JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Relationship between OprD porin downregulation and in-vitro interactions in *Pseudomonas aeruginosa*

Tuba Müderris[1], Ufuk Akbayırlı[2], Rıza Durmaz[3], Selçuk Kaya[1], Tuba Dal[3], Ziya C Açıkgöz[3], Ayşegül Aksoy Gökmen[1]

[1] *İzmir Katip Çelebi University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Türkiye*
[2] *Muğla Seydikemer State Hospital, Medical Microbiology Laboratory, Muğla, Türkiye*
[3] *Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Türkiye*

Abstract

Introduction: OprD porin plays a critical role in antibiotic uptake. However, the role of OprD in antimicrobial interactions is not yet fully understood. This study investigated the relationship between OprD downregulation and in vitro interactions of meropenem (MEM) with combinations of colistin (CT), ertapenem (ETP), and fosfomycin (FF) in *Pseudomonas aeruginosa*.

Methodology: A total of 40 *P. aeruginosa* isolates were included in the study and divided into two groups (group-1, 20 isolates with confirmed OprD suppression; group-2; 20 isolates without OprD suppression). Antimicrobial interactions were examined using the checkerboard method.

Results: The synergy rates of MEM/CT, MEM/ETP, and MEM/FF combinations in group-1/group-2 isolates were determined as 45%/30%, 5%/5%, and 15%/10% respectively. The minimum inhibitory concentration (MIC) of antimicrobials in combinations were examined for group-1 and group-2 isolates, and a decrease of 90%, 90% for MEM and 70%, 40% for CT was detected in the MEM/CT combination, 50%, 45% for MEM and 35%, 40% for ETP in the MEM/ETP combination, and 35%, 20% for MEM and 55%, 45% for FF in the MEM/FF combination, respectively.

Conclusions: While significant synergy was observed between MEM/CT in OprD-deficient isolates, the limited synergistic effects observed with other combinations, such as MEM/ETP and MEM/FF, suggest that the impact of OprD downregulation on antimicrobial resistance is multifactorial and complex. The findings suggest that CT, when combined with MEM, may overcome certain resistance mechanisms mediated by OprD downregulation. Further investigations incorporating molecular diagnostics and in vivo validation are warranted to fully elucidate the therapeutic potential of these combinations.

Key words: *Pseudomonas aeruginosa*; OprD; porin; antimicrobial synergy.

J Infect Dev Ctries 2026; 20(7):1031-1037. doi:10.3855/jidc.22217

(Received 11 August 2025 – Accepted 28 November 2025)

Introduction

Pseudomonas aeruginosa is an aerobic Gram-negative bacillus and one of the important opportunistic pathogens [1]. It is widely found in nature [1]. Due to its widespread role in infections and high antibiotic resistance rates, limited options and treatment failure have recently become an important global problem [1]. It shows high rates of intrinsic resistance to many antibiotic groups, including beta-lactams, fluoroquinolones, and aminoglycosides. This resistance contributes significantly to the increased morbidity and mortality rates associated with infections caused by this pathogen [1,2]. Furthermore, overuse of antibiotics during treatment accelerates the development of multidrug resistant/extensive drug resistant (MDR/XDR) *P. aeruginosa* strains [3].

Carbapenems are antibiotics with a five-membered ring and are frequently used as a last-line treatment option in MDR/XDR *P. aeruginosa* infections. However, increasing carbapenem resistance in recent years has paved the way for use of antibiotic combinations [4]. Different mechanisms contribute to resistance to carbapenems. These mechanisms include overexpression of the efflux pump, decreased outer membrane permeability, production of antibiotic inactivating enzymes and acquisition or mutation of resistance genes. Carbapenem resistance arises through a complex interplay of mechanisms, often involving multiple pathways simultaneously [2,3,5]. However, one of the most studied resistance mechanisms is OprD porin downregulation. This porin plays a role in the uptake of positively charged amino acids from the outer membrane, as well as being the main channel for carbapenem entry [3]. However, the interactions of these resistance mechanisms with antimicrobial treatment remains poorly understood. To date, only a few studies have investigated the association between OprD porin downregulation and in vitro antimicrobial interactions in MDR/XDR *P. aeruginosa* isolates [6].

In this context, this study aimed to investigate the

relationship between OprD porin downregulation and the in vitro synergistic interactions of meropenem (MEM) in combination with colistin (CT), ertapenem (ETP), and fosfomycin (FF) in clinical isolates of *P. aeruginosa*.

