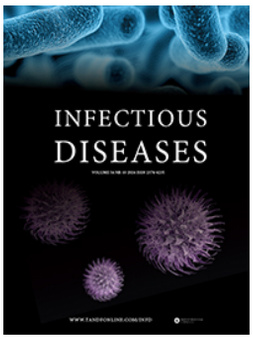

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Synergistic efficacy of ceftazidime/avibactam and aztreonam against carbapenemase-producing *Pseudomonas aeruginosa*: insights from the hollow-fiber infection model

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RESEARCH ARTICLE

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Synergistic efficacy of ceftazidime/avibactam and aztreonam against carbapenemase-producing *Pseudomonas aeruginosa*: insights from the hollow-fiber infection model

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ABSTRACT

Background: Combination therapy is an attractive therapeutic option for extensively drug-resistant (XDR) *Pseudomonas aeruginosa* infections. Existing data support the combination of aztreonam and ceftazidime/avibactam (CZA) against class serine-β-lactamase (SBL)- and metallo-β-lactamase (MBL) - producing *Enterobacterales*. However, data about that combination against SBL- and MBL-producing *P. aeruginosa* are scarce. The objective of the study was to assess the *in vitro* activity of CZA and aztreonam alone and in combination against SBL- and MBL-producing XDR *P. aeruginosa* isolates

Methods: The combination was analyzed by means of the hollow-fiber infection model in three selected carbapenemase-producing *P. aeruginosa* isolates that were representative of the three most common XDR*P. aeruginosa* high-risk clones (ST175, ST111, ST235) responsible for global nosocomial infection outbreaks.

Results: The three isolates were nonsusceptible to CZA and nonsusceptible to aztreonam. In the dynamic hollow-fiber infection model, the combination of CZA plus aztreonam exerts a bactericidal effect on the isolates, regardless of their resistance mechanism and demonstrates synergistic interactions against three isolates, achieving a bacterial reduction of 5.07 $\log_{10}$ CFU/ml, 5.2 $\log_{10}$ CFU/ml and 4 $\log_{10}$ CFU/ml, respectively.

Conclusion: The combination of CZA and aztreonam significantly enhanced the *in vitro* efficacy against XDR *P. aeruginosa* isolates compared to each monotherapy. This improvement suggests that the combination could serve as a feasible treatment alternative for infections caused by carbapenemase-producing XDR *P. aeruginosa*, especially in scenarios where no other treatment options are available.

KEYWORDS
Pseudomonas aeruginosa
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Introduction

The escalating challenge of antimicrobial resistance in Gram-negative bacteria has critically constrained treatment options. Among this group, *Pseudomonas aeruginosa* stands out for its remarkable ability to develop resistance [1–3]. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* have become leading causes of nosocomial infections and are independently linked to in-hospital mortality [4]. Certain high-risk clones of XDR *P. aeruginosa,* notably ST111, ST175 and ST235, have spread across hospital settings worldwide, presenting significant treatment challenges [1,5–8]. *P. aeruginosa* acquired resistance mechanisms are varied, including chromosomally encoded AmpC β-lactamases, changes in outer membrane porins, numerous efflux pumps, and novel PBP3 insertions. Additionally, the growing occurrence of horizontally transmitted class A carbapenemases (serine-β-lactamases, SBLs) and metallo-β-lactamases (MBLs) in *P. aeruginosa*is a grave concern [8]. Given the rapid spread of these carbapenemases and their link to increased mortality [9], finding effective treatments against this pathogen is imperative. In this context, combining ceftazidime-avibactam (CZA) with aztreonam emerges as a promising strategy for the treatment of these infections [10].

Amid the rising prevalence of MDR/XDR *P. aeruginosa* showing resistance to all first-line agents, with limited available treatments, CZA presents itself as a potential agent to treat these infections. CZA is a β-lactam/β-lactamase inhibitor combination (a third-generation cephalosporin that is combined with a new potent broad-spectrum β-lactamase inhibitor) with activity against type A and type C β-lactamases but does not possess activity against MBL-producing organisms [11].

