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Activity of aztreonam in combination with ceftazidime–avibactam against serine- and metallo-β-lactamase–producing *Pseudomonas aeruginosa*☆,☆☆,★

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ABSTRACT

Existing data support the combination of aztreonam and ceftazidime–avibactam against serine-β-lactamase (SBL)– and metallo-β-lactamase (MBL)–producing Enterobacterales, although there is a paucity of data against SBL- and MBL-producing *Pseudomonas aeruginosa*. In this study, 5 SBL- and MBL-producing *P. aeruginosa* (1 IMP, 4 VIM) were evaluated against aztreonam and ceftazidime–avibactam alone and in combination via broth microdilution and time-kill analyses. All 5 isolates were nonsusceptible to aztreonam, aztreonam–avibactam, and ceftazidime–avibactam. Combining aztreonam with ceftazidime–avibactam at subinhibitory concentrations produced synergy and restored bactericidal activity in 4/5 (80%) isolates tested. These results suggest that the combination of aztreonam and ceftazidime–avibactam may be a viable treatment option against SBL- and MBL-producing *P. aeruginosa*.

1. Introduction

The rapid global dissemination of Ambler class B metallo-β-lactamase (MBL) enzymes along with their increasing diversity across bacterial species is a cause for significant public health concern (Khan and Nordmann, 2012). Moreover, the dearth of safe and effective treatment options for these pathogens results in mortality rates >45% for patients with serious infections (Falcone et al., 2020). Although the combination of aztreonam and avibactam (via ceftazidime–avibactam) has demonstrated promise against serine-β-lactamase (SBL)– and MBL-producing Enterobacterales (Biagi et al., 2019; Marshall et al., 2017), the myriad of intrinsic and acquired resistance mechanisms among multidrug resistant *Pseudomonas aeruginosa* severely limits the ability of avibactam to restore the activity of aztreonam (Bush and Bradford, 2020). In addition to resistance mediated by chromosomally encoded AmpC β-lactamases, changes in outer membrane porins, numerous efflux pumps, and novel PBP3 insertions, the type and frequency of SBLs and MBLs harbored by *P. aeruginosa* are fundamentally different than those in Enterobacterales (Alm et al., 2015; Kazmierczak et al., 2016; Periasamy et al., 2020; Poirel et al., 2000; Watanabe et al., 1991). These differences therefore inhibit the ability to translate the activity of aztreonam–avibactam against SBL- and MBL-producing Enterobacterales to *P. aeruginosa*. This is evidenced by previous data demonstrating that the MIC_{90} of aztreonam–avibactam against MBL-producing Enterobacterales is typically approximately 1 mg/L, while against MBL-producing *P. aeruginosa*, it ranges from 32 to 64 mg/L (Biedenbach et al., 2015; Kazmierczak et al., 2016), leaving the polymyxins as the only agents with reliable in vitro activity against these organisms. Given the dramatic increases in the prevalence of MBLs in *P. aeruginosa* (Bush and Fisher, 2011) and their association with mortality compared to non–MBL-producing strains (Matos et al., 2018), there is a desperate need to identify reliable treatment options against this pathogen. Available in vitro synergy studies are restricted to gradient-strip–based methods (Emeraud et al., 2019; Wenzler et al., 2017), and in vivo studies have utilized isolates harboring only MBL enzymes with correspondingly low aztreonam–avibactam MICs (≤16 mg/L) (Crandon and Nicolau, 2013) despite that up to 95% of MBL-producing *P. aeruginosa* co-harbor at least 1 or more SBLs (Kazmierczak et al., 2016). As such, the true synergistic effect of aztreonam in combination with ceftazidime–avibactam against SBL- and MBL-producing *P. aeruginosa* remains unknown. The objective of this study was to evaluate the in vitro activity of aztreonam and ceftazidime–avibactam alone

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and in combination via time-kill analyses against SBL- and MBL-producing *P. aeruginosa* nonsusceptible to both aztreonam and ceftazidime–avibactam.

