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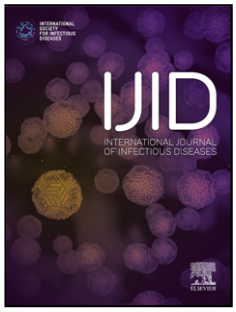

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PII: S1201-9712(21)00489-6

DOI: https://doi.org/10.1016/j.ijid.2021.05.085

Reference: IJID 5470

To appear in: *International Journal of Infectious Diseases*

Received Date: 29 April 2021

Revised Date: 25 May 2021

Accepted Date: 31 May 2021

Please cite this article as: Mularoni A, Mezzatesta ML, Pilato M, Medaglia AA, Cervo A, Bongiorno D, Aprile A, Luca A, Stefani S, Grossi P, Combination of aztreonam, ceftazidime-avibactam and amikacin in the treatment of VIM-1 *Pseudomonas aeruginosa* ST235 osteomyelitis, *International Journal of Infectious Diseases* (2021), doi: https://doi.org/10.1016/j.ijid.2021.05.085

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

Running title: Treatment of VIM-1 *Pseudomonas aeruginosa*

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Highlights

- Metallo-beta-lactamase-producing *P. aeruginosa* osteomyelitis is difficult to treat
- Aztreonam/ceftazidime-avibactam combined with amikacin was strongly synergic
- Time kill curves showed that triple combination achieved 99.9% killing until 48 hours
- Antipseudomonal synergistic agents plus debridment resolved the infection

ABSTRACT

We describe a challenging case of a patient with MBL-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 X MIC was strongly synergic after 8 hours, achieving 99.9% killing, and maintaining this until 48 hours.

Keywords: *Pseudomonas aeruginosa*, ST235, VIM-1, ceftazidime-avibactam, aztreonam, osteomyelitis

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 available, which has shown potent *in vitro* activity against MBL and 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 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.

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 to ascending aorta aneurism and three-vessel coronary artery disease underwent aortic valve replacement with biological prosthesis, ascending aorta replacement, and coronary artery 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 VAC therapy plus ertapenem and amikacin for 4 weeks.

At month +10 she was readmitted because of fever and elevated inflammatory markers. A chest CT 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 then treated with VAC therapy plus 3 weeks of antibiotic therapy with ceftazidime-avibactam 2.5g/8h, aztreonam 2g/8h, and amikacin 15 mg/Kg/24h, 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.

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 PCR 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. (Curran et al.,2004), and sequence types were analyzed using the *P.aeruginosa* MLST Web site (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 4mg/l), ceftazidime/avibactam (MIC 1024 mg/l) and aztreonam (MIC 32 mg/l) [2], 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.

The killing activity of ceftazidime/avibactam + aztreonam showed a synergism at 24 h but re-growth was occured at 48 h, probably due to ceftazidime/avibactam (Keepers et al., 2014). The triple combination of ceftazidime/avibactam + amikacin and aztreonam at 1 X MIC was strongly synergic after 8 hours, achieving 99.9% killing, and maintaining this until 48 hours (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. She 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 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 12th-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-PA 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: these case report have 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 hours. 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-PA 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., 2012).

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 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 difficult infections such as osteomyelitis.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Source

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval statement

Informed consent was obtained from the patient at admission

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.

References

1. Nguyen L, Garcia J, Gruenberg K, MacDougall C. Multidrug-resistant Pseudomonas Infections: hard to treat, but Hope on the horizon? Curr Infect Dis Rep 2018;20:23.

2. Karakonstantis S, Kritsotakis EI, Gikas A. Treatment options for K. pneumoniae, P. aeruginosa and A. baumannii co-resistant to carbapenems, aminoglycosides, polymyxins and tigecycline: an approach based on the mechanisms of resistance to carbapenems. Infection 2020;48:835-851.