Methodology

Selection of isolates

P. aeruginosa isolates included in this study were selected based on the presence or absence of specific resistance mechanisms, including OprD porin downregulation (analyzed with real-time reverse transcription polymerase chain reaction (PCR)), overexpression of efflux pumps (MexAB - OprM, MexCD - OprJ, MexXY – OprN; analyzed with real-time reverse transcription PCR), production of carbapenemase ((*Klebsiella pneumoniae* carbapenemase (KPC), imipenemase (IMP), verona integrin-encoded metallo-β-lactamases (VIM), oxacillinases (OXA), New Delhi metallo-β-lactamases (NDM); analyzed with PCR), and clonal relatedness (analyzed with pulsed-field gel electrophoresis), as previously characterized [7].

The isolates were divided into two groups for the purposes of this study: group 1, included isolates exhibiting OprD porin downregulation as the sole resistance mechanism; and group 2, included isolates lacking any known resistance mechanisms. Wherever feasible, clonally unrelated isolates were preferentially included to avoid bias due to clonal propagation.

A total of 40 clinical *P. aeruginosa* isolates were included in the study. Group 1 consisted of 20 isolates with confirmed OprD porin downregulation, while group 2 included 20 isolates without OprD porin downregulation.

Identification and antimicrobial susceptibility

All the isolates were identified using matrix-assisted laser desorption ionization - time of flight (MALDI - TOF) (Bruker, BD, Blomberg, Germany). Antimicrobial susceptibility was assessed using both the disk diffusion method and an automated system (Phoenix, BD, Blomberg, Germany). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [8].

MDR was defined as acquired insensitivity to at least one agent in at least three antimicrobial categories, while XDR was defined as insensitivity to at least one agent in all but two antimicrobial categories [9].

Determining the minimum inhibitory concentrations (MICs)

The powdered forms of the antimicrobials colistin (CT) sulfate (Chem-Impex, IL, USA), meropenem (MEM) trihydrate (Chem-Impex, IL, USA), ertapenem (ETP) sodium (Biosynth Carbosynth, Hampshire, UK), and fosfomycin (FF) (Kocak Farma, Istanbul, Türkiye) were used. The potency of each antimicrobial agent was calculated using formula 1 (below) in accordance with the manufacturer's recommendations. The volume of solvent required to reach the targeted concentrations was calculated using formula 2 (below).

$$Formula\ 1:\ Potency = Purity \times Active\ fraction \times (1 - Water\ content)$$

$$Formula\ 2: Volume\ (mL) = Weight\ (mg) \times Potency\ (\mu g/mg) \div Concentration\ (\mu g/mL)$$ [10].

The MIC values were determined using the broth dilution method for MEM, CT, and ETP; and the agar dilution method for FF. All results were interpreted in accordance with the EUCAST guidelines [8].

Checkerboard assays

The antimicrobial stock solutions were prepared at a concentration of 4096 µg/mL, and bacterial suspensions were prepared at approximately 5×10^5 CFU/mL. FF containing combinations were tested by adding 25 µg/mL glucose-6-phosphate to the medium. The antimicrobial concentration range was calculated as 0.031 × MIC - 4 × MIC. The checkerboard test was performed as previously described in the literature [11].

The fractional inhibitor concentration index (FICI) was used to evaluate the effect of the combinations, and calculated as follows [11];

$$FICI = \frac{MIC\ of\ drug\ A\ in\ combination}{MIC\ of\ drug\ A\ alone} \oplus \frac{MIC\ of\ drug\ B\ in\ combination}{MIC\ of\ drug\ B\ alone}$$

All wells with no growth in the combination plate were scanned, and the well with the lowest FICI was evaluated. The FICIs were interpreted as follows:

≤ 0.5	synergistic effect
> 0.5 to ≤ 1.0	additive effect
> 0.1 to ≤ 4.0	indifferent effect
> 4.0	antagonistic effects [11,12].

Ethics approval

This study was approved by the İzmir Katip Çelebi University ethics committee of clinical research (6 December 2018, Approval Number 139) and was

conducted by following the guidelines of the Declaration of Helsinki.

