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Comparison of Meropenem and Meropenem-Amikacin Combination against Carbapenem-resistant and Multidrug-resistant *Pseudomonas aeruginosa* Isolates In vitro

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Abstract

Infections caused by carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) and multidrug-resistant *P. aeruginosa* (MDR-PA) pose major therapeutic challenges. The limited availability of new antibiotics necessitates evaluating rational combination therapies. This study aimed to compare the in vitro efficacy of meropenem versus meropenem-amikacin combination against carbapenem-resistant *P. aeruginosa* isolates (including CRPA and MDR-PA). An in vitro experimental study using the checkerboard method was performed on 10 clinical isolates (3 CRPA and 7 MDR-PA) obtained from Dr. Soetomo General Hospital, Surabaya (June-October 2025). Three isolates that showed a decrease in meropenem Minimum Inhibitory Concentration (MIC) to the susceptible range upon confirmatory testing were excluded from the combination synergy analysis. The effectiveness of the combination was evaluated by comparing the MIC of meropenem with that of the combination. Antibiotic interactions were analyzed using the Fractional Inhibitory Concentration Index. Data were analyzed using the Mann-Whitney U test, with the primary analysis focusing on the combined group of resistant isolates. The median MIC of meropenem for the combined CRPA and MDR-PA isolates was 32 µg/mL. The checkerboard test revealed an additive interaction as the dominant effect (71.4%), followed by synergistic (14.3%) and indifferent (14.3%) effects. No antagonistic effects were observed. Statistical analysis of the combined group showed that the meropenem-amikacin combination was significantly more effective in reducing the MIC compared to monodrug (median MIC combination: 4 µg/mL vs. monodrug: 32 µg/mL; P = 0.009). These findings support the consideration of this combination as a therapeutic option for such infections.

Keywords: *Pseudomonas aeruginosa,* Multidrug-resistant, Carbapenem-resistant, Antibiotic Combination, Meropenem, Amikacin, Checkerboard, FIC

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INTRODUCTION

Pseudomonas aeruginosa is a notorious nosocomial pathogen. Its resistance to carbapenems, which places it on the World Health Organization's critical priority list, represents a significant global health threat.[1] Infections caused by carbapenem-resistant *P. aeruginosa* (CRPA) and its multidrug-resistant (MDR) variants are associated with poor clinical outcomes, prolonged hospital stays, and increased mortality.[2]

The therapeutic options for these resistant infections are limited, particularly in resource-constrained settings where access to newer agents such as ceftolozane-tazobactam or cefiderocol is restricted.[3] This scarcity underscores the critical need to optimize the use of available antibiotics. Combination therapy, employing agents with distinct mechanisms of action such as a β-lactam (meropenem) and an aminoglycoside (e.g., amikacin), represents a strategic approach to potentially overcome resistance, lower effective antibiotic concentrations, and delay the emergence of further resistance.[4]

Previous studies have investigated similar combination strategies against resistant gram-negative pathogens. Avent et al. evaluated meropenem-amikacin combination therapy against *P. aeruginosa* bacteremia using a hollow-fiber infection model, demonstrating its potential utility in specific clinical scenarios.[5] Furthermore, recent research has shown that combination approaches yield variable results for different bacterial species and resistance mechanisms. Recent studies have indicated that the efficacy of β-lactam and aminoglycoside combinations against *Acinetobacter baumannii* is significantly influenced by specific resistance mechanisms, such as efflux pump overexpression.[6] Similarly, research on carbapenem-resistant *Enterobacteriaceae* has highlighted how the presence of different β-lactam enzymes can dramatically affect combination therapy outcomes.[7] These findings indicate that the success of combination therapy is highly dependent on both bacterial species and specific molecular resistance determinants.