2. Materials and methods

2.1. Bacteria and susceptibility testing

Five clinical SBL- and MBL-producing (1 IMP and 4 VIM) *P. aeruginosa* strains acquired from the FDA-CDC Antimicrobial Resistance Isolate Bank were utilized for all experiments (Lutgring et al., 2018). Strains were maintained at −80 °C in cation-adjusted Mueller–Hinton broth (CAMHB) (Teknova, Hollister, CA) with 20% glycerol and were subcultured twice on 5% sheep blood tryptic soy agar plates prior to testing. Analytical-grade ceftazidime, avibactam, and aztreonam (Sigma-Aldrich, St. Louis, MO) were obtained commercially. Stock solutions of each agent were freshly prepared as single-use aliquots at the beginning of each week and kept frozen at −80 °C. Minimum inhibitory concentrations (MICs) were performed in triplicate via broth microdilution using the same 0.5 McFarland suspension according to Clinical and Laboratory Standards Institute (CLSI) guidelines with avibactam fixed at 4 mg/L for all experiments. Quality control was completed with *P. aeruginosa* ATCC 27853, and modal MIC values are reported. Susceptibility interpretations for aztreonam–avibactam were based on CLSI interpretive criteria for *P. aeruginosa* against aztreonam Clinical and Laboratory Standards Institute (CLSI), 2020. Complete genomes were downloaded from the NCBI nucleotide database where available, and β-lactam resistance genes were identified by BLAST searching the derived contigs against the ResFinder 3.1 (Zankari et al., 2012) and CARD-RGI (Jia et al., 2016) databases.

2.2. Time-kill experiments

Time-kill analyses were performed in triplicate on the same day as previously described (Biagi et al., 2019). Briefly, experiments proceeded stepwise as follows: First, aztreonam and ceftazidime–avibactam (avibactam fixed at 4 mg/L) were tested alone at ¼, ½, 1, 2, and 4× the MIC unless any of these concentrations exceeded the respective drug's fC_{max} value, in which case the fC_{max} was used. The fC_{max} values were chosen to simulate a 2-g dose of aztreonam 112 mg/L (Scully et al., 1983; Swabb et al., 1983) and ceftazidime 80 mg/L (Das et al., 2015; O'Callaghan et al., 1980). For organisms with off-scale MICs, the highest (256 mg/L) concentration observed was utilized to determine multiplicative values. Second, the highest concentrations of aztreonam and ceftazidime–avibactam alone from step 1 that showed no meaningful activity compared to the drug-free control strain (≤1 $\log_{10}$ CFU/mL decrease from the starting inoculum at 24 h) were combined. A growth control without any antibiotic was included with each experiment. Aliquots were removed from each sample at 0, 2, 4, 6, and 24 h and serially diluted in $\log_{10}$ dilutions in sterile saline. A 50-μL aliquot was subsequently plated onto Mueller–Hinton agar plates using an automated spiral plater (Don Whitley WASP Touch) and incubated at 35 °C for 24 h. Colony counts were performed the following day using a colony counter (ProtoCOL 3 Plus). The theoretical lower limit of quantification was 100 CFU/mL. Time-kill curves were generated by plotting the average $\log_{10}$ CFU/mL versus time to compare the 24-h killing effects of single agents alone and in combination. Bactericidal activity was defined as ≥3 $\log_{10}$ CFU/mL reduction at 24 h compared to the starting inoculum, and synergy was defined as a ≥2 $\log_{10}$ reduction in CFU/mL between the combination and the most active single agent alone. Antagonism was defined as ≥2 $\log_{10}$ increase in CFU/mL between the combination and the most active single agent alone.

Table 1
β-Lactamase profile and susceptibility of tested clinical SBL- and MBL-producing *Pseudomonas aeruginosa* isolates.

Isolate	β-Lactamase(s)	MIC (mg/L), interpretive category[a]		
		ATM	ATM-AVI[b]	CAZ-AVI
UIC1	IMP-14, OXA-10, OXA-488, VEB-9, PDC-2	>128, R	64, R	>128, R
UIC2	VIM-4, OXA-396, PDC-3	16, I	16, I	128, R
UIC3	VIM-2, OXA-488, PDC-2	64, R	64, R	64, R
UIC4	VIM-2, OXA-488, PDC-3	64, R	32, R	64, R
UIC5	VIM-2, PDC-8	16, I	32, R	128, R

SBL = serine-β-lactamase; MBL = metallo-β-lactamase; MIC = minimum inhibitory concentration; ATM = aztreonam; ATM-AVI = aztreonam–avibactam; CAZ-AVI = ceftazidime–avibactam; R = resistant; I = intermediate; IMP = imipenemase; OXA = oxacillinase; PDC = *Pseudomonas*-derived cephalosporinase; VEB = Vietnamese extended-spectrum β-lactamase; VIM = Verona integron-encoded metallo-β-lactamase.
[a] Avibactam was tested at a fixed concentration of 4 mg/L.
[b] Susceptibility interpreted according to CLSI interpretative criteria against aztreonam.