3. Laghmouche N, Compain F, Jannot AS, Guigui P, Mainardi JL, Lonjon G, Bouyer B, Fernandez-Gerlinger MP. Successful treatment of Pseudomonas aeruginosa osteomyelitis with antibiotic monotherapy of limited duration. J Infect 2017;75:198-206.

4. Marshall S, Hujer AM, Rojas LJ, Papp-Wallace KM, Humphries RM, Spellberg B, Hujer KM, Marshall EK, Rudin SD, Perez F, Wilson BM, Wasserman RB, Chikowski L, Paterson DL, Vila AJ, van Duin D, Kreiswirth BN, Chambers HF, Fowler VG, Jr, Jacobs MR, Pulse ME, Weiss WJ, Bonomo RA. Can ceftazidime–avibactam and aztreonam overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae? Antimicrob Agents Chemother 2017;61(4):e02243. 16.

5. Oliver A, Mulet X, Lopez-Causape C, Juan C. The increasing threat of Pseudomonas aeruginosa high-risk clones. Drug Resist Updat 2015;21–22:41–59.

6. Gugliandolo A, Caio C, Mezzatesta ML, Rifici C, Bramanti P, Stefani S, Mazzon E. Successful ceftazidime-avibactam treatment of MDR-KPC-positive Klebsiella pneumoniae infection in a patient with traumatic brain injury: A case report. Medicine (Baltimore) 2017; 96(31):e7664.

7. Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a multilocus sequence typing scheme for the opportunistic pathogen Pseudomonas aeruginosa. J Clin Microbiol 2004;42(12):5644–5649

8. Aprile A, Caio C, Gona F, Stefani S, Mezzatesta ML. In vitro evidence of the synergistic interaction of ceftopibrole and other antibiotics against multidrug-resistant Gram-negative isolates. Diagn Microbiol Infect Dis 2019;7:114884.

9. Toor R, Garcia E. Ceftazidime-avibactam and aztreonam against multidrug- resistant pseudomonas aeruginosa. Critical Care Medicine 2020;48:284.

10. Lee M, Abbey T, Biagi M, Wenzler E. Activity of aztreonam in combination with ceftazidime–avibactam against serine- and metallo-β-lactamase–producing-Pseudomonas aeruginosa. Diagn Microbiol Infect Dis 2021;99:115227.

11. Falcone M, Daikos GL, Tiseo G, Bassoulis D, Giordano C, Galfo V, Leonildi A, Tagliaferri E, Barnini S, Sani S, Farcomeni A, Ghiadoni L, Menichetti F. Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by MBL- producing Enterobacterales. Clin Infect Dis 2020;19:ciaa586.

12. Li, H., Estabrook, M., Jacoby, G.A., Nichols, W.W., Testa, R.T., Bush, K. In vitro susceptibility of characterized β-lactamase-producing strains tested with avibactam combinations. Antimicrob Agents Chemother 2015;59:1789-1793.

13. Biedenbach, D.J., Kazmierczak, K., Bouchillon, S.K., Sahm, D.F., Bradford, P.A. In vitro activity of aztreonam-avibactam against a global collection of Gram-negative pathogens from 2012 and 2013. Antimicrob Agents Chemother 2015;59:4239-4248.

14. Kaur A, Singh S. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing Pseudomonas aeruginosa and Acinetobacter baumannii Isolated from Various Clinical Samples. J Pathog 2018;2018:6845985.

15. Pollini S, Mugnaioli C, Dolce D, Campana S, Neri AS, Taccetti G, Rossolini GM. Chronic infection sustained by a Pseudomonas aeruginosa High-Risk clone producing the VIM-1 metallo-β-lactamase in a cystic fibrosis patient after lung transplantation. J Cyst Fibros 2018; 17:470–474.

16. Keepers TR, Gomez M, Celeri C, Nichols WW, Krause KM. Bactericidal activity, absence of serum effect, and time-kill kinetics of ceftazidime-avibactam against β-lactamase-producing

Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother 2014; 58:5297-305.

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