Results

Twelve isolates in group 1 were obtained from tracheal aspirates, 3 from blood samples, 2 from urine, 2 from wound samples, and 1 from a bronchoalveolar lavage specimen. In group 2, 11 isolates were obtained from tracheal aspirates, 3 from urine, 3 from wound samples, 2 from blood, and 1 from an endotracheal aspirate (Table 1).

The rates of synergistic interactions in group 1 isolates were 45% for MEM/CT, 5% for MEM/ETP, and 15% for MEM/FF combinations. No antagonistic effects were observed in the MEM/CT combination. However, antagonistic interactions were detected in 20% and 35% of isolates with MEM/ETP and MEM/FF combinations, respectively (Table 2). The synergistic interaction rates in group 2 isolates were 30%, 5%, and 10% for MEM/CT, MEM/ETP, and MEM/FF combinations, respectively. Similarly, no antagonistic effects were noted in the MEM/CT combination, whereas antagonistic interactions were observed in 20% and 50% of isolates treated with MEM/ETP and MEM/FF, respectively (Table 2).

When the relationship between interaction types and resistance phenotypes was assessed, the synergy rates were generally comparable between group 1 and group 2 isolates (Table 3).

When the changes in MIC values of antimicrobials in antimicrobial combinations were examined, significant decreases were observed in group 1 isolates.

Table 1. Characteristic features of the isolates.

	Isolate number	Sample	Antimicrobial susceptibility												Resistance phenotype
			CTZ	CPM	AZT	PIP	PPT	TIC	GEN	AMI	TOB	IMI	CIP	OFL	
Group 1	1	Blood	I	S	I	R	R	R	S	S	S	I	I	S	-
	2	TA	S	S	I	R	R	R	S	S	S	S	R	S	-
	3	TA	S	S	S	S	S	S	S	S	S	R	I	R	-
	4	TA	I	S	I	R	R	R	S	S	S	I	I	S	-
	5	TA	S	S	I	R	R	R	S	S	S	S	R	S	-
	6	TA	S	S	S	S	S	S	S	S	S	R	R	R	-
	7	TA	I	S	I	S	S	R	S	S	S	R	I	S	-
	8	TA	S	S	S	S	S	R	S	S	S	I	R	R	-
	9	Urine	I	S	I	S	S	R	S	S	S	R	I	S	-
	10	TA	S	S	S	S	S	S	S	S	S	R	I	R	-
	11	TA	S	S	S	S	S	R	S	S	S	I	R	R	-
	12	Blood	S	S	S	S	S	R	S	S	S	I	R	R	-
	13	TA	I	S	I	R	R	R	R	S	R	R	R	R	XDR
	14	TA	S	S	I	R	S	S	R	S	S	R	R	S	XDR
	15	Wound	S	S	S	R	R	R	R	R	R	R	R	R	XDR
	16	Urine	I	S	I	R	R	R	R	S	R	R	R	R	XDR
	17	Blood	S	S	I	R	S	S	R	S	S	R	R	S	MDR
	18	TA	R	I	R	R	R	R	S	S	S	R	R	S	XDR
	19	BAL	R	I	R	R	R	R	S	S	S	R	R	S	XDR
	20	Wound	S	S	S	R	R	R	R	R	R	R	R	R	XDR
Group 2	21	TA	I	S	I	R	R	R	S	S	S	I	I	S	-
	22	TA	S	S	I	R	R	R	S	S	S	S	R	S	-
	23	TA	I	S	I	S	S	R	S	S	S	R	I	S	-
	24	TA	S	S	S	S	S	R	S	S	S	I	R	R	-
	25	Blood	I	S	I	R	R	R	S	S	S	I	I	S	-
	26	ETA	S	S	S	S	S	R	S	S	S	I	R	R	-
	27	Urine	S	S	I	R	R	R	S	S	S	S	R	S	-
	28	TA	I	S	I	S	S	R	S	S	S	R	I	S	-
	29	TA	S	S	S	S	S	S	S	S	S	R	I	R	-
	30	Wound	S	S	S	S	S	S	S	S	S	R	I	R	-
	31	Urine	S	S	S	S	S	S	S	S	S	R	R	R	-
	32	Wound	S	S	S	S	S	R	S	S	S	I	R	R	-
	33	Wound	I	S	I	R	R	R	R	S	R	R	R	R	XDR
	34	TA	S	S	I	R	S	S	R	S	S	R	R	S	XDR
	35	TA	S	S	S	R	R	R	R	R	R	R	R	R	XDR
	36	Blood	R	I	R	R	R	R	S	S	S	R	R	S	XDR
	37	Urine	I	S	I	R	R	R	R	S	R	R	R	R	XDR
	38	TA	S	S	I	R	S	S	R	S	S	R	R	S	XDR
	39	TA	S	S	S	R	R	R	R	R	R	R	R	R	XDR
	40	TA	R	I	R	R	R	R	S	S	S	R	R	S	XDR

