Investigation of mechanisms responsible for decreased susceptibility of aztreonam/avibactam activity in clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019

Rodrigo E. Mendes1*, Timothy B. Doyle1, Jennifer M. Streit1, Francis F. Arhin2, Helio S. Sader1 and Mariana Castanheira1

1JMI Laboratories, North Liberty, IA 52317, USA; 2Pfizer, Inc, Kirkland, Quebec, Canada

*Corresponding author. E-mail: rodrigo-mendes@jmilabs.com

Received 2 April 2021; accepted 6 July 2021

Background: The combination aztreonam/avibactam is currently under Phase 3 trials for the treatment of serious infections caused by Gram-negative bacteria including those with MBLs.

Objectives: To investigate the resistance mechanisms in Enterobacterales exhibiting aztreonam/avibactam MICs of ≥4 mg/L.

Methods: Among 8787 Enterobacterales, 17 (0.2%) isolates exhibited an aztreonam/avibactam MIC of ≥4 mg/L. Isolates were sequenced and screened for ß-lactamases. Sequences of porins, penicillin-binding protein 3 (PBP3) and expression levels of AmpC and AcrA were evaluated.

Results: Eleven (11/4154 isolates; 0.26%) Escherichia coli, three (3/1981; 0.15%) Klebsiella pneumoniae and three (3/628; 0.5%) Enterobacter cloacae were identified. All E. coli showed either an ‘YRIK’ or ‘YRIN’ insertion in PBP3. In general, these isolates carried blaCMY and/or blaCTX-M variants, except for one isolate from Korea that also produced NDM-5 and one isolate from Turkey that produced OXA-48. Two DHA-1-producing K. pneumoniae overexpressed acrA and had a premature stop codon in either OmpK35 or OmpK36, whereas a third K. pneumoniae carried blaPER-2 and had a premature stop codon in OmpK35. All three E. cloacae expressed AmpC at levels ≥570-fold, but sequence analysis did not reveal known amino acid alterations associated with decreased avibactam binding or increased hydrolysis of ß-lactams. Minor amino acid polymorphisms within OmpC, OmpF and PBP3 were noted among the E. cloacae.

Conclusions: A small number of isolates (0.2%) met the inclusion criteria. E. coli showed altered PBP3 as the most relevant resistance mechanism, whereas K. pneumoniae had multiple resistance mechanisms. Further investigations are needed to clarify resistance in E. cloacae.

Introduction

Antimicrobial resistance remains a great concern worldwide, especially among Gram-negative bacteria. The latest report from the US CDC estimated 197,400 cases and 9100 deaths caused by Enterobacterales resistant to expanded-spectrum cephalosporins (ESC), and 13,100 cases and 1100 deaths caused by Enterobacterales resistant to carbapenems (CRE).1 In Europe, 31.7% of Klebsiella pneumoniae were reported as resistant to ESC and 7.5% of K. pneumoniae were reported as resistant to carbapenems in 2018. Resistance rates varied greatly (0%–78%) among the 30 European countries, but most countries (18) reported resistance rates for ESC higher than 20%. Moreover, carbapenem resistance among K. pneumoniae remained below 4% in most countries, but occurrences between 8% and 30% were reported in seven countries, and a rate as high as 64% was reported in Greece.2

The occurrences of resistance phenotypes to ESC and carbapenems are considered serious and urgent threats, respectively.1 These threats prompted the development of new therapeutic options and/or strategies as part of a global action plan against antimicrobial resistance.3 Aztreonam/avibactam, a monobactam/ß-lactamase inhibitor (BLI) combination, is undergoing Phase 3 clinical trials for treating infections caused by Gram-negative organisms including those producing MBLs.4 In contrast to most ß-lactams, monobactams are not substrates for MBLs, whereas avibactam reversely inactivates most Class A and C and some D ß-
lactamase enzymes. Thus, this combination mitigates resistance caused by most ESBL, including carbapenemases.