Unfortunately, the emergence of resistance to CZA is very troublesome [12]. Given the risk for the emergence of CZA-resistant mutants, there is clearly a need to investigate new therapeutic options [7], which would allow to widen the spectrum of coverage, and to suppress resistance emergence [13]. It is also well known that monotherapy carries a significant risk of selecting resistant strains in *P. aeruginosa* [13].

Aztreonam, a β-lactam antibiotic not hydrolyzed by MBLs, remains active against many Gram-negative pathogens due to its potent affinity for PBP2. However, aztreonam susceptibility to hydrolysis by most serine β-lactamases renders it ineffective as monotherapy [14].

Existing evidence supports the use of CZA and aztreonam combination against class A carbapenemases- and MBL-producing Enterobacterales [10]. However, data about that combination against SBL- and MBL-producing XDR*P. aeruginosa* are scarce.

The aim of this study was to assess the *in vitro* activity of CZA and aztreonam alone and in combination against SBL- and MBL-producing XDR *P. aeruginosa* isolates by hollow-fiber infection model.

Material and methods

Bacterial isolates and resistance mechanisms

Three clinical carbapenemase-producing XDR *P. aeruginosa* (1 GES and 2 VIM) isolates were evaluated. These isolates were previously collected as a part of a multicenter trial [8,15]. These isolates had been previously characterized at a molecular level using pulsed-field gel electrophoresis, multi-locus sequence typing, and whole genome sequencing [8,15].

Antibiotics

The antipseudomonal antibiotics used in the experiments were aztreonam, ceftazidime (Sigma-Aldrich) and avibactam (Pfizer). Antibiotics solutions were prepared according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [16].

Antibiotics were administered in the hollow-fiber to simulate free plasma concentrations in critically ill patient under treatment for several infections. The simulated CZA dosing regimen was 2/0.5 g every 8 h by intravenous infusion over 2 h to achieve a free maximum concentration of 74 mg/L (avibactam fixed at 4 mg/L), with a simulated elimination half-life of 2 h. Regarding the area-under-the-curve (AUC) serum levels, an AUC24 of 800 μg h/ml was simulated for ceftazidime [17]; and an AUC24 of 147 μg h/ml for avibactam [17].

The simulated aztreonam dosing regimen was 2 g every 6 h by intravenous infusion over 1 h to achieve a free maximum concentration of 110 mg/L, with an area under the concentration-time curve for 24 h (AUC24) of 1,050 μg · h/ml [18]; and a simulated elimination half-life of 2 h [10,19,20].

Antibiotic susceptibility testing *(AST)*

Susceptibility testing studies were performed using EUCASTfor broth microdilution using cation-adjusted Mueller–Hinton broth (CAMHB) for CZA and aztreonam [16]. Ceftazidime susceptibility tests were performed alone and in combination with a fixed avibactam

concentration (4 mg/L). AST results were categorized according to EUCAST breakpoints [16].

Pharmacodynamics endpoints

Bactericidal effect was defined as a reduction of $\geq$3-log$_{10}$ CFU/ml in colony count at the end of the experiment from that in the initial inoculum of the time-kill curve. Synergy was defined as $a \geq$ 2-log$_{10}$ CFU/ml reduction in the colony count at the end of the experiment when comparing the combination with the most active single agent. Additive effect was defined as a 1- to 2-log$_{10}$ CFU/ml colony count reduction in the combination compared with the most active single agent at the end of the experiment [21,22].

