Success of ceftazidime–avibactam and aztreonam in combination for a refractory biliary infection with recurrent bacteraemia due to \textit{blaIMP-4} carbapenemase-producing \textit{Enterobacter hormaechei} subsp. \textit{oharae}

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**CASE REPORT**

A 45-year-old woman was admitted to the intensive care unit (ICU) for 5 months in December 2017 with protracted status epilepticus due to limbic encephalitis. This was complicated by diffuse intra-hepatic biliary duct dilatation and marked derangement of liver function tests, presumed to be due to antiepileptic medication, with a liver biopsy demonstrating non-inflammatory, non-steatotic hepatocyte injury of unclear aetiology. She also developed sacral pressure ulcers, deep venous thrombosis, an upper gastrointestinal bleed and cardiomyopathy. After resolution of status epilepticus, she had ongoing cognitive impairment and intermittent seizures. She was treated initially with high-dose corticosteroids.

**INTRODUCTION**

We present a case where treatment with ceftazidime–avibactam and aztreonam in combination was effective in a patient with recurrent and sustained \textit{blaIMP-4}+ \textit{Enterobacter cloacae} complex bacteraemia from an undrainable biliary source, where alternative antibiotic treatment had failed over a 5-month period.
Table 1. Antibiotic treatment regimens, blaIMP-4 status and interval between septic episodes for the first nine E. cloacae complex bloodstream infections

| Isolate no. | Date         | E. cloacae complex antibiotic resistance | Antibiotic                          | Days off treatment until recurrence |
|-------------|--------------|-----------------------------------------|-------------------------------------|-------------------------------------|
| ECI01       | 23 December 2017 | Wild-type                               | Gentamicin → meropenem, 14 days     | 29                                  |
| ECI02*      | 7 February 2018   | blaIMP-4, CIP intermediate, MDR†         | Ciprofloxacin, 12 days              | -2                                  |
| ECI03*      | 18 February 2018  | MDR, except cefepime-susceptible        | Meropenem, 5 days                   | 3                                   |
| ECI04       | 2 March 2018     | blaIMP-4, MDR                            | PTZ† and amikacin, 19 days          | -6                                  |
| ECI05*      | 10 March 2018    | blaIMP-4, MDR                            | Aztreonam, 7 days                   | 4                                   |
| ECI06       | 24 March 2018    | blaIMP-4, MDR                            | Amikacin 5 days, PTZ† 4 days, aztreonam 14 days | 6                               |
| ECI07       | 5 April 2018     | MDR                                      | Amikacin 16 days                    | 9                                   |
| ECI08       | 1 May 2018       | MDR                                      | Amikacin 2 days → meropenem 12 days | 13                                  |
| ECI09       | 28 May 2018      | blaIMP-4, MDR (MER MIC 4 mg l⁻¹)         | Amikacin and meropenem 14 days      | 36                                  |
| ECI10       | 18 July 2018     | blaIMP-4, MDR                            | Amikacin 7 days → aztreonam and ceftazidime–avibactam 14 days | No recurrence |

*Sequenced isolates. †MDR, resistant to ciprofloxacin, gentamicin, cefepime, trimethoprim–sulphamethoxazole, tigecycline, nitrofurantoin. ‡Piperacillin–tazobactam.

Table 1. Antibiotic treatment regimens, blaIMP-4 status and interval between septic episodes for the first nine E. cloacae complex bloodstream infections

From 2 weeks after admission, over an 8-month period, she had 10 discrete episodes of Gram-negative bacteraemia, all culturing E. cloacae complex (MALDI Biotyper, Bruker) (see Table 1, isolates ECI01–10). These were attributed to cholangitis. She had an endoscopic retrograde cholangiopancreatogram with stenting of the mildly dilated common bile duct, but this did not improve biliary drainage. A magnetic resonance cholangiogram demonstrated a gallstone, gallbladder wall thickening, and moderate irregularity and dilatation of the intrahepatic ducts. Computed tomography demonstrated contrast enhancement of the major ducts consistent with cholangitis. Histopathology of the common bile duct revealed a mild acute inflammatory infiltrate of the mucosa and stroma, with no malignant cells. Positron-emitted tomography revealed diffuse, moderate-to-markedly increased metabolism outlining the biliary tree in both lobes of the liver, consistent with cholangitis, without other abnormalities. She was treated for cholestasis with cholestyramine and ursodeoxycholic acid. Liver transplantation was not considered an option.