The in vitro activity of aztreonam/avibactam was assessed against a large collection of contemporary (2019) clinical Enterobacterales recovered from patients hospitalized in medical centres located in Europe, Latin America and the Asia-Pacific region. In this previous study, a total of 18 Enterobacterales displayed an aztreonam/avibactam MIC of >4 mg/L. These isolates were selected for molecular characterization to investigate the resistance mechanisms associated with this phenotype. This study expands on the previous publication to report on the epidemiological typing and resistance mechanisms observed among these select pathogens.

Materials and methods

The original study included 8787 Enterobacterales collected consecutively in 2019 from 64 medical centres in Europe, Russia, and Turkey (n = 6170), the Asia-Pacific region (n = 1456), and Latin America (n = 1161). Information related to these organisms can be obtained in Sader et al. (2021). Within this collection, 18 (0.2%) isolates exhibited an aztreonam/avibactam MIC of >4 mg/L (Table 1). Isolates were selected for the Asia-Pacific region. In this previous study, a total of 18 Enterobacterales displayed an aztreonam/avibactam MIC of >4 mg/L. These isolates were selected for molecular characterization to investigate the resistance mechanisms associated with this phenotype. This study expands on the previous publication to report on the epidemiological typing and resistance mechanisms observed among these select pathogens.

DNA extraction was performed with the ThermoScientificTM KingFisherTM Flex Magnetic Particle Processor (Cleveland, OH, USA) and used as input material for library construction. Libraries were normalized using the bead-based normalization procedure (Illumina) and then sequenced on MiSeq (Reagent Kit v2; 2 × 250 paired reads; 500 cycles). De novo assembled FASTQ files were screened for β-lactamases, as previously described. Gene sequences encoding for penicillin-binding protein 3 (PBP3) OmplOmpK36 and OmplOmp35 were sequenced. Sequence analysis comparison was performed using sequences from a control strain. The control strain showed expression of acrA and acrB (AcrAB-ToIc) expression.

DNA extraction was performed with the ThermoScientificTM KingFisherTM Flex Magnetic Particle Processor (Cleveland, OH, USA) and used as input material for library construction. Libraries were normalized using the bead-based normalization procedure (Illumina) and then sequenced on MiSeq (Reagent Kit v2; 2 × 250 paired reads; 500 cycles). De novo assembled FASTQ files were screened for β-lactamases, as previously described. Gene sequences encoding for penicillin-binding protein 3 (PBP3) OmplOmpK36 and OmplOmp35 were sequenced. Sequence analysis comparison was performed using sequences from a control strain. The control strain showed expression of acrA and acrB (AcrAB-ToIc) expression.

Results

Eleven (11/4154 surveillance isolates; 0.26%) E. coli had elevated aztreonam/avibactam MICs (4–16 mg/L). Ceftazidime/avibactam MICs of 1–8 mg/L were obtained against these isolates, except for one E. coli from Korea that carried blaNDM-1 and blaOXA-181 (MIC, >32 mg/L) (Tables 1 and 2). Elevated MIC results for aztreonam and ESC (≥8 mg/L) were obtained against E. coli, whereas low MIC values were noted for meropenem (0.03–0.12 mg/L) and imipenem (≤0.12–1 mg/L), except against the isolate from Korea that carried blaNDM-1 and blaOXA-181 (imipenem and meropenem MIC, >8 mg/L) and one isolate from Turkey with a BLOXO-48 (imipenem MIC, 4 mg/L) (Tables 1 and 2).