Hollow-fiber infection model

Duplicate 7-day hollow-fiber infection model assays were conducted to compare the antibiotic combinations of CZA with aztreonam against the selected XDR *P. aeruginosa* isolates, as described previously [23]. Polyethersulfone hemofilters were used as hollow-fiber cartridges with a volume of 50 mL (Aquamax HF03, Nikkiso, Belgium) [24]. The assays were conducted at 37 °C. Each antibiotic regimen was pumped directly into the corresponding reservoir with a separate infusion pump to achieve the target concentration. Treatment regimens were compared with a no-treatment control. Fresh drug-free culture medium (CAMHB) was continuously infused into the central reservoir to dilute and simulate drug elimination in humans. An equal volume of drug-containing medium was removed from the central reservoir to maintain an isovolumetric system. Bacterial suspensions were inoculated into the extracapillary compartment of the cartridge, where they were exposed to fluctuating drug concentrations. Bacterial densities (log$_{10}$ CFU/mL) in the cartridges were measured at 0, 8, 24, 48, 72, 96, 144 and 168 h; samples were previously washed in saline solution to minimize drug carryover. Serial decimal dilutions were cultured onto drug-free Trypticase Soy Agar (BBLTM TSA II, Becton Dickinson) plates to determine the total bacterial population (log$_{10}$ CFU/mL). The lower limit of detection (LLOD) was 1.3 log$_{10}$ CFU/ml.

Drug concentrations

Antibiotic samples were collected at different points in time over the first 48 h (0, 3, 5, 7, 9, 23, 25 27, 29 and 47 h) and once a day until the end of the study. Samples were stored at −80 °C until their analysis. Antibiotic samples were taken to report free peak and trough values. The pharmacokinetic concentrations were analyzed by high-performance liquid chromatography [25].

Statistical analysis

Differences in bacterial concentrations (at logarithmic phase) among antibiotic regimens for each included isolate were assessed using analysis of variance (ANOVA). To ensure the appropriateness of ANOVA application, data normality was verified *via* the Shapiro-Wilk test, and variance homogeneity was evaluated using Levene's test. Upon demonstration of statistically significant differences between antibiotic regimens within an isolate by ANOVA, a *post hoc* analysis employing Tukey's test was conducted to delineate these differences. The means of differences (x Δ), their respective 95% confidence intervals (CI) and the adjusted p-values for multiple comparisons, were reported. A p-value $<$0.05 was considered statistically significant. The analysis was performed using the R programming language in the RStudio software version 2023.12.1.

Results

Antimicrobial susceptibility testing

The antibiotic susceptibility profiles and previously characterized antibiotic resistance of the three XDR *P. aeruginosa* isolates are shown in Table 1. The three carbapenemase-harboring isolates were nonsusceptible to CZA (MIC $>$8/4 mg/L) and nonsusceptible to aztreonam (MIC $>$16 mg/L). All were categorized as XDR isolates, resistant to most available options, with susceptibility only to colistin in two of them.

Hollow-fiber infection model

The total CFU/ml reduction observed during the 7-day *in vitro* hollow-fiber infection model for the three selected isolates is shown in Figure 1. The dynamic hollow-fiber model showed synergistic interactions for ST175, ST111 and ST235 isolates. Compared with the most active single drug, the corresponding bacterial reduction caused by this antibiotic combination was 5.07 log$_{10}$ CFU/ml, 5.2 log$_{10}$ CFU/ml and 4 log$_{10}$ CFU/ml, for ST175, ST111 and ST235 isolates, respectively.

A starting inoculum of 6.6 ± 0.21 log$_{10}$ CFU/ml was used in the GES-5-carrying ST175 isolate. CZA

Table 1. Characteristics of the three *P. aeruginosa* isolates. Antibiotic resistance mechanisms and minimal inhibitory concentrations (MIC) (mg/L) of aztreonam and ceftazidime/avibactam antibiotics are included.

Sequence Type (ST)	Acquired β-lactamases	AmpC hyper-production	OprD deficiency	AZT MIC	CZA MIC	AMI MIC	MER MIC	CST MIC	C/T MIC
111	VIM-2	YES	YES	>128	>32	32	>32	4	>64/4
175	GES-5	NO	YES	>16	32	16	>32	2	16/4
235	VIM-47	NO	NO	32	32	64	>32	2	>64/4

*Abbreviations: MIC, minimuminhibitoryconcentration (mg/L); AZT, aztreonam; CZA, ceftazidime/avibactam; MER, meropenem; CST, colistin; C/T, ceftolozane/tazobactam.