The first episode of bacteraemia occurred in December 2017. A wild-type E. cloacae complex isolate was cultured. This episode was followed by six further episodes of blaIMP-4+ E. cloacae complex bloodstream infection, over 5 months beginning in February 2018, interspersed with three episodes where ESBL-producing, but blaIMP-4-negative, E. cloacae complex was isolated. All blaIMP-4+ isolates remained amikacin-susceptible (Vitek2, BioMérieux); meropenem Etest MICs (Biomérieux) were >16 mg l⁻¹ for all isolates except for one (ECI09), at 4 mg l⁻¹.

Throughout these episodes she received multiple treatments of varied duration with combinations of antibiotics, including meropenem, amikacin, aztreonam, piperacillin–tazobactam, ciprofloxacin, gentamicin and trimethoprim–sulphamethoxazole (Table 1). Despite responding clinically on each occasion, particularly when amikacin was included in treatment, the septic episodes recurred regularly, usually within 1–2 weeks of antibiotic therapy ceasing. These presented clinically with slight worsening of cognitive status, low-grade fever and gradual increase in C-reactive protein and transaminase levels, without other overt signs or symptoms of typical sepsis.

A 10th episode of E. cloacae complex bacteraemia (blaIMP-4+) occurred on 18 July 2018. To aid treatment of this episode, in vitro synergy testing for the combination of ceftazidime–avibactam (CAZ–AVI) and aztreonam was performed. The patient then received 14 days of CAZ–AVI 2g/0.5g 8-hourly in combination with aztreonam 1g 8-hourly. She had also received amikacin 900mg daily for the previous 7 days. Aztreonam was initially dosed at 2g, but after a seizure, a lower dosage was used because of the risk of provoking seizures with double β-lactam therapy. She tolerated the treatment course without complications.

She has had no further recurrences during 12 months of follow-up, which included 16 separate blood culture collections. Her rectal screening samples continue to culture blaIMP-4+E. cloacae complex.

**PHENOTYPIC TESTING AND WHOLE-GENOME SEQUENCING (WGS)**

Phenotypic antimicrobial susceptibility testing was performed with Vitek2. The colistin broth microdilution MIC was 0.25 mg l⁻¹ (MERLIN Diagnostika GmbH). Supplementary testing was performed with Etest strips and the individual MIC results were 6mg l⁻¹ for tigecycline, 128mg l⁻¹ for aztreonam and >256mg l⁻¹ for CAZ–AVI. A layered Etest method for synergy between CAZ–AVI and aztreonam demonstrated an MIC of 2mg l⁻¹ for the combination.
WGS was performed on the Illumina MiSeq platform and analysed with the Nullarbor pipeline [1] for three of the isolates. ECI02 was the first non-wild-type isolate, and was blaIMP-4 PCR-positive but still MDR isolate, but notably, ceftipime-susceptible. ECI05 was again blaIMP-4 PCR-positive, and was chosen because treatment with amikacin had failed. Trimmed reads from ECI02, ECI03 and ECI05 were aligned to infer core SNP phylogeny (maximum-likelihood GTR+G4 model) with IQTree (see Fig. 1). ECI05 and ECI03 are more closely related to ECI02 (590 and 645 core-genome SNP differences, respectively) than they are to each other (1120 core-genome SNP difference). Of note, ECI02 and ECI05 are the blaIMP-4-positive isolates, despite their core-genome differences. Species identification (Kraken) [2] for all three isolates was consistent with Enterobacter hormaechei subsp. oharae (part of the E. cloacae complex). They were found to belong to multilocus sequence type 114 (mlst 2.6, http://pubmlst.org/). Sequence data are available in the European Nucleotide Archive (accession PRJEB39176).

Plasmid replicons were detected by uploading assembled contigs (from SPAdes v3.12.0) [3] to PlasmidFinder [4]. Contigs were uploaded to the CARD database to detect antimicrobial resistance genes [5]. The isolates were MDR, and multiple antibiotic resistance genes were detected. All three isolates had a blaTEM-4 class A extended-spectrum beta-lactamase, blaOXA-1 and blaACT-25 beta-lactamases. Two of the isolates (ECI02 and ECI03) carried the IncH2 plasmid replicon, along with blaIMP-4, and a number of plasmid-associated antimicrobial resistance genes [6], which were missing from the carbapenem-susceptible isolate ECI03 (Table 2). This isolate (ECI03), like the two others, carried an IncL/M and colRNAI plasmid replicon, but not IncH2, suggesting that blaIMP-4 was carried on the IncH2 plasmid, whilst the other antimicrobial resistance genes were carried on IncL/M and possibly colRNAI (see Table 3).