TA: tracheal aspirate; BAL: bronchoalveolar lavage; ETA: endotracheal aspirate; CTZ: ceftazidime; CPM: cefepim; AZT: aztreonam; PIP: piperacillin; PPT: piperacillin/tazobactam; TIC: tikarcillin; GEN: gentamicin; AMI: amikacin; TOB: tobramycin; IMI: imipenem; CIP: ciprofloxacin; OFL: ofloxacin; MDR: multi drug resistance; XDR: extensive drug resistance

Table 2. MIC's of isolates alone and in combination, and the combination effects of the tested antibiotics.

	Isolate number	MIC of single antimicrobial (µg/mL)				MIC of antibiotic combinations (µg/mL) + FICI + INT								
						MEM/CT			MEM/ETP			MEM/FF		
		MEM	ETP	FF	CT	MIC	FICI	INT	MIC	FICI	INT		FICI	INT
Group 1	1	32	32	64	1	4/0.25	0.37	SYN	64/32	3	IND	8/8	0.37	SYN
	2	32	32	16	0.125	8/0.25	2.25	IND	16/64	2.5	IND	128/16	5	ANT
	3	12	32	64	0.5	2/0.25	0.66	ADD	16/64	3.33	IND	64/64	6.33	ANT
	4	6	32	16	0.5	0.5/0.125	0.33	SYN	8/32	2.33	IND	8/8	1.83	IND
	5	4	32	1024	1	0.5/0.125	0.25	SYN	2/8	0.75	ADD	2/128	0.62	ADD
	6	2	32	64	0.25	1/0.5	2.5	IND	0.25/1	0.15	SYN	0.125/1	0.07	SYN
	7	4	32	32	1	0.5/0.25	0.37	SYN	2/64	2.5	IND	4/64	3	IND
	8	32	32	8	0.5	1/0.25	0.53	ADD	4/16	0.62	ADD	4/4	0.62	ADD
	9	3	32	32	0.5	0.25/0.25	0.58	ADD	8/64	4.66	ANT	16/64	7.33	ANT
	10	1	32	128	0.5	0.25/0.25	0.75	ADD	4/64	6	ANT	4/128	5	ANT
	11	32	32	128	0.5	0.25/0.125	0.25	SYN	64/32	3	IND	64/64	2.5	IND
	12	4	32	32	0.5	4/0.5	2	IND	16/64	6	ANT	16/64	6	ANT
	13	32	32	128	0.125	4/0.062	0.62	ADD	32/64	3	IND	64/512	6	ANT
	14	3	32	128	1	0.25/0.25	0.33	SYN	1/8	0.58	ADD	8/128	3.66	IND
	15	8	32	8	0.5	1/0.125	0.37	SYN	4/8	0.75	ADD	4/2	0.75	ADD
	16	32	32	8	0.25	16/0.5	2.5	IND	8/16	0.75	ADD	4/4	0.62	ADD
	17	3	32	64	0.125	4/0.25	3.33	IND	8/16	3.16	IND	8/32	3.16	IND
	18	16	32	128	0.125	4/0.25	2.25	IND	8/32	1.5	IND	32/64	2.5	IND
	19	0.5	16	128	1	0.125/0.0625	0.31	SYN	2/32	6	ANT	0.125/4	0.28	SYN
	20	32	32	128	0.5	2/0.15	0.31	SYN	16/64	2.5	IND	128/256	6	ANT
Group 2	21	32	32	16	0.125	16/0.031	0.74	ADD	8/16	0.75	ADD	8/8	0.75	ADD
	22	32	32	32	1	4/0.25	0.37	SYN	8/32	1.25	IND	128/32	5	ANT
	23	32	32	16	0.125	16/0.25	2.5	IND	32/16	1.5	IND	32/8	1.5	IND
	24	32	32	128	1	2/0.15	0.21	SYN	16/64	2.5	IND	32/64	1.5	IND
	25	16	32	4	0.125	4/0.25	2.25	IND	32/16	2.5	IND	16/2	1.5	IND
	26	32	32	64	0.125	16/0.25	2.5	IND	64/32	3	IND	128/128	6	ANT
	27	32	32	32	1	0.5/0.25	0.26	SYN	8/16	0.75	ADD	128/64	6	ANT
	28	6	32	4	1	8/2	3.33	IND	4/16	1.16	IND	8/2	1.83	IND
	29	12	32	16	0.125	2/0.25	2.16	IND	16/4	1.45	IND	32/32	4.66	ANT
	30	32	32	16	0.125	8/0.25	2.25	IND	32/64	3	IND	64/64	6	ANT
	31	2	32	32	0.25	0.5/0.5	2.25	IND	8/64	6	ANT	0.5/64	2.25	IND
	32	1.5	32	64	0.125	2/0.25	3.33	IND	8/64	7.33	ANT	8/64	6.33	ANT
	33	32	32	4	1	0.5/0.015	0.3	SYN	8/64	2.25	IND	64/16	6	ANT
	34	8	32	128	0.25	4/0.5	2.5	IND	4/64	2.5	IND	32/256	6	ANT
	35	8	32	256	1	4/0.25	0.75	ADD	32/64	6	ANT	8/32	1.12	IND
	36	32	32	64	0.125	16/0.25	2.5	IND	16/32	1.5	IND	128/64	5	ANT
	37	32	4	32	0.125	8/0.25	2.25	IND	2/0.25	0.12	SYN	1/0.25	0.03	SYN
	38	32	32	64	1	1/0.125	0.15	SYN	16/32	1.5	IND	128/128	6	ANT
	39	32	32	128	1	0.25/0.015	0.02	SYN	32/16	1.5	IND	2/2	0.78	SYN
	40	4	32	64	0.125	1/0.25	2.25	IND	4/64	3	IND	8/32	2.5	IND