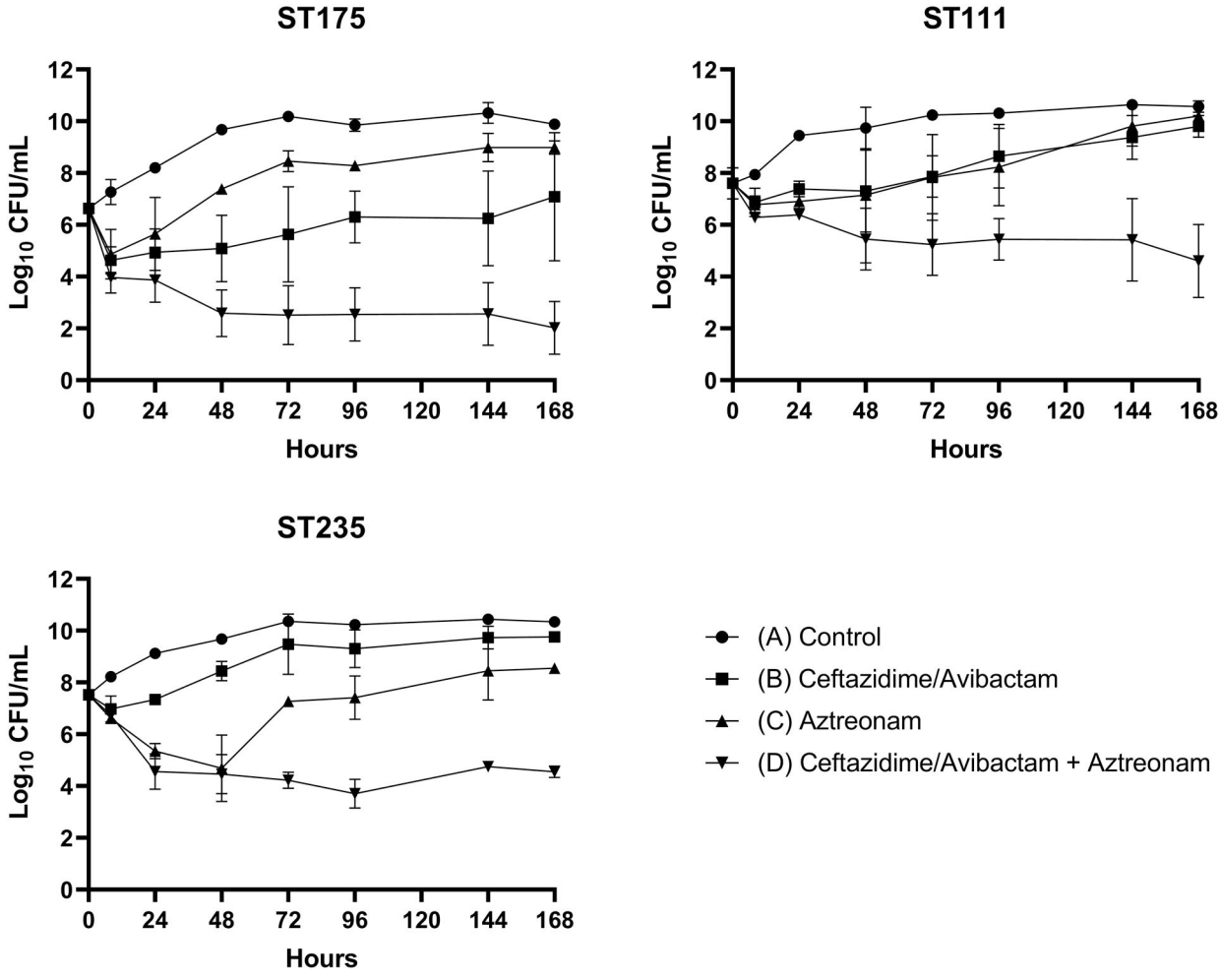

Figure 1. *In vitro* hollow-fiber infection model against the three selected *P. aeruginosa* isolates. The values correspond to the mean numbers of CFU/mL throughout each experimental condition.

monotherapy caused an initial decrease of 2 $\log_{10}$ CFU/ml at 24 h, followed by regrowth; the final bacterial load was similar to the starting inoculum. Aztreonam monotherapy caused a 2 $\log_{10}$ CFU/ml decrease at 8 h, followed by regrowth; the final bacterial load was similar to the control without antibiotic. The combination of CZA with aztreonam caused an overall bacterial reduction of 4.61 $\log_{10}$ CFU/ml at day 7 compared to the beginning of the experiment.