However, the antibacterial activity of such combinations is not universal and is highly dependent on the specific resistance profile of the infecting strain.[8] Robust local data on the interaction between meropenem and amikacin against contemporary clinically relevant CRPA and MDR-PA isolates in Indonesia are lacking. Most existing studies have focused on Western strains or utilized different methodologies that may not fully capture local epidemiological patterns. Furthermore, emerging evidence suggests unique regional patterns of antimicrobial resistance in Southeast Asia compared to other parts of the world,[9] indicating that combination therapy outcomes may differ substantially in local clinical settings. Recent methodological comparisons have also shown that different in vitro testing approaches can yield varying assessments of antibiotic interactions,[10] emphasizing the need for standardized evaluations in local contexts. Furthermore, recent surveillance data indicate unique patterns of carbapenemase distribution in Southeast Asia compared to other regions,[11] suggesting that combination therapy outcomes may differ substantially in local settings.

This study aimed to conduct a comparative in vitro evaluation of meropenem monodrug versus meropenem-amikacin combination against a combined group of CRPA and MDR-PA isolates from a tertiary care hospital in Surabaya. The novelty and originality of this study lie in its focus on contemporary, locally prevalent clinical isolates from Indonesia in 2025, employing the standardized checkerboard method to provide detailed Fractional Inhibitory Concentration Index (FICI) analysis specifically for this understudied population. This approach addresses a critical gap in regional antimicrobial resistance data and offers evidence tailored to guide local clinical practice, where newer antibiotics are often inaccessible, contributing to antimicrobial stewardship efforts in resource-limited settings.

MATERIALS AND METHODS

Study design and isolates

This in vitro experimental study was conducted at the Clinical Microbiology Laboratory of Dr. Soetomo General Hospital, Surabaya, Indonesia, from June to December 2025. Ethical approval was obtained from the hospital's Institutional Review Board (No. 2065/LOE/301.4.2/VII/2025).

Table 1. Minimum Inhibitory Concentration of Carbapenem-resistant and Multidrug-resistant *Pseudomonas aeruginosa* Isolates

Isolate	Amikacin		Meropenem	
	MIC (µg/mL)	Interpretation	MIC (µg/mL)	Interpretation
CRPA 1	≤8	Sensitive	>8	Resistant
CRPA 2	≤8	Sensitive	>8	Resistant
CRPA 3	≤8	Sensitive	>8	Resistant
MDR-PA 1	≤8	Sensitive	>8	Resistant
MDR-PA 2	16	Sensitive	8	Resistant
MDR-PA 3	≤8	Sensitive	>8	Resistant
MDR-PA 4	≤8	Sensitive	>8	Resistant
MDR-PA 5	≤8	Sensitive	>8	Resistant
MDR-PA 6	≤8	Sensitive	>8	Resistant
MDR-PA 7	≤8	Sensitive	>8	Resistant

Interpretation of MIC values based on Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition breakpoints:[12] amikacin (susceptible ≤16 µg/mL, resistant ≥64 µg/mL); meropenem (susceptible ≤2 µg/mL, resistant ≥8 µg/mL) for *Pseudomonas aeruginosa*

Bacterial isolates

A purposive sample of 10 non-duplicate, stored clinical isolates of *P. aeruginosa* from inpatient specimens (collected from June to October 2025) was used. Isolates were identified, and their susceptibility profiles were determined using the BD Phoenix™ automated system. The collection included 3 CRPA (meropenem-resistant) and 7 MDR-PA (defined as non-susceptible to ≥1 agent in ≥3 antimicrobial categories, including meropenem resistance).[13] It should be noted that of the initial 10 isolates, 3 samples showed a decrease in the Minimum Inhibitory Concentration (MIC) of meropenem to within the sensitive range upon confirmatory retesting prior to the combination assay; consequently, these three samples were not evaluated for antibiotic combination synergy. A key inclusion criterion was in vitro susceptibility to amikacin. *P. aeruginosa* ATCC 27853 served as the quality control strain.