MIC: minimal inhibitory concentration; MEM: meropenem; ETP: ertapenem; FF: fosfomycin; CT: colistin; FICI: fractional inhibitory concentration index; INT: interpretation; SYN: synergy; IND: indifference; ADD: additivity; ANT: antagonist.

Table 3. Distribution of interactions in antimicrobial combinations according to resistance phenotypes and the presence of OprD downregulation.

		MEM/CT				MEM/ETP				MEM/FF			
		SYN	ADD	IND	ANT	SYN	ADD	IND	ANT	SYN	ADD	IND	ANT
Group 1 n: 20	MDR/XDR (+) n: 8	4	1	3	-	-	3	4	1	1	2	3	2
	MDR/XDR (-) n: 12	5	4	3	-	1	2	6	3	2	2	3	5
	Total	9	5	6	-	1	5	10	4	3	4	6	7
Group 2 n: 20	MDR/XDR (+) n: 8	3	1	4	-	1	-	6	1	2	-	2	4
	MDR/XDR (-) n: 12	3	1	8	-	-	2	8	2	-	1	5	6
	Total	6	2	12	-	1	2	14	3	2	1	7	10

MDR: multi drug resistance; XDR: extensive drug resistance; MEM: meropenem; ETP: ertapenem; FF: fosfomycin; CT: colistin; SYN: synergy: IND: indifference; ADD: additivity; ANT: antagonist.

In the MEM/CT combination, MEM MIC values decreased in 90% of isolates, while CT MIC values decreased in 70% of isolates. In the case of the MEM/ETP combination, MEM MIC values decreased in 50% of isolates and ETP MIC values decreased in 35% of the isolates. In the case of the MEM/FF combination, reductions in MEM and FF MIC values were observed in 35% and 55% of isolates, respectively (Table 2).

Similarly, in the case of Group 2 isolates, the MEM/CT combination resulted in a decrease of MEM MIC values in 90% of isolates and CT MIC values in 40% of isolates. In the MEM/ETP combination, MEM and ETP MIC values decreased in 45% and 40% of isolates, respectively. In the MEM/FF combination, reductions in MEM and FF MIC values were detected in 20% and 45% of the isolates, respectively (Table 2).

