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Case Report

Combination of aztreonam, ceftazidime–avibactam and amikacin in the treatment of VIM-1 *Pseudomonas aeruginosa* ST235 osteomyelitis

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ABSTRACT

We describe a challenging case of patient with metallo-beta-lactamase-producing *Pseudomonas aeruginosa* sternal osteomyelitis following aortic valve replacement with biological prosthesis. The strain exhibited a multidrug-resistance phenotype carrying the bla_{VIM-1} gene and belonged to the high-risk clone sequence type ST235. The patient was successfully treated with surgical debridement plus antibiotic therapy with ceftazidime/avibactam, aztreonam, and amikacin. Time-kill curves showed that this triple antibiotic combination at 1 × MIC was strongly synergic after 8 h, achieving 99.9% killing and maintaining this until 48 h.

Introduction

Metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* is an important cause of nosocomial infections and often requires treatment with novel or 'last resort' agents (Nguyen et al., 2018; Karakonstantis et al., 2020). *P. aeruginosa* osteomyelitis is associated with a high rate of treatment failure and poor prognosis (Laghmouche et al., 2017). Aztreonam/avibactam is a new monobactam/beta-lactamase-inhibitor, not yet clinically available, which has shown potent *in vitro* activity against MBL and *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains. Though aztreonam is active against many Gram-negative bacteria, including some isolates of Enterobacteriaceae that produce MBLs alone, it is inactive against isolates that produce additional β-lactamases, which can include extended-spectrumbeta-lactamases (ESBLs), AmpC enzymes, and serine carbapenemases (Marshall et al., 2017).

Avibactam, a non-β-lactam β-lactamase inhibitor, is being developed in combination with aztreonam to restore this drug's activity against isolates expressing MBLs in combination with one or more additional serine β-lactamases. P. MBL-producing *P. aeruginosa* often belongs to epidemic high-risk clones that are widespread in hospital settings worldwide, such as those of sequence type (ST) 111, 175, and 235 (Oliver et al., 2015).

Case report

A 78-year-old diabetic and obese woman with chronic heart failure secondary to severe aortic stenosis, associated with ascending aorta aneurism and three-vessel coronary artery disease, underwent aortic valve replacement with biological prosthesis, ascending aorta replacement, and coronary artery

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bypass. Her post-operative course was regular except for lung interstitial infiltrates, for which she received levofloxacin. On day +6, she was transferred to rehabilitation where she continued the antibiotic therapy until day +20. She was then discharged home in good clinical condition.

On day +42, she was admitted, because of superficial sternal wound dehiscence, to undergo surgical wound revision, which allowed debridement up to the sternum; ESBL-producing *K. pneumoniae* and pan-susceptible *Proteus* spp were isolated from soft tissue. She was treated with vacuum assisted closure (VAC) therapy plus ertapenem and amikacin for 4 weeks.

At month +10, she was readmitted because of fever and elevated inflammatory markers. A chest computed tomography scan showed sternal osteomyelitis and retro-sternal fluid collection. She underwent soft tissue and xiphoidal process surgical debridement. Bone biopsy culture was positive for *P. aeruginosa*, identified using the Vitek-2 system (bioMerieux Inc.), susceptible to amikacin and colistin, and resistant to carbapenems, ceftolozane–tazobactam and ceftazidime–avibactam. She was treated with VAC therapy plus 3 weeks of antibiotic therapy with ceftazidime–avibactam 2.5 g/8 h, aztreonam 2 g/8 h, and amikacin 15 mg/ kg/24 h, with trough-level monitoring.

P. aeruginosa isolate was sent to the Laboratory of Molecular Microbiology and Antibiotic Resistance of the University of Catania for confirmation of resistance profile, *in vitro* studies of antibiotic combinations, and molecular epidemiology. Minimum inhibitory concentrations (MICs) of antibiotics were determined by broth microdilution method as described by Clinical and Laboratory Standards Institute 2015. Susceptibility and resistance categories were assigned according to the European Committee on Antimicrobial Susceptibility Testing v.6.1, 2016 (http://www.eucast.org). The isolate was susceptible to amikacin (MIC 4 mg/l), colistin (MIC 2 mg/l), and was resistant to aztreonam (MIC 32 mg/l), piperacillin–tazobactam (MIC 128 mg/l), meropenem (MIC >32 mg/l), gentamicin (MIC >256 mg/l), ciprofloxacin (MIC 4 mg/l), cefepime (>256 mg/l), ceftazidime (MIC >256 mg/l), ceftazidime/avibactam (MIC 1024 mg/l), and ceftolozane/tazobactam (MIC >256 mg/l).

Analysis of carbapenemase genes by polymerase chain reaction and sequencing revealed the presence of the bla_{VIM-1} gene (Gugliandolo et al., 2017). Multilocus sequence typing (MLST) was carried out according to the protocol of Curran et al. (2004), and sequence types were analyzed using the *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/). Analyses revealed that the isolate belonged to the epidemic clone sequence type 235. The activity of the antibiotic combinations, with amikacin (MIC 4 mg/l), ceftazidime/avibactam (MIC 1024 mg/l) and aztreonam (MIC 32 mg/l), was evaluated by time-kill curves performed in duplicate, as described previously (Aprile et al., 2019).