Checkerboard assay and MIC determination

The checkerboard broth microdilution method was performed using 2-fold serial dilution of meropenem (0.5-32 µg/mL) and amikacin (0.25-256 µg/mL), prepared in Mueller-Hinton Broth in a 96-well microtiter plate. Each well received 50 µL of each antibiotic dilution. The final inoculum was standardized to approximately 5×10^5 CFU/mL, and 100 µL was added to each well. Plates were incubated at 35 °C for 18-20 hrs. The MIC was defined as the lowest concentration that inhibited visible growth. The MIC for meropenem alone was determined concurrently with the standard broth microdilution.

The interactions between meropenem and amikacin were evaluated using the checkerboard method. The FICI was calculated as follows:

$$FICI = \frac{\text{MIC of drug A combination}}{\text{MIC of drug A alone}} + \frac{\text{MIC of drug B combination}}{\text{MIC of drug B alone}}$$

The FICI was interpreted as follows: synergy (FICI $\leq$ 0.5), additive (0.5 < FICI $\leq$ 1.0), indifferent (1.0 < FICI $\leq$ 4.0), antagonism (FICI > 4.0).[8] For each isolate, the lowest FICI ($\sum$FIC min) representing the most effective combination was recorded.

RESULTS

Characteristics of isolates

All 10 clinical isolates (3 CRPA and 7 MDR-PA) were confirmed to be meropenem-resistant and amikacin-susceptible, thereby fulfilling the study's inclusion criteria (Table 1).

Meropenem monodrug

Three isolates that showed a decrease in meropenem MIC to the susceptible range upon

Table 2. Minimum Inhibitory Concentration Results of Meropenem Monodrug on Carbapenem-resistant and Multidrug-resistant isolates of *Pseudomonas aeruginosa* using the checkerboard method

Isolate Group	Isolate	MIC Meropenem Monodrug (µg/mL)
Carbapenem-resistant	CRPA-1	32
P. aeruginosa	CRPA-2	<8
	CRPA-3	<8
Multidrug-resistant	MDR-PA 1	64
P. aeruginosa	MDR-PA 2	<8
	MDR-PA 3	16
	MDR-PA 4	16
	MDR-PA 5	16
	MDR-PA 6	32
	MDR-PA 7	32
ATCC *P. aeruginosa*	-	<8

confirmatory testing were excluded from the combination synergy analysis.

Meropenem showed high MIC values against the tested isolates. ranging from 16-64 µg/mL, with a median of 32 µg/mL. This confirmed the high level of resistance in the combined isolate group (Table 2).

Interaction of the meropenem-amikacin combination

The checkerboard assay results for the combined isolates are summarized in Table 3. The predominant interaction observed was additive (71.4% of evaluable interaction points), followed by synergistic (14.3%) and indifferent (14.3%) interactions. No antagonistic interactions were observed.

Three isolates (CRPA-2, MDR-PA 2, and MDR-PA 3) could not be evaluated for FICI because of specific methodological constraints. For CRPA-2 and MDR-PA 2, the meropenem monodrug MIC was below the lowest tested concentration (<0.5 µg/mL), indicating susceptibility that contradicted the initial CRPA/MDR-PA classification upon retesting during the checkerboard assay. This likely represents the phenotypic reversion of resistance following subculturing from frozen storage, a phenomenon previously described in *P. aeruginosa* isolates stored under similar conditions.[14] For MDR-PA 3, the amikacin monodrug MIC was below

Table 3. Distribution of Antibiotic Interaction Types Based on Fractional Inhibitory Concentration Index for the Combined Isolates

Interaction	Criteria ∑FIC	Number of Test Points	Percentage
Synergy	≤0.5	1	14.3%
Additive	0.5<∑FIC≤1 1	5	71.4%
Indifferent	1<∑FIC≤4	1	14.3%
Antagonism	>4	0	0%

the lowest tested concentration (<0.25 µg/mL), whereas meropenem remained resistant (MIC = 16 µg/mL). In this case, the combination could not be evaluated properly because the denominator for calculating the FIC amikacin approached zero, making the FICI calculation mathematically unreliable. Therefore, these isolates were excluded from the FICI analysis, and were retained in the comparative MIC analysis between monotherapy and combination therapy.