Discussion

Only one study in previously published literature has examined the relationship between OprD porin downregulation and in vitro synergistic interactions of antimicrobial combinations. In this study, the in vitro interactions of the combination of imipenem/relebactam plus aztreonam in 10 OprD porin downregulated *P. aeruginosa* isolates were investigated using the checkerboard method. Synergistic interactions were reported in 5 isolates, 3 of which were metallo-beta-lactamase positive. Although isolates with OprD porin downregulation harboring inducible *Pseudomonas*-derived cephalosporinases did not show synergy, MICs of aztreonam were reported to be significantly reduced by the combination [6]. To our knowledge, this is the first study to examine the relationship between OprD porin downregulation and the antimicrobial combinations tested. A previous study examined all major resistance mechanisms responsible for carbapenem resistance, and in this study, the study groups were created from isolates analyzed in the previous study. A control group was formed from isolates without any of the resistance mechanisms and the study group was formed from isolates containing only OprD porin downregulation. In this way, the relationship between antimicrobial combinations and OprD porin downregulation was examined in a more rational way. For all these reasons, this study is the first of its kind in the literature.

A significant finding of this study was the higher rate of synergistic interaction observed with the MEM/CT combination in group 1 compared to group 2. This enhanced synergy, primarily reflected by a more pronounced decrease in CT MIC values, can be mechanistically explained by colistin's ability to disrupt the outer membrane through electrostatic interactions with lipopolysaccharides [13]. While OprD porin downregulation impairs carbapenem entry, membrane disruption by CT likely facilitates the intracellular penetration of membrane-impermeable agents such as MEM [14]. These findings align with prior studies suggesting that colistin can restore carbapenem activity by compromising outer membrane integrity [15,16]. Interestingly, despite the decrease in MEM MIC values being comparable between groups, the differential reduction in CT MIC values may be attributed to its larger molecular size (molecular weight of 1155.4 g/mol) relative to MEM (383.5 g/mol), making the entry of CT more dependent on membrane disruption [17,18]. The selected isolates were resistant to most antimicrobials tested except CT, suggesting that these drugs may be a salvage therapy to eliminate bacterial activity. However, concerns about poor pharmacokinetics and nephrotoxicity have limited the use of such treatments. Until effective antibiotics are developed, combination therapies may be an acceptable strategy because they can mitigate the side effects of CT with the same lethal effect at lower concentrations and restore carbapenem efficacy against these isolates.

In contrast, no substantial differences in synergy rates between groups were noted for MEM/ETP and MEM/FF combinations. Both MEM and ETP exert their antibacterial effects by inhibiting cell wall synthesis but do not significantly alter membrane permeability, potentially explaining their limited synergistic effects in the context of OprD porin downregulation. Similarly, the modest reduction in MIC values for these combinations across groups raises the possibility of alternative resistance mechanisms in Group 2 isolates.

Regarding FF, although OprD has been implicated in FF uptake resistance, the primary entry routes for this antibiotic involve OprO, and, to a lesser extent, OprP [19]. Despite previous reports indicating that OprD loss may increase FF resistance, synergy rates for MEM/FF were similar between groups in this study. It is plausible that compensatory overexpression of alternative porins, such as OprO, may have mitigated the impact of OprD loss on FF entry, partially explaining the observed findings.

CT is used to treat MDR *P. aeruginosa*, usually in combination with MEM [20]. The use of aminoglycosides is also a valid alternative in combination therapy, but the high risk of renal toxicity generally limits the combination of an aminoglycoside with colistin in clinical practice [21]. A meta-analysis

published in 2021 reported that the combination of a carbapenem and an aminoglycoside showed advantages in terms of bactericidal activity and regrowth rates compared to monotherapies. However, CT used as monotherapy has been reported to have similar results in terms of bactericidal activity compared to other CT-based combinations [22]. However, in this study, the MEM/CT combination was the antibiotic combination with the highest synergistic effect rates in both groups. This result needs to be supported by time-kill and pharmacokinetic/pharmacodynamic studies.

This study has several limitations. First, the presence of oprD mutation and other resistance pathways such as overexpression of other efflux pumps or AmpC β-lactamase hyperproduction were not evaluated. However, porin downregulation, overexpression of MexAB-OprM, MexCD-OprJ, and MexXY-OprN efflux pumps and carbapenemase production are the main mechanisms that are effective in carbapenem resistance; and the presence of these resistance mechanisms was investigated in all isolates [23]. In addition, mutations in the oprD gene are mostly effective in carbapenem resistance when they result in oprD porin downregulation [24]. The presence of oprD porin downregulation in the isolates is known. Secondly, although in vitro synergy studies offer important preliminary insights, clinical validation through in vivo models is essential, as laboratory-observed synergistic effects may not always translate into therapeutic success.