3. Results

The β-lactamase profile and phenotypic susceptibilities of the 5 *P. aeruginosa* isolates are displayed in Table 1. All isolates were nonsusceptible to aztreonam, aztreonam–avibactam, and ceftazidime–avibactam. The addition of avibactam to aztreonam did not reduce the aztreonam MIC or restore susceptibility against any of the 5 isolates. Isolate UIC1 carried the IMP-14 MBL along with 4 other SBLs, including 2 class D OXA enzymes, 1 class A VEB, and the chromosomal class C enzyme PDC and had the highest MIC (>128 mg/L) to both aztreonam and ceftazidime–avibactam of the 5 isolates tested. Isolates UIC2–4 each harbored a VIM MBL along with a class D OXA β-lactamase and class C cephalosporinase, while UIC5 harbored only the VIM-2 MBL and a class C cephalosporinase.

Results of time-kill experiments of aztreonam and ceftazidime–avibactam alone against each strain at the highest concentration tested are shown in Fig. 1. Aztreonam had no activity against UIC1 at fC_{max} but was bactericidal against UIC2 at ≥2× MIC. Against UIC3, aztreonam achieved a 2.78 $\log_{10}$ CFU/mL reduction at 24 h at fC_{max} and was bactericidal against UIC4 at fC_{max} and against UIC5 at 4× MIC. Ceftazidime–avibactam alone had no activity against any isolate at any concentration tested (Fig. 1).

Based on results from monotherapy time-kill experiments, aztreonam was added to all combination experiments at 1/2× MIC, except against UIC1 in which fC_{max} was used (Fig. 2). Ceftazidime–avibactam was tested at fC_{max} against all 5 isolates in combination experiments. The combination of aztreonam and ceftazidime–avibactam achieved synergy and was bactericidal against all 5 isolates tested except UIC2. The mean (±SD) $\log_{10}$ CFU/mL reduction at 24 h across the other 4 strains was 5.11 ± 1.33. Against UIC2, the combination achieved a 1 $\log_{10}$ CFU/mL decrease by 6 h followed by regrowth up to 7.3 $\log_{10}$ CFU/mL at 24 h (Fig. 2). No antagonism was observed in any experiment.

4. Discussion

To our knowledge, this is the first study to evaluate the time-killing profile of the combination of aztreonam and ceftazidime–avibactam against SBL- and MBL-producing *P. aeruginosa*. Despite all 5 isolates demonstrating MICs ≥16 mg/L against aztreonam–avibactam, aztreonam and ceftazidime–avibactam combined at subinhibitory concentrations restored bactericidal activity and achieved synergy against 4/5 (80%) strains tested, with an average reduction of more than 5 $\log_{10}$ CFU/mL. Our work adds to existing data supporting the utility of aztreonam with ceftazidime–avibactam against SBL- and MBL-producing Enterobacterales and expands on these data by evaluating challenging clinical strains of *P. aeruginosa*. Strengths of this study include the use of isolates with a variety of increasingly complex arrays of SBL enzymes