The starting inoculum for the VIM-carrying ST111 isolate was 7.6 ± 0.6 $\log_{10}$ CFU/ml. Both CZA and aztreonam in monotherapy causeda final bacterial count that was similar to the control without antibiotic. Compared to the initial inoculum, the combination of CZA plus aztreonam caused a final bacterial reduction of 3 $\log_{10}$ CFU/ml.

The starting inoculum for the VIM-carrying ST235 isolate was 7.5 ± 0.19 $\log_{10}$ CFU/ml. After an initial lag, CZA monotherapy caused a similar behavior in this isolate as the control without antibiotic. Aztreonam alone caused a decrease of 3 $\log_{10}$ CFU/ml at 48h, followed by regrowth that reached 8.5 $\log_{10}$ CFU/ml at day 7. Compared to the starting inoculum, the combination of CZA plus aztreonam caused a decrease of 3 $\log_{10}$ CFU/ml

Table 2. Comparative analysis of bacterial concentrations among antibiotic regimens of isolates included in the hollow-fiber infection model.

	ST111 (10-009)			ST175 (07-016)			ST235 (04-042)		
	x Δ[a]	CI[a] 95%	p[b]	x Δ[a]	CI[a] 95%	p[b]	x Δ[a]	CI[a] 95%	p[b]
Aztreonam vs. Control	−1.5	−3.09 0.01	0.07	−1.49	−3.09 0.1	0.072	−2.51	−4.21 −0.81	0.002*
CZA vs. Control	−1.45	−3.04 0.14	0.082	−1.45	−3.05 0.14	0.083	−0.92	−2.62 0.78	0.464
CZA + Aztreonam vs. Control	−3.72	−5.31 −2.13	<0.001*	−3.72	−5.31 −2.12	<0.001*	−4.43	−6.13 −2.73	<0.001*
CZA vs. Aztreonam	0.05	−1.54 1.64	0.1	0.04	−1.55 1.63	0.1	1.59	−0.11 3.29	0.074
CZA + Aztreonam vs. Aztreonam	−2.21	−3.81 −0.63	0.004*	−2.22	−3.81 −0.63	0.004*	−1.92	−3.62 −0.22	0.022*
CZA + Aztreonam vs. CZA	−2.26	−3.85 −0.67	0.003*	−2.26	−3.86 −0.67	0.003*	−3.51	−5.21 −1.81	<0.001*

[a]Mean of differences in bacterial concentration (in logarithmic phase) between antibiotic regimens and 95% Coefficient Interval (CI).
[b]Tukey-adjusted p-value: * demonstrated significant results.
Abbreviations: CZA, ceftazidime/avibactam; ST, sequence type.

in the bacterial load at the end of 7 days of the experiment.

The statistical analysis of the three bacterial isolates demonstrated significant results through analysis of variance (ANOVA). A *post hoc* Tukey analysis was conducted to delineate the differences among treatments (Table 2). The combined therapy of CZA and aztreonam significantly diverged from the control treatment in the three isolates. The superiority of the binary combination was corroborated when compared with each of its individual components; significant differences favoring the combined therapy were noted. Specifically, aztreonam monotherapy exhibited a significant reduction in the bacterial concentration of ST235 isolate compared to the control but the magnitude of this difference was smaller when contrasted with the reduction observed between the combined therapy and the control. These findings underscore the potential synergy between CZA and aztreonam and highlight the significance of the combined therapy in the effective management of infections caused by the examined bacterial isolates.