Comparative: Meropenem vs. Combination

The primary statistical analysis comparing the MICs of the two treatment groups for all isolates combined showed a significant difference. The median MIC for the meropenem-amikacin combination (4 µg/mL) was substantially and statistically significantly lower than the median MIC for meropenem (32 µg/mL) (Mann-Whitney U test, P = 0.009). Detailed FICI results are presented in Table 4.

DISCUSSION

This study provides in vitro evidence that combining meropenem with amikacin results in significantly greater antibacterial activity against a combined population of clinically relevant carbapenem-resistant and multidrug-resistant *P. aeruginosa* isolates than meropenem monodrug. The median MIC was reduced 8-fold, from 32 µg/mL to 4 µg/mL, a finding of considerable pharmacodynamic relevance.

The observed effect was predominantly additive (71.4%), which aligns with the theoretical basis for combining a cell wall-active agent with an aminoglycoside.[4,5] The additive effect is clinically meaningful as it indicates that the activity of the combination is at least equal to the cumulative

Table 4. Fractional Inhibitory Concentration Index of Meropenem-Amikacin Combination in Carbapenem-resistant and *Pseudomonas aeruginosa* isolates

Isolate	Monodrug MIC (µg/mL)		Optimal MIC Combination (µg/mL)		FICI	Interpretation
	Mem	Ak	Mem	Ak		
CRPA-1	16	1	2	0,5	0.625	Additive
CRPA-2	<0.5	1	-	-	-	Cannot be evaluated
CRPA-3	1	2	0.5	0.5	0.75	Additive
MDR-PA 1	32	64	32	0.25	1.003	Indifferent
MDR-PA 2	<0.5	0.5	-	-	-	Cannot be evaluated
MDR-PA 3	16	<0.25	-	-	-	Cannot be evaluated
MDR-PA 4	16	1	4	0.25	0.50	Synergy
MDR-PA 5	16	2	4	1	0.75	Additive
MDR-PA 6	16	2	2	1	0.625	Additive
MDR-PA 7	16	4	4	2	0.75	Additive

Mem: meropenem, Ak: Amikacin; FIC Index (FICI) = FIC Meropenem+ FIC Amikacin; Criteria: Synergy (FICI ≤0.5), Additive (0.5<FICI≤1.0), Indifferent (1.0<FICI≤4.0), Antagonism (FICI>4.0)[8]

effect of the individual drugs, potentially allowing the use of lower individual drug doses to achieve target exposures, thereby possibly reducing toxicity risks.[4,8] The finding of synergy in only one isolate underscores the well-documented heterogeneity in resistance mechanisms among *P. aeruginosa* clinical isolates, which can significantly influence combination outcomes.[1,15]

This variability may also be attributed to heteroresistance, a phenomenon where subpopulations of bacteria exhibit differential susceptibility to antibiotics, potentially affecting in vitro testing outcomes and complicating therapeutic decision-making.[16]

Carbapenem-resistance in *P. aeruginosa* is multifactorial and often arises from a confluence of mechanisms that create a formidable therapeutic barrier. The primary mechanisms include: (1) production of carbapenemase enzymes, particularly metallo-β-lactamases such as VIM, IMP, or NDM, which hydrolyze the β-lactam ring; (2) decreased outer membrane permeability due to the loss or mutation of the *OprD* porin, a specific channel for carbapenem uptake; (3) hyperexpression of efflux pump systems (e.g., MexAB-OprM, MexEF-OprN) that actively expel carbapenems from the bacterial cell; and (4) in some cases, overexpression of AmpC β-lactamase coupled with porin loss.[2] The isolates used in this study, confirmed as CRPA, likely carried one or more of these mechanisms, explaining the high baseline MICs for meropenem (16-32 µg/mL).