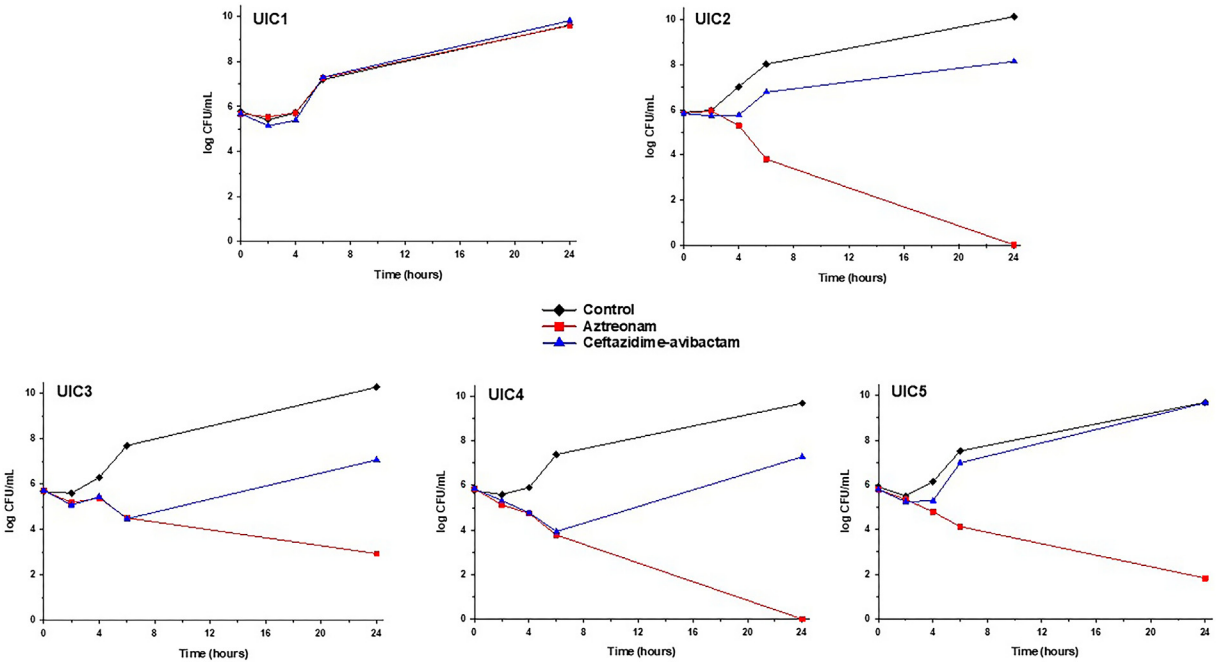

Fig. 1. Mean $\log_{10}$ CFU/mL versus time profile for each drug at the highest concentration tested against the 5 *P. aeruginosa* strains (UIC1–5). Aztreonam is shown at fC_{max} against UIC1, UIC3, and UIC4 and at 4× MIC against UIC2 and UIC5. Ceftazidime–avibactam is shown at fC_{max} against all strains. Curves represent average concentrations for triplicate experiments.

in conjunction with MBLs. Limitations to this study include the 24-h static nature of time-kill experiments and the limited number of strains and drug concentrations tested. Additionally, although the isolates were phenotypically similar, SBL- and MBL-producing *P. aeruginosa* isolates remain rare in the United States, and the included isolates were selected for their β-lactamase profiles and nonsusceptibility to aztreonam and ceftazidime–avibactam, which would prompt consideration of combination therapy in the clinical arena. While whole genome sequencing was not performed and therefore we cannot exclude the influence of other resistance mechanisms, the results of our current time-kill analyses correlate well with the known β-lactamase profile, similar to our previous work with Enterobacterales (Biagi et al., 2019). Further, the bactericidal activity of aztreonam and ceftazidime–avibactam may have been underestimated in time-kill experiments by using multiplicatives of the MIC in place of serum achievable concentrations, but this strategy allowed us to evaluate synergy and compare differences between strains that were not exclusively due to differences in the concentration:MIC ratio. Finally, dual β-lactam combinations were excluded from our time-kill experiments as previous data generated by our group suggest that the activity of aztreonam plus β-lactam/β-lactamase