Conclusions

The findings of this study suggest that colistin, when combined with MEM, may overcome certain resistance mechanisms mediated by OprD porin downregulation. Overall, the study underscores the critical role of OprD porin downregulation in shaping the synergistic interactions of antimicrobial combinations in *P. aeruginosa*. While significant synergy was observed between MEM and CT in OprD-deficient isolates, the limited synergistic effects observed with other combinations, such as MEM/ETP and MEM/FF, suggest that the impact of OprD porin downregulation on antimicrobial resistance is multifactorial and complex. Further investigations focusing on pharmacokinetic factors are warranted to better elucidate the clinical relevance of these findings.

Acknowledgements

This study was conducted at the Department of Medical Microbiology, Faculty of Medicine, Izmir Katip Çelebi University, İzmir, Türkiye. This article was presented as an oral presentation at the XLI Turkish Microbiology Congress (13–17 November 2024, Antalya, Türkiye).

Funding

This work was supported by the İzmir Katip Çelebi University Scientific Research Projects Coordinatorship (Project No: 2020-GAP-TIPF-0009).

Corresponding author

Tuba Müderris, MD.
Basın Sitesi, Atatürk Eğitim ve Araştırma Hastanesi,
35360 Karabağlar, İzmir/Türkiye.
Tel: +905055025143
Email: tubamuderris@yahoo.com

Conflict of interest

No conflict of interest is declared.

References

1. Chegini Z, Khoshbayan A, Taati Moghadam M, Farahani I, Jazireian P, Shariati A (2020) Bacteriophage therapy against *Pseudomonas aeruginosa* biofilms: a review. Ann Clin Microbiol Antimicrob 1 Suppl 19: 45. doi: 10.1186/s12941-020-00389-5.
2. Mancuso G, Midiri A, Gerace E, Biondo C (2021) Bacterial antibiotic resistance: the most critical pathogens. Pathogens 10 Suppl 10: 1310. doi: 10.3390/pathogens10101310.
3. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z (2019) Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv 1 Suppl 37: 177–192. doi: 10.1016/j.biotechadv.2018.11.013.
4. El-Sherbiny GM, Basha AM, Mabrouk MI (2022) Control of extensively drug-resistant *Pseudomonas aeruginosa* co-harboring metallo-β-lactamase enzymes with *oprD* gene downregulation. Indian J Med Microbiol 1 Suppl 40: 51–56. doi: 10.1016/j.ijmmb.2021.11.002.
5. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ (2022) Infectious Diseases Society of America 2022 guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR-*P. aeruginosa*). Clin Infect Dis 2 Suppl 75: 187–212. doi: 10.1093/cid/ciac268.
6. O'Donnell JN, Putra V, Belfiore GM, Maring BL, Young K, Lodise TP (2022) In vitro activity of imipenem/relebactam plus aztreonam against metallo-β-lactamase-producing, OprD-deficient *Pseudomonas aeruginosa* with varying levels of *Pseudomonas*-derived cephalosporinase production. Int J Antimicrob Agents 6 Suppl 59: 106595. doi: 10.1016/j.ijantimicag.2022.106595.
7. Muderris T, Durmaz R, Ozdem B, Dal T, Unaldı O, Aydogan S, Celikbilek N, Acıkgoz ZC (2018). Role of efflux pump and OprD porin expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. J Infect Dev Ctries 1 Suppl 12: 1–8. doi: 10.3855/jidc.9486.