The combination of ceftazidime/avibactam + amikacin was ineffective, while the combination of amikacin + aztreonam exhibited bactericidal synergism, with a 3-log decrease in CFU/ml, at 4 h., at 4 h. The killing activity of ceftazidime/avibactam + aztreonam showed a synergism at 24 h, but re-growth occurred at 48 h, probably due to ceftazidime/avibactam (Keepers et al., 2014). The triple combination of ceftazidime/avibactam + amikacin and aztreonam at 1 × MIC was strongly synergic after 8 h, achieving 99.9% killing and maintaining this until 48 h (Figure 1). After 3 weeks of treatment, follow up cultures were negative, her general condition improved, she became afebrile, and she was discharged home.

Unfortunately, at month +12, a visible fistula extended up to the sub mammary region. The patient underwent further surgical wound revision with debridement of the fistula. The necrotic tissues involving ribs and soft tissue were detected and removed, culture was positive for MBL-producing *P. aeruginosa*. VAC therapy

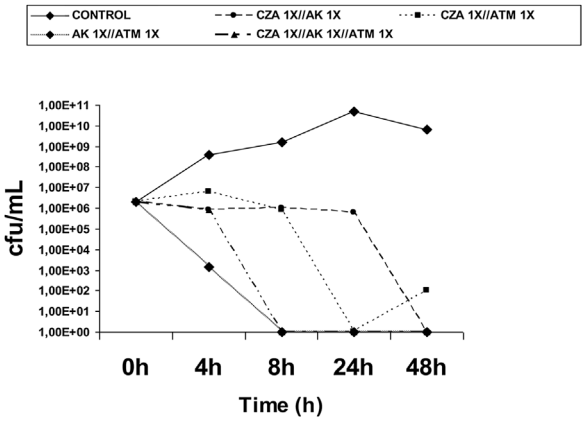

Figure 1. Time-kill curves of ceftazidime/avibactam (CZA) at 1 × MIC (1024 mg/l) in combination with amikacin (AK) at 1 × MIC (4 mg/l), and aztreonam (ATM) at 1 × MIC (32 mg/l) against *Pseudomonas aeruginosa*.

was applied, and another 4-week cycle of ceftazidime–avibactam + aztreonam and amikacin was started. The patient was discharged home with VAC therapy, follow up cultures were negative. At 12 month follow-up after withdrawal of antibiotics, the patient had no evidence of recurrence of infection.

Osteomyelitis is a major therapeutic challenge and is associated with a high rate of relapse despite apparently successful treatment. Bone localization makes the treatment of MBL-*P. aeruginosa* infection even more complex. In our case, surgical debridement was crucial both to allow the rapid detection of MBL positive isolate and to help control the source of infection. The possibility of evaluating the susceptibility of the isolate to different molecules, and the synergy, supported the choice of the treatment.

There are still few clinical case series reported in the literature of MBL-*P. aeruginosa* infections treated by ceftazidime–avibactam + aztreonam One of these has demonstrated synergistic effects against *P. aeruginosa* when combining ceftazidime–avibactam with aztreonam due to its stability against hydrolysis by MBLs (Toor and Garcia, 2020; Lee et al., 2021). Our study demonstrates the synergistic effect of the triple combination of ceftazidime/avibactam + aztreonam and amikacin at 1 × MIC after 8 h. This result allowed us to treat the patient successfully, even with a difficult infection such as osteomyelitis.

Considering the relapse after 3 weeks of treatment, a prolonged course should be required for MBL-*P. aeruginosa* osteomyelitis, especially in cases where surgical source control is inadequate or not feasible. Despite prolonged treatment and multiple comorbidities, our patient did not develop any side effect related to amikacin, ceftazidime/avibactam or aztreonam. Our case adds to existing data supporting the utility and safety of aztreonam with ceftazidime–avibactam against MBL-producing Enterobacterales (Falcone et al., 2020; Li et al., 2015) and expands on these data by evaluating challenging clinical strains of *P. aeruginosa* (Biedenbach et al., 2015).

The emergence of multidrug-resistant, MBL-positive *P. aeruginosa* represents an increasing therapeutic challenge worldwide (Kaur and Singh, 2018). These resistant elements are strongly linked to the high-risk clones, sequence type (ST) 175, ST235 and ST111, disseminated worldwide. In particular, the ST235 clone is very rarely associated with the VIM-1 carbapenemase gene (Pollini et al., 2018). The strain described in this report belonged to ST235, which is likely the most widespread *P. aeruginosa* epidemic clonal lineage.

In conclusion, our results confirm the efficacy of ceftazidime–avibactam and aztreonam association, especially with amikacin for MBL *P. aeruginosa*, even in challenging infections such as osteomyelitis.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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Ethical approval statement

Informed consent was obtained from the patient at admission.

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