections (cUTIs) and bloodstream infections. Its good safety profile and non-inferiority to piperacillin-tazobactam in treating cUTIs were shown in a recent Phase 3 trial (ALLEVIATE research)[18][19]. Our in vitro findings support the translation of such efficacy into broader applications, especially in institutions facing high ESBL and MDR prevalence.

CONCLUSION:

In conclusion, cefepime-enmetazobactam exhibits notable in vitro activity against MDR Gram-negative bacteria, especially Enterobacterales that produce ESBL. This medication may close a crucial gap in the antibiotic arsenal against growing resistance, even if more clinical research is required to determine its role in treating infections brought on by organisms that produce carbapenemase. Future directions should include real-world clinical outcome studies, resistance surveillance, and the potential inclusion of cefepime-enmetazobactam in empiric therapy algorithms in high-risk settings.

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