These 11 E. coli isolates carried multiple ESBL and plasmid AmpC-encoding genes, mostly consisting of CTX-M and CMY variants (Table 2). Four ST types were observed, with five isolates from two sites in Turkey belonging to ST410. Additionally, the NDM-5-producing E. coli strain from Korea belonged to ST410 (Table 2). All E. coli showed amino acid alterations in the PBP3 sequence either as an ‘YRIK’ or ‘YRIN’ insertion after position 333 of PBP3. These insertions were previously described by Alm et al. to cause decreased aztreonam binding at the target site and were further evaluated by Sadek et al. (2020). Isolates possessing an altered PBP3 and blaNDM would be refractory to aztreonam/avibactam and any clinically available β-lactams and β-lactam/BLI combinations. Recent studies reported a high prevalence of NDM-producing E. coli with PBP3 insertions, which seem to be more prevalent in India. However, other surveillance studies reported a low proportion (<0.3%) of Enterobacterales with aztreonam/avibactam MICs of ≥4 mg/L; these isolates tended to be carbapenem susceptible. A narrow aztreonam/avibactam MIC range (4–16 mg/L) was obtained against E. coli as well as for ceftazidime/avibactam (1–8 mg/L), except against the NDM-5-producing E. coli (>32 mg/L). These results indicate that the PBP3 mutations are essentially driving the higher aztreonam/avibactam MICs and the MIC variation (4–16 mg/L) may be caused by the β-lactamase background, as demonstrated previously.

The three K. pneumoniae had aztreonam/avibactam MICs of ≥8 mg/L and ceftazidime/avibactam MICs of 4–16 mg/L. The A total of 11 E. coli isolates were selected for this study; of these isolates, 9 isolates had the ‘YRIK’ insertion and 2 isolates had the ‘YRIN’ insertion after position 333 of PBP3. These insertions were previously described by Alm et al. to cause decreased aztreonam binding at the target site and were further evaluated by Sadek et al. (2020). Isolates possessing an altered PBP3 and blaNDM would be refractory to aztreonam/avibactam and any clinically available β-lactams and β-lactam/BLI combinations. Recent studies reported a high prevalence of NDM-producing E. coli with PBP3 insertions, which seem to be more prevalent in India. However, other surveillance studies reported a low proportion (<0.3%) of Enterobacterales with aztreonam/avibactam MICs of ≥4 mg/L; these isolates tended to be carbapenem susceptible. A narrow aztreonam/avibactam MIC range (4–16 mg/L) was obtained against E. coli as well as for ceftazidime/avibactam (1–8 mg/L), except against the NDM-5-producing E. coli (>32 mg/L). These results indicate that the PBP3 mutations are essentially driving the higher aztreonam/avibactam MICs and the MIC variation (4–16 mg/L) may be caused by the β-lactamase background, as demonstrated previously.

Discussion

A total of 11 E. coli isolates were selected for this study; of these isolates, 9 isolates had the ‘YRIK’ insertion and 2 isolates had the ‘YRIN’ insertion after position 333 of PBP3. These insertions were previously described by Alm et al. to cause decreased aztreonam binding at the target site and were further evaluated by Sadek et al. (2020). Isolates possessing an altered PBP3 and blaNDM would be refractory to aztreonam/avibactam and any clinically available β-lactams and β-lactam/BLI combinations. Recent studies reported a high prevalence of NDM-producing E. coli with PBP3 insertions, which seem to be more prevalent in India. However, other surveillance studies reported a low proportion (<0.3%) of Enterobacterales with aztreonam/avibactam MICs of ≥4 mg/L; these isolates tended to be carbapenem susceptible. A narrow aztreonam/avibactam MIC range (4–16 mg/L) was obtained against E. coli as well as for ceftazidime/avibactam (1–8 mg/L), except against the NDM-5-producing E. coli (>32 mg/L). These results indicate that the PBP3 mutations are essentially driving the higher aztreonam/avibactam MICs and the MIC variation (4–16 mg/L) may be caused by the β-lactamase background, as demonstrated previously.

