



IN VITRO EFFICACY OF CEFEPIME-ENMETAZOACTAM AMONG MULTIDRUG-RESISTANT INCLUDING BIOFILM-PRODUCING ACINETOBACTER SPECIES

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ABSTRACT Antimicrobial resistance among *Acinetobacter baumannii* presents a significant therapeutic challenge, particularly in critical care settings where biofilm production enhances persistent survival on medical devices [1]. Cefepime-enmetazobactam is a novel β -lactam/ β -lactamase inhibitor combination targeting extended-spectrum β -lactamase (ESBL)-producing organisms [2, 3]. This prospective cross-sectional study evaluated the in vitro efficacy of cefepime-enmetazobactam against 90 multidrug-resistant (MDR) and biofilm-producing *Acinetobacter baumannii* clinical isolates. Biofilm formation was quantified using the Tissue Culture Plate (TCP) assay [4], and antimicrobial susceptibility was evaluated alongside minimum inhibitory concentration (MIC) determination for cefepime-enmetazobactam using the broth microdilution method and E-test [2]. High rates of resistance were observed for conventional agents, including meropenem (88.9%) and cefepime (85.7%). Biofilm production was detected in 7.8% ($n = 7$) of isolates (all moderate intensity). Against EUCAST 2024 criteria (≤ 4 mg/L susceptible, > 4 mg/L resistant), all 90 isolates (100%) had MIC values ranging from 0.041 mg/L to 0.217 mg/L [2]. These findings indicate the need for careful breakpoint application when evaluating cefepime-enmetazobactam against hospital-derived MDR *Acinetobacter* cohorts.

KEYWORDS : *Acinetobacter Baumannii*, Cefepime-enmetazobactam, Multidrug Resistance, Biofilm Formation, Tissue Culture Plate Method, Broth Microdilution

1. INTRODUCTION

Acinetobacter baumannii is a prominent healthcare-associated pathogen capable of causing severe ventilator-associated pneumonia, bloodstream infections, and post-surgical wound infections in critically ill patients [1]. The organism's inherent capacity to form robust biofilms on biotic and abiotic surfaces significantly reduces antibiotic penetration and protects bacterial cells from host immune defense mechanisms, contributing to treatment failures and hospital outbreaks [1, 4].

Cefepime-enmetazobactam combines an established fourth-generation cephalosporin with a novel penicillanic acid sulfone β -lactamase inhibitor designed to penetrate Gram-negative outer membranes and inhibit Class A β -lactamases efficiently [2]. While currently approved for complicated urinary tract infections and hospital-acquired pneumonia caused by ESBL-producing Enterobacterales [2, 3], its activity against carbapenem-resistant *A. baumannii*—where OXA-type carbapenemases dominate—requires rigorous evaluation [1, 2].

2. MATERIALS AND METHODS

Following Institutional Human Ethics Committee approval, 90 non-duplicate clinical isolates of *Acinetobacter baumannii* were collected from respiratory specimens (95.6% endotracheal secretions), bronchoalveolar lavage (BAL), and sputum at KMCH Institute of Health Sciences and Research. Isolates were identified using standard microbiological techniques and biochemical profiles (Oxidase-negative, Citrate-positive, Non-motile, Oxidative OF glucose).

- Antimicrobial Susceptibility Testing (AST):** Performed using the Kirby-Bauer disk diffusion technique according to CLSI guidelines.
- Biofilm Detection:** Quantified by the Tissue Culture Plate (TCP) method at OD₅₇₀ nm in 96-well microtiter plates [4]. Cut-offs were classified as Non/weak (< 0.120), Moderate (0.120 – 0.240), and Strong (> 0.240) [4].
- Cefepime-Enmetazobactam MIC:** Determined via E-test gradient strips and broth microdilution, interpreted according to EUCAST criteria (Susceptible ≤ 4 mg/L, Resistant > 4 mg/L) [2, 3].

3. RESULTS

The study cohort comprised 90 clinical isolates, predominantly recovered from male patients (75.6%) admitted to Intensive Care Units (72.2%).

Table 1: Demographic and Clinical Parameters of Clinical Isolates (N = 90)

Parameter / Characteristic	Distribution / Value (N = 90)
Mean Age $\pm$ Standard Deviation	52.0 $\pm$ 17.5 years
Gender Distribution	Male: 68 (75.6%) Female: 22 (24.4%)
Specimen Sources	Endotracheal Secretions: 86 (95.6%), BAL: 3 (3.3%), Sputum: 1 (1.1%)
Primary Ward / ICU Location	ICU: 65 (72.2%), SICU: 11 (12.2%), MICU/General: 10 (11.2%), NICU: 4 (4.4%)
Multidrug Resistance (MDR) Rate	82 / 90 isolates (91.1%)

Table 2: Antibiotic Susceptibility Profile of *Acinetobacter baumannii* Isolates

Antibiotic Agent	Tested (n)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Cotrimoxazole	76	7 (9.2%)	0 (0.0%)	69 (90.8%)
Meropenem	90	10 (11.1%)	0 (0.0%)	80 (88.9%)
Ciprofloxacin	87	10 (11.5%)	0 (0.0%)	77 (88.5%)
Piperacillin-Tazobactam	85	10 (11.8%)	0 (0.0%)	75 (88.2%)
Imipenem	85	8 (9.4%)	3 (3.5%)	74 (87.1%)
Cefepime	84	9 (10.7%)	3 (3.6%)	72 (85.7%)
Minocycline	79	56 (70.9%)	7 (8.9%)	16 (20.3%)
Tigecycline	77	36 (46.8%)	36 (46.8%)	5 (6.5%)
Colistin	84	4 (4.8%)	79 (94.0%)	1 (1.2%)

Summary of Cefepime-Enmetazobactam Efficacy & Biofilm Results

All 90 isolates (100.0%) demonstrated MIC values ranging between 0.041 mg/L and 0.217 mg/L [2]. Biofilm screening via the TCP assay identified 83 isolates (92.2%) in the non/weak category and 7 isolates (7.8%) with moderate biofilm production [4]. No statistically significant association was observed between biofilm strength and MDR status ($p = 0.491$) or patient ward distribution ($p = 0.400$).

4. DISCUSSION

The high rate of multidrug resistance (91.1%) observed among ICU lower-respiratory isolates aligns with global surveillance reports emphasizing carbapenem resistance trends in *Acinetobacter baumannii* [1]. While enmetazobactam effectively protects cefepime against Class A ESBL producers [2, 3], it provides limited inhibition against OXA-type carbapenemases (such as OXA-23) and metallo- β -lactamases, which predominantly mediate carbapenem resistance in

Acinetobacter spp. [1, 2].

The spectrum of activity observed in this study underscores that novel β -lactamase inhibitor combinations cannot be empirically relied upon for *Acinetobacter* infections without prior antimicrobial susceptibility confirmation [2, 3]. Polymyxins (Colistin) and tetracycline derivatives (Minocycline, Tigecycline) continue to demonstrate superior *in vitro* preservation against local clinical strains.

5. CONCLUSION

Cefepime-enmetazobactam susceptibility testing requires careful evaluation alongside local enzymatic profiles in multidrug-resistant clinical isolates of *Acinetobacter baumannii* [1,2]. Colistin, minocycline, and tigecycline retained high levels of activity. Surveillance and strict infection prevention strategies remain critical in controlling the dissemination of resistant hospital pathogens [1].

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