Drug concentrations

The relation between observed and predicted CZA and aztreonam concentrations over seven days is shown in Table 3. Overall, the observed versus predicted drug exposures achieved in this model were considered satisfactory for all regimens based on r^2 values of 0.953 and 0.957 ceftazidime and aztreonam, respectively.

Table 3. Observed concentrations and calculated pharmacokinetic parameters for the antibiotic regimens in the hollow-fiber model.

	Free peak concn (mg/L)		Free trough concn (mg/L)	
	Target	Observed [mean (SD)]	Target	Observed [mean (SD)]
CZA	73	64 (2.19)	9.2	14 (0.84)
Aztreonam	110	97 (7)	20	28 (1.8)

*Data are presented as the means and standard deviations. Abbreviations: CZA, ceftazidime/avibactam; concn, concentration; SD, standard deviation.

Discussion

The therapeutic options available to treat infections caused by XDR *P. aeruginosa* must be optimized and individualized to achieve the best clinical outcome. The high mutation rates, along with the acquisition of carbapenemases, may render this bacterium into an almost untreatable nosocomial menace. Widely used against SBL- and MBL-carrying *Enterobacterales*, the combination of CZA and aztreonam against XDR *P. aeruginosa* might be seem limited due its broad range of intrinsic and acquired mechanisms of resistance, especially compromised by its efflux pump (MexAB-OprM) activity. The types and prevalence of SBLs and MBLs harbored by *P. aeruginosa* differ significantly from those in *Enterobacterales* [26,27]. Due to these disparities, translating the effectiveness of this combination against *Enterobacterales* to *P. aeruginosa* could be difficult. Nevertheless, the increasing prevalence of this type of XDR *P. aeruginosa* emphasizes the need to urgently identify effective treatment options against this pathogen. Currently, only a few *in vitro*studies have investigated CZA-containing combinations against *P. aeruginosa*, using either static methods or time-kill studies [10,20]. The aim of our study was to evaluate the combination CZA plus aztreonam against carbapenemase-producing *P. aeruginosa* isolates by means ofhollow-fiber infection model. To our knowledge this is the first study to evaluate this antibiotic combination against XDR*P. Aeruginosa* using this dynamic infection model.

The *in vitro* hollow-fiber infection model provides invaluable insights into antibiotic treatments by simulating *in vivo* interactions.This robust pharmacokinetic/pharmacodynamic (PK/PD) model allows the optimization of antibiotic treatments. This model has yet to be applied in assessing the CZA and aztreonam combination against *P. aeruginosa* [28]. This antibiotic combination achieved an overall reduction in the bacterial load of 4-5 $\log_{10}$ CFU/ml when compared with the most active single drug. However, the mechanisms of resistance and the antibiotic MICs are paramount parameters to be considered. As expected, the GES-carrying isolate had the largest overall reduction from the beginning of the experiment. Despite an initial decrease across all the three regimens, only CZA plus aztreonam managed to achieve bacterial reduction in the final bacterial load of this isolate (4.61 $\log_{10}$ CFU/ml). Final regrowth was observed in the monotherapies potentially due to emerging resistance during treatment. Despite their resistance to CZA and aztreonamalone, both VIM-carrying isolates also showed a decrease in final bacterial load with the combination of these two antibiotics compared to the initial inoculum, albeit not so relevant as in the SBL-carrying isolate. Overall, these hollow-fiber experiments support that CZA and aztreonam in combination may be an effective therapeutic approach against carbapenemase-producing *P. aeruginosa*, especially in the presence of GES-type enzymes.