8. European Committee on Antimicrobial Susceptibility Testing (2024) Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. Available: http:// www. eucast. org. Accessed: 17 April 2024.
9. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 3 Suppl 18: 268–281. doi: 10.1111/j.1469-0691.2011.03570.x.
10. Clinical and Laboratory Standards Institute, CLSI (2012) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition. CLSI document M07-A9. USA.
11. Lertsrisatit Y, Santimaleeworagun W, Thunyaharn S, Traipattanakul J (2017) In vitro activity of colistin mono- and combination therapy against colistin-resistant *Acinetobacter baumannii*, mechanism of resistance, and clinical outcomes of patients infected with colistin-resistant *A. baumannii* at a Thai university hospital. Infect Drug Resist 10: 437–443. doi: 10.2147/IDR.S148185.
12. Pankey G, Ashcraft D, Kahn H, Ismail A (2014) Time-kill assay and etest evaluation for synergy with polymyxin B and fluconazole against *Candida glabrata*. Antimicrob Agents Chemother 10 Suppl 58: 5795–5800. doi: 10.1128/AAC.03035-14.
13. El-Sayed Ahmed MAE, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB (2020) Colistin and its role in the era of antibiotic resistance: an extended review (2000–2019). Emerg Microbes Infect 1 Suppl 9: 868–885. doi: 10.1080/22221751.2020.1754133.
14. Maifiah MH, Creek DJ, Nation RL, Forrest A, Tsuji BT, Velkov T, Li J (2017) Untargeted metabolomics analysis reveals key pathways responsible for the synergistic killing of colistin and doripenem combination against *Acinetobacter baumannii*. Sci Rep 7: 45527. doi: 10.1038/srep45527.
15. Montero MM, Domene Ochoa S, López-Causapé C, VanScoy B, Luque S, Sorlí L, Campillo N, Padilla E, Prim N, Segura C, Pomar V, Rivera A, Grau S, Ambrose PG, Oliver A, Horcajada JP (2019) Colistin plus meropenem combination is synergistic in vitro against extensively drug-resistant *Pseudomonas aeruginosa*, including high-risk clones. J Glob Antimicrob Resist 18: 37–44. doi: 10.1016/j.jgar.2019.04.012.
16. Mohamed AF, Kristoffersson AN, Karvanen M, Nielsen EI, Cars O, Friberg LE (2016) Dynamic interaction of colistin and meropenem on a WT and a resistant strain of *Pseudomonas aeruginosa* as quantified in a PK/PD model. J Antimicrob Chemother 5 Suppl 71: 1279–1290. doi: 10.1093/jac/dkv488.
17. National Center for Biotechnology Information (2026) PubChem compound summary for CID 5311054, colistin. https://pubchem.ncbi.nlm.nih.gov/compound/Colistin. Accessed: 13 February 2025.
18. National Center for Biotechnology Information (2026) PubChem compound summary for CID 441130, meropenem. https://pubchem.ncbi.nlm.nih.gov/compound/Meropenem. Accessed: 13 February 2025.
19. Golla VK, Piselli C, Kleinekathöfer U, Benz R (2022) Permeation of fosfomycin through the phosphate-specific channels OprP and OprO of *Pseudomonas aeruginosa*. J Phys Chem B 7 Suppl 126: 1388–1403. doi: 10.1021/acs.jpcb.1c08696.
20. Linden PK, Kusne S, Coley K, Fontes P, Kramer DJ, Paterson D (2003) Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant *Pseudomonas aeruginosa*. Clin Infect Dis 37 Suppl 11: e154–160. doi: 10.1086/379611.
21. Falagas ME, Rafailidis PI, Kasiakou SK, Hatzopoulou P, Michalopoulos A (2006) Effectiveness and nephrotoxicity of colistin monotherapy vs. colistin-meropenem combination therapy for multidrug-resistant Gram-negative bacterial infections. Clin Microbiol Infect 12 Suppl 12: 1227–1230. doi: 10.1111/j.1469-0691.2006.01559.x.
22. Scudeller L, Righi E, Chiamenti M, Bragantini D, Menchinelli G, Cattaneo P, Giske CG, Lodise T, Sanguinetti M, Piddock LJV, Franceschi F, Ellis S, Carrara E, Savoldi A, Tacconelli E (2021) Systematic review and meta-analysis of in vitro efficacy of antibiotic combination therapy against carbapenem-resistant Gram-negative bacilli. Int J Antimicrob Agents 57 Suppl 5: 106344. doi: 10.1016/j.ijantimicag.2021.106344.
23. De Rosa A, Mutters NT, Mastroianni CM, Kaiser SJ, Günther F (2019) Distribution of carbapenem resistance mechanisms in clinical isolates of XDR *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis 8 Suppl 38: 1547–1552. doi: 10.1007/s10096-019-03585-0.
24. Fang ZL, Zhang LY, Huang YM, Qing Y, Cao KY, Tian GB, Huang X (2014) *OprD* mutations and inactivation in imipenem-resistant *Pseudomonas aeruginosa* isolates from China. Infect Genet Evol 21: 124–128. doi: 10.1016/j.meegid.2013.10.027.