**DISCUSSION**

This case contributes to the literature on the use of ceftazidime–avibactam and aztreonam combination therapy in the treatment of serious infections due to metallo-β-lactamase (MBL)-producing organisms, in the presence of other beta-lactamases. The distinguishing feature of this case is that our patient had limited surgical options for source control. Despite multiple recurrences of infection due to a persistent biliary focus over more than 7 months, and sustained treatment failure using alternative active antibiotics, the patient was successfully treated with a single limited 14-day course of CAZ–AVI–AZT treatment.

Ceftazidime–avibactam is a successful option for treatment of carbapenemase-producing enterobacteria (CPE) infections, especially those caused by blaKPC- and blaOXA-48-producing...
organisms. Avibactam is a beta-lactamase inhibitor with activity against Ambler class A ESBLs and carbapenemases, Ambler class C-producing AmpC beta-lactamases and blaOXA-48-like carbapenemases, but not MBLs [7]. Thus, the management of sepsis caused by MBL-producing CPE, such as blaNDM and blaIMP-4, remains unsatisfactory. Aztreonam is a beta-lactam antibiotic that also inhibits MBLs, and its addition to another beta-lactam antibiotic may overcome this problem [7].

Although MBLs do not hydrolyze aztreonam, which then retains activity, MBL-producing isolates may also co-produce ESBLs that confer resistance to aztreonam. The use of aztreonam–avibactam may potentially counter this, but as this drug combination is not commercially available, the combination of ceftazidime–avibactam and aztreonam has increasingly been utilized in the treatment of infection caused by MBL-producing organisms [7–10]. In this case, phenotypic detection of ESBL was confirmed by genomic analysis.

In vitro data using the layered Etest and chequerboard methodology has demonstrated reduction in ceftazidime–avibactam MICs by the addition of aztreonam in enterobacterales isolates with MBLs and ESBLs [11]. Isolates included in the published literature have harboured blaNDM or blaVIM alone, or in combination with blaOXA-48, blaOXA-181 or blaKPC-2, as well as various ESBLs. Synergy has also been demonstrated in disc diffusion assays, in agar dilution, in time–kill studies and in mouse neutropenic thigh infection models [7].

In vivo, the utility of this combination has been demonstrated in a prospective observational study, individual case studies and series with a range of treatment duration from 10 days to greater than 6 weeks [8]. The combination was curative in cases of blaNDM-1-producing Enterobacter cloacae and ESBL-producing Klebsiella pneumoniae arthroplasty infection [7], blaOXA-48 and blaNDM-1-producing persistent Klebsiella pneumoniae bacteraemia [9], blaNDM-1-producing Pseudomonas aeruginosa lung abscess [9] and extensive osteomyelitis due to blaNDM-1- and blaOXA-181-producing Klebsiella pneumoniae, which also required aggressive surgical management [12]. There was a 60% reduction in the risk of mortality compared to treatment with other active antibiotics [10].

### Table 3. Plasmid replicons detected in the three sequenced isolates and their blaIMP4 status

| Isolate  | Antibiotic susceptibility profile | IncI/M | coI/1A | IncHI2 | blaIMP4 gene |
|----------|-------------------------------|--------|--------|--------|-------------|
| ECI02    | blaIMP-4, CIP intermediate, MDR* | Positive | Positive | Positive | Positive |
| 7 February 2018 | | | | | |
| ECI03    | MDR, except cefepime-susceptible | Positive | Positive | Negative | Negative |
| 18 February 2018 | | | | | |
| ECI05    | blaIMP-4, MDR                  | Positive | Positive | Positive | Positive |
| 10 March 2018 | | | | | |

*MDR, resistant to ciprofloxacin, gentamicin, cefepime, trimethoprim-sulphamethoxazole, tigecycline, nitrofurantoin.

**CONCLUSION**

Infections due to MBLs are becoming a significant problem, with organisms producing blaNDM and blaIMP-4 causing increasing numbers of community- and healthcare-associated infections, and antibiotic treatment options are limited. Aztreonam combined with avibactam presents an increasingly useful therapeutic choice, and though currently not commercially available as a combination, the use of CAZ–AVI with aztreonam provided a safe and effective cure in this difficult biliary infection.

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**Author contributions**

G.M.: conceptualization, formal analysis, investigation, data curation, writing – original draft preparation, review and editing. J.M.: investigation. A.B.: investigation. S.v.H.: investigation, resources, writing – review and editing. T.G.: conceptualization, writing – review and editing, supervision.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

The Sydney Local Health District Human Research Ethics Committee – Concord Repatriation and General Hospital approved this work.
(CH62/6/20201-001). Informed consent was obtained from the patient’s next of kin.

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