Antibiotic combination therapies aim to achieve an effective treatment through the synergistic activity of the two antimicrobials and by preventing the potential emergence of resistance [20,29,30]. Aβ-lactam and an aminoglycoside combination is recommended for empirical treatment of *P. aeruginosa* infections [31–33], ensuring that at least one agent has activity against the infecting isolate to improve clinical outcome. Synergy with this antibiotic combination has been reported by other authors against several MDR Gram-negative bacilli [34–36]. While CZA exhibits potent activity against many XDR *P. aeruginosa* isolates, it lacks efficacy against MBL-producing organisms. Given the multiple resistance mechanisms and the higher mutant prevention concentration values in most isolates, clinical failures may eventually result from resistance development in CZA monotherapies, even at elevated doses [37]. The combination of CZA and aztreonam had demonstrated efficacy against Gram-negative bacilli carrying MBL and SBL, including *Enterobacterales* and *P. aeruginosa* isolates [10]. In contrast, other studies suggest that the combination of CZA and aztreonam was not active against *P. aeruginosa* isolates that showed MIC values >16 mg/L [38]. Overall, hyperexpression of efflux pump activity combined with other resistance mechanisms might compromise the activity of this combination. However, our study demonstrates that even with resistant aztreonam (MIC >16 mg/L), the combination of CZA and aztreonam can achieve synergy against the tested isolates. In the case of GES-producing isolate, aztreonam is less affected by GES-5 than ceftazidime, so aztreonam plus avibactam would be more active than ceftazidime plus avibactam. Additionally, the inhibition of AmpC by avibactam could enhances the activity of aztreonam.

The combination of CZA and aztreonam exerted a bactericidal effect and synergistic interactions on the three carbapenemase-carrying *P. aeruginosa* isolates over a 7-day model, regardless of their resistance mechanism. In view of our data, the combination of CZA and aztreonam should be carefully considered as a therapeutic opportunity to treat complicated *P. aeruginosa* infections where no alternative treatments are available. Ensuring effective antibiotic therapy in the initial hours is crucial, particularly in high inoculum infections, and could help monitor and prevent resistance selection during treatment.

One of the main strengths of this study is the evaluation of XDR *P. aeruginosa* isolates from the most prevalent high-risk clones (ST175, ST111 and ST235) responsible for global nosocomial infection outbreaks and with different mechanisms of resistance [39]. Additionally, employing the hollow-fiber infection model represents a novel approachto evaluate the potential of the combination of CZA and aztreonam against this pathogen. However, our study had also some limitations. Although the *in vitro* dynamic hollow-fiber model may simulate the PK/PD interactions *in vivo*, it does not account for factors such as toxicity, the contribution of the immune system, or the different PK/PD effects occurring at the specific site of infection. Although avibactam monitoring was not conducted, its pharmacokinetic characteristics were presumed to be like those of ceftazidime, including distribution volume, time-dependent activity, minimal plasma protein binding, and renal excretion.

The dynamic *in vitro* PK/PD model showed that combining CZA with aztreonam significantly enhanced efficacy against XDR *P. aeruginosa* isolates, outperforming each monotherapy and regardless of the carbapenemases type involved. Our results suggest that this antibiotic combination could serve as a feasible treatment option for infections caused by carbapenemases-producing XDR *P. aeruginosa*, especially in scenarios where no other treatment options are available.

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Informed consent statement

Not applicable.

Authorship

All authors have seen and approved the manuscript. We confirm that the manuscript has not been published and it is not under consideration for publication elsewhere. Conceptualization: MMM; Data curation: MMM, SDO; Formal analysis: MMM, SDO, MFAM; Funding acquisition: JPH; Investigation: MMM, SDO, NP, EF, MFAM, VVT; Methodology; MMM, SDO, NP, CLC; Project administration: MMM, JPH; Resources: MMM, JPH, EP; Software: MFAM; Supervision: JPH, AO; Validation: JPH, AH; Visualization: SDO; Roles/Writing - original draft: MMM, SDO; and Writing - review and editing: LS, SL.

Disclosure statement

MMM has received consulting fees and participated in educational activities from Pfizer, MSD, Shionogi, and Biomerieux. JPH has received consulting fees from Gilead, Tillots, Menarini and TFT Pharmaceuticals, and participated in educational activities from MSD, Pfizer and Angelini. A.O. has received fees as a speaker and/or research grants from MSD, Shionogi, Pfizer and Wockhardt. All other authors have no potential conflict of interest.

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