The predominantly additive effect observed in this study (FICI 0.5-1.0 in 80% of isolates) represents a combined inhibitory outcome where the effect of the combination equals the sum of each drug's individual effects. The mechanistic rationale for this effect lies in the distinct and complementary targets of the two antibiotics. Meropenem, a β-lactam, inhibits the synthesis of the peptidoglycan cell wall by binding to penicillin-binding proteins. This action not only inhibits cell wall formation but also compromises cell wall integrity. Amikacin, an aminoglycoside, exerts its bactericidal effect by irreversibly binding to the 30S ribosomal subunit, causing mRNA misreading and protein synthesis inhibition.[17] It has been hypothesized that the initial damage to the cell wall inflicted by meropenem may facilitate increased intracellular penetration of amikacin, allowing aminoglycosides to reach their ribosomal target more effectively. This sequential action, although not potentiating each other to a dramatic degree (synergy), resulted in a greater cumulative inhibitory effect than that of either agent alone.[4,8]

The significant reduction in MIC for the combination group suggests that the meropenem-amikacin combination could be a valuable therapeutic strategy in settings where newer antibiotics are unavailable. It may help "rescue"

the activity of meropenem against otherwise resistant strains, as indicated by reductions in MIC to potentially susceptible or intermediate ranges in several cases. This is particularly important for empirical or directed therapies in critically ill patients with suspected or confirmed resistant *P. aeruginosa* infections.[18]

The absence of antagonism is a reassuring finding, indicating that this specific combination is unlikely to be detrimental from a direct pharmacodynamic interaction perspective in this isolated set.

No antagonistic interactions were observed. Antagonism between a bactericidal drug (such as meropenem) and a bacteriostatic agent is a recognized phenomenon. However, both meropenem and amikacin are rapidly bactericidal, which may explain the absence of antagonism. Furthermore, their different modes of action minimize direct interference.[19] The finding that higher concentrations of the combination sometimes resulted in an indifferent effect (as seen in some wells during the checkerboard analysis) is also consistent with pharmacodynamic principles. Beyond the optimal ratio, increasing the concentration of one drug may not yield additional benefits if the other has already achieved its maximal effect at the target site.[20]

Although these in vitro results are promising, they must be interpreted with caution regarding direct clinical translation. The checkerboard assay measures the inhibitory concentrations under static conditions that do not replicate the dynamic pharmacokinetic/pharmacodynamic (PK/PD) parameters in vivo, such as tissue penetration, protein binding, and time-dependent activity of meropenem versus the concentration-dependent activity of amikacin.[21] Achieving and maintaining the synergistic or additive drug concentrations identified in vitro at the site of infection is critical for clinical success. Therefore, this study provides a strong rationale for considering meropenem-amikacin combination therapy for the treatment of carbapenem-resistant infections. However, their application should be guided by therapeutic drug monitoring and clinical judgment.

These findings are consistent with those of previous studies supporting combination therapy for multidrug-resistant Gram-negative infections. However, the variability in responses underscores the importance of individualized susceptibility testing.

Limitations

This study has some inherent limitations. Firstly, it was an in vitro investigation and did not account for the complex PK/PD interplay, host factors, or clinical outcomes in vivo. Second, the sample size, which was adequate to show a statistically significant difference in the combined group, was modest. Third, the study did not include the genotypic characterization of resistance mechanisms (e.g., carbapenemase genes and efflux pump overexpression), which may have provided deeper mechanistic insights into the observed interactions.[22] Future studies should incorporate such analyses and consider PK/PD modeling to better predict the clinical utility.

CONCLUSION

The meropenem-amikacin combination was significantly superior in vitro than meropenem against a combined group of carbapenem-resistant *P. aeruginosa* isolates from a tertiary hospital in Indonesia. This interaction was primarily additive. These findings provide a rationale for considering this combination as a potential treatment option for infections caused by carbapenem-resistant *P. aeruginosa* in similar settings, especially when therapeutic choices are limited. Further research involving larger prospective cohorts and integrated PK/PD assessments is warranted to validate these findings and inform clinical practice.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, under approval number No. 2065/LOE/301.4.2/VII/2025.

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