The three K. pneumoniae had aztreonam/avibactam MICs of ≥8 mg/L and ceftazidime/avibactam MICs of 4–16 mg/L.
| Collection number | Site code | Country | City   | Organism | MIC (mg/L) |
|-------------------|-----------|---------|--------|----------|------------|
|                   |           |         |        |          | ATM       | ATM/AVI  | ATM/CLA | CAZ | CAZ/AVI | CAZ/CLA | COZ/TZB | CRO | FEP | SAM | TZP | MEM | IPM | ETP |
| 1108470           | 68        | Turkey  | Ankara | E. coli | >64  8  32 | >128 2 >128 16 >8 >256 >64 >128 0.03 | 0.25 | 0.12 |
| 1108523           | 68        | Turkey  | Ankara | E. coli | 32  8  32 | >128 2 >128 16 >8 >256 >64 >128 0.03 | 0.25 | 0.12 |
| 1108694           | 68        | Turkey  | Ankara | E. coli | >64  8  64 | >128 2 >128 16 >8 8 64 128 0.06 | 0.5  | 0.12 |
| 1114251           | 69        | Turkey  | İstanbul | E. coli | >64 16 32 | >128 8 >128 16 >8 >256 >64 >128 0.06 | 1   | 0.5  |
| 1114255           | 69        | Turkey  | İstanbul | E. coli | >64  8  64 | >128 8 >128 16 >8 128 >64 >128 1 | 4   | 2    |
| 1118669           | 606       | Korea   | Kangwondo | E. coli | >64  4  32 | >128 >32 >128 16 >8 >256 >64 >128 32 | >8  | 2    |
| 1116284           | 603       | Thailand | Bangkok | E. coli | >64  8  16 | >128 4 16 >16 >8 >256 >64 >128 0.03 | ≤0.12 | 0.5 |
| 1128667           | 380       | France  | Rennes Cedex | E. coli | >64  4  8 | >128 1 8 >16 >8 >256 64 64 0.03 | ≤0.12 | 0.25 |
| 1130864           | 86        | Italy   | Rome   | E. coli | >64  8  16 | >128 8 16 >16 >8 >256 >64 >128 0.12 | 0.25 | 2    |
| 1116957           | 263       | Australia | Sydney | E. coli | 16  8  32 | >128 2 >128 16 >8 64 >64 >128 0.03 | 0.25 | 2    |
| 1126350           | 283       | Vietnam | Hanoi  | E. coli | >64  16 64 | >128 4 >128 16 >8 >256 >64 >128 0.12 | 0.25 | 2    |
| 1122568           | 40        | Argentina | Buenos Aires | K. pneumoniae | >64  8  0.25 | >128 16 1 >16 >8 32 >64 >128 1 | 0.5  | >2   |
| 1125511           | 215       | Taiwan  | Taipei  | K. pneumoniae | >64  8  >64 | >128 4 >128 16 >8 8 >64 >64 >128 0.5 | 1   | >2   |
| 1116221           | 603       | Thailand | Bangkok | K. pneumoniae | >64 >16 >64 | >128 16 >128 16 >8 16 >64 >128 4 | 4   | >2   |
| 1102685           | 614       | Australia | Melbourne | E. cloacae | 64  4  >64 | >128 1 >128 16 >8 32 16 128 >64 >128 0.03 | ≤0.12 | 0.03 |
| 1108008           | 81        | Poland  | Warsaw  | E. cloacae | 64  4  64 | >128 4 128 16 >8 128 >64 >128 2 | 2   | >2   |
| 1118254           | 81        | Poland  | Warsaw  | E. cloacae | >64  16 >64 | >128 2 >128 16 >8 32 >64 >128 0.12 | 0.5  | 2    |

ATM, aztreonam; ATM/AVI, aztreonam/avibactam (at fixed concentration of 4 mg/L); ATM/CLA, aztreonam/clavulanate (at fixed concentration of 4 mg/L); CAZ, ceftazidime; CAZ/AVI, ceftazidime/avibactam; CAZ/CLA, ceftazidime/clavulanate; COZ/TZB, ceftolozane/tazobactam (at fixed concentration of 4 mg/L); CRO, ceftriaxone; FEP, cefepime; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; MEM, meropenem; IMP, imipenem; ETP, ertapenem.
Table 2. Isolates exhibiting ATM/AVI MIC results $\geq 4$ mg/L selected for further characterization and main resistance mechanisms documented