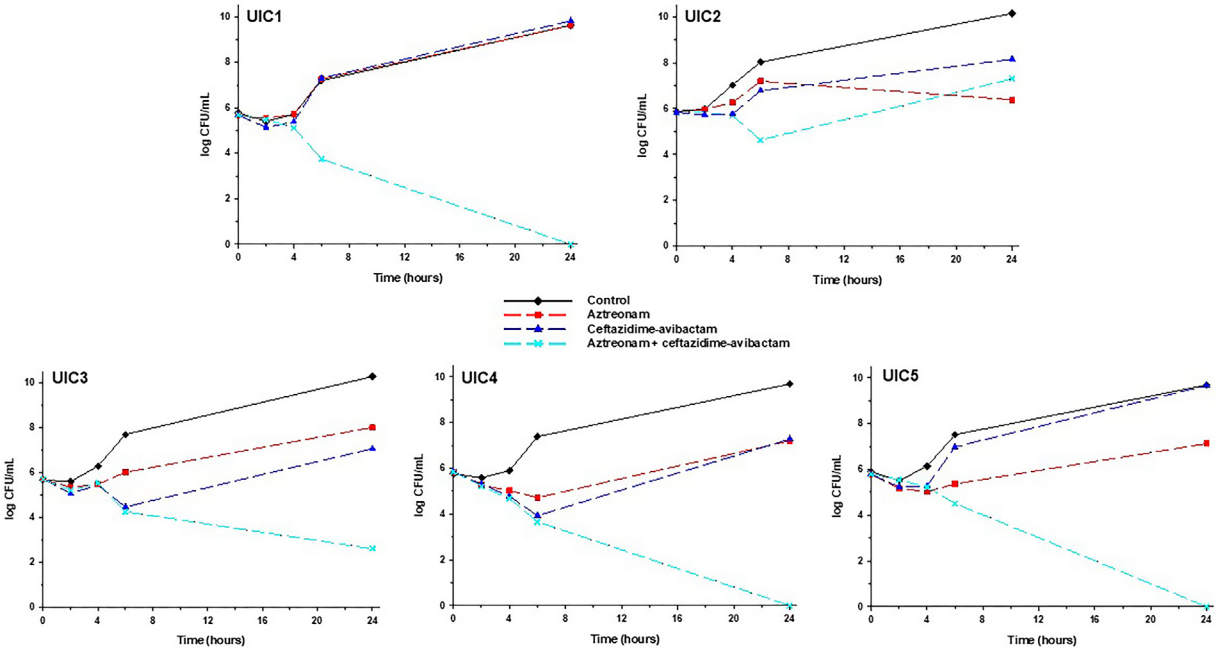

Fig. 2. Mean $\log_{10}$ CFU/mL versus time profile for the combination of aztreonam and ceftazidime–avibactam against the 5 *P. aeruginosa* strains (UIC1–5). Aztreonam is shown alone and in combination at fC_{max} against UIC1, and at 1/2× MIC alone and in combination against UIC2–5. Ceftazidime–avibactam is shown at fC_{max} alone and in combination against UIC1–5. Curves represent average concentrations for triplicate experiments.

inhibitor combinations against MBL producers is primarily driven by the interaction between aztreonam and the β-lactamase inhibitor (Biagi et al., 2019, 2020).

Given the enigmatic nature of the relationship of both substrate and protein to the mechanism of MBLs and the challenges in performing mechanistic studies (Bush and Fisher, 2011), it is unlikely that a novel β-lactamase inhibitor with reliable activity against class B enzymes will be commercially available in the foreseeable future. Furthermore, MBLs such as VIM-4 have demonstrated the ability to efficiently catalyze avibactam hydrolysis (Abboud et al., 2016), potentially explaining the lack of synergy against UIC2 observed in our study. This further underscores the crucial need to develop novel agents with activity against these challenging pathogens and optimizing those that are currently available. Finally, the future availability of aztreonam–avibactam is uncertain as the Phase 3 trial for the treatment of serious Gram-negative infections (NCT03329092) has been ongoing since November 2017 but is currently suspended. An additional trial specifically against MBL producers (NCT03580044) has not yet started recruiting but is limited to Enterobacterales and *Stenotrophomonas maltophilia.* As such, in the absence of available clinical data and more reliable therapeutic agents, our preclinical results support the combination of aztreonam and ceftazidime–avibactam against SBL- and MBL-producing *P. aeruginosa,* although further dynamic in vitro, in vivo, and clinical outcomes studies are needed to confirm these findings.

References

Abboud MI, Damblon C, Brem J, Smargiasso N, Mercuri P, Gilbert B, et al. Interaction of avibactam with class B metallo-β-lactamases. Antimicrob Agents Chemother 2016;60(10):5655–62.

Alm RA, Johnstone MR, Lahiri SD. Characterization of Escherichia coli NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. J Antimicrob Chemother 2015;70(5):1420–8.

Biagi M, Lamm D, Meyer K, Vialichka A, Jurkovic M, Patel S, et al. Activity of aztreonam in combination with avibactam, clavulanate, relebactam, and vaborbactam against multidrug resistant Stenotrophomonas maltophilia. Antimicrob Agents Chemother 2020. https://doi.org/10.1128/AAC.00297-20.

Biagi M, Wu T, Lee M, Patel S, Butler D, Wenzler E. Searching for the optimal treatment for Metallo- and serine-beta-lactamase producing Enterobacteriaceae: aztreonam in combination with ceftazidime–avibactam or meropenem–vaborbactam. Antimicrob Agents Chemother 2019;63(12):e01426-19.

Biedenbach DJ, Kazmierczak K, Bouchillon SK, Sahm DF, Bradford PA. In vitro activity of aztreonam–avibactam against a global collection of Gram-negative pathogens from 2012 and 2013. Antimicrob Agents Chemother 2015;59(7):4239–48.