| Collection number | Organism | MIC (mg/L) | ATM/AVI | CAZ/AVI | MLST | β-lactamase genes | mRNA expression$^a$ | Amino acid alterations |
|-------------------|----------|------------|---------|---------|------|-------------------|---------------------|-----------------------|
| 1108470           | *E. coli* | 8          | 2       | 410     |      | CMY-42, CTX-M-15, OXA-1 | 3.6                 | 1.2                   | WT                    | WT                    | R333insYRIK           |
| 1108523           | *E. coli* | 8          | 2       | 410     |      | CMY-42, OXA-1 | 3.8                 | <1                    | WT                    | L14Q                  | R333insYRIK           |
| 1108694           | *E. coli* | 8          | 2       | 410     |      | CMY-141 | 2.7                 | <1                    | WT                    | G137D                 | R333insYRIK           |
| 1114251           | *E. coli* | 16         | 8       | 410     |      | CMY-42, CTX-M-15, OXA-1, TEM-1 | 3.5                 | <1                    | WT                    | WT                    | R333insYRIK           |
| 1114255           | *E. coli* | 8          | 8       | 410     |      | OXA-48, CMY-42, CTX-M-14, TEM-190 | 2.3                 | <1                    | WT                    | WT                    | R333insYRIK           |
| 1118669           | *E. coli* | 4          | >32     | 410     |      | NDM-5, OXA-181, CMY-2, CTX-M-15, OXA-1, TEM-1 | 2.4                 | <1                    | WT                    | R199L                 | R333insYRIK           |
| 1116284           | *E. coli* | 8          | 4       | 405     |      | CTX-M-15, OXA-1 | <1                 | <1                   | N258X                 | WT                    | R333insYRIK           |
| 1128667           | *E. coli* | 4          | 1       | 405     |      | CTX-M-55, OXA-1 | <1                 | <1                   | N258X                 | WT                    | R333insYRIK           |
| 1130864           | *E. coli* | 8          | 8       | 405     |      | CTX-M-15, OXA-1 | <1                 | <1                   | N258X                 | WT                    | R333insYRIK           |
| 1119657           | *E. coli* | 8          | 2       | 38      |      | CMY-42, OXA-1 | <1                 | <1                   | WT                    | WT                    | R333insYRIK           |
| 1126350           | *E. coli* | 16         | 4       | 617     |      | CMY-42, CTX-M-27 | 4.5                 | <1                    | WT                    | WT                    | R333insYRIK           |
| 1122568           | *K. pneumoniae$^a$* | 8          | 16     | 872     |      | PER-2, SHV-11 | 1.1                 | NA                   | Y286X                 | WT                    | M6T, A33V, V411, L370I, Q374K, H396R, E434A, I447M, N455S, L577Q, A578G |
| 1125511           | *K. pneumoniae$^a$* | 8          | 4       | 15      |      | DHA-1, SHV-28 | 5.5                 | NA                   | A119X                 | WT                    | Y432C                 |
| 1116221           | *K. pneumoniae$^a$* | 16         | 16     | 273     |      | DHA-1, LAP-2, SHV-11, TEM-1 | 6.2                 | NA                   | WT                    | Y43X                  | WT                    |
| 1102685           | *E. cloacae* | 4          | 1       | 350     |      | ACT-27 | <1                 | 3667                  | A19S, S159L, Y199F, E200D, Y208L, E224K, G225A, G234E, L235M, Y236H, T243K, N276A, Q276A, F277H, D278_F279insENT | P177A                 | G306V                 |
| 1108008           | *E. cloacae* | 4          | 4       | 121     |      | ACT-25, CTX-M-15, OXA-1, TEM-1 | <1                 | 3060                  | WT                    | WT                    | E258_S259insE         |
| 1118254           | *E. cloacae* | 16         | 2       | 78      |      | ACT-24, SHV-12, TEM-1 | <1                 | 570                   | WT                    | P177A, D188E          