Bush K, Bradford PA. Epidemiology of beta-lactamase-producing pathogens. Clin Microbiol Rev 2020;33(2).

Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. Annu Rev Microbiol 2011;65(1):455–78.

Clinical and Laboratory Standards Institute (CLSI). Methods for determining bactericidal activity of antimicrobial agents. CLSI document M26-A. Wayne, PA: CLSI; 1999.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: thirtieth informational supplement M100-S30. CLSI, Wayne, PA, USA, 2020.

Crandon JL, Nicolau DP. Human simulated studies of aztreonam and aztreonam–avibactam to evaluate activity against challenging gram-negative organisms, including metallo-beta-lactamase producers. Antimicrob Agents Chemother 2013;57(7):3299–306.

Das S, Li J, Armstrong J, Learoyd M, Edeki T. Randomized pharmacokinetic and drug–drug interaction studies of ceftazidime, avibactam, and metronidazole in healthy subjects. Pharmacol Res Perspect 2015;3(5):e00172.

Emeraud C, Escaut L, Boucly A, Fortineau N, Bonnin RA, Naas T, et al. Aztreonam plus clavulanate, tazobactam, or avibactam for treatment of infections caused by metallo-beta-lactamase-producing Gram-negative bacteria. Antimicrob Agents Chemother 2019;63(5).

Falcone M, Tiseo G, Antonelli A, Giordano C, Di Pilato V, Bertolucci P, et al. Clinical features and outcomes of bloodstream infections caused by New Delhi metallo-β-lactamase-producing Enterobacterales during a regional outbreak. Open forum infectious diseases 2020;7(2):ofaa011–ofaa.

Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 2016;45(D1):D566. D73.

Kazmierczak KM, Rabine S, Hackel M, McLaughlin RE, Biedenbach DJ, Bouchillon SK, et al. Multiyear, multinational survey of the incidence and global distribution of metallo-beta-lactamase-producing Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother 2016;60(2):1067–78.

Khan AU, Nordmann P. Spread of carbapenemase NDM-1 producers: the situation in India and what may be proposed. Scand J Infect Dis 2012;44(7):531–5.

Lutgring JD, Machado MJ, Benahmed FH, Conville P, Shawar RM, Patel J, et al. FDA-CDC antimicrobial resistance isolate bank: a publicly available resource to support research, development, and regulatory requirements. J Clin Microbiol 2018;56(2).

Marshall S, Hujer AM, Rojas LJ, Papp-Wallace KM, Humphries RM, Spellberg B, et al. Can ceftazidime–avibactam and aztreonam overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae? Antimicrob Agents Chemother 2017;61(4):e02243. 16.

Matos ECO, Andriolo RB, Rodrigues YC, Lima PDL, Carneiro I, Lima KVB. Mortality in patients with multidrug-resistant Pseudomonas aeruginosa infections: a meta-analysis. Rev Soc Bras Med Trop 2018;51(4):415–20.

O'Callaghan CH, Acred P, Harper PB, Ryan DM, Kirby SM, Harding SM. GR 20263, a new broad-spectrum cephalosporin with anti-pseudomonal activity. Antimicrob Agents Chemother 1980;17(5):876–83.

Periasamy H, Joshi P, Palwe S, Shrivastava R, Bhagwat S, Patel M. High prevalence of Escherichia coli clinical isolates in India harbouring four amino acid inserts in PBP3 adversely impacting activity of aztreonam/avibactam. Journal of Antimicrobial Chemotherapy 2020;75(6):1650–1.

Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo J-D, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. Antimicrob Agents Chemother 2000;44(4):891–7.

Scully BE, Swabb EA, Neu HC. Pharmacology of aztreonam after intravenous infusion. Antimicrob Agents Chemother 1983;24(1):18–22.

Swabb EA, Singhvi SM, Leitz MA, Frantz M, Sugerman A. Metabolism and pharmacokinetics of aztreonam in healthy subjects. Antimicrob Agents Chemother 1983;24(3):394–400.

Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1991;35(1):147–51.

Wenzler E, Deraedt MF, Harrington AT, Danizger LH. Synergistic activity of ceftazidime–avibactam and aztreonam against serine and metallo-beta-lactamase–producing gram-negative pathogens. Diagn Microbiol Infect Dis 2017;88(4):352–4.

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. Journal of Antimicrobial Chemotherapy 2012;67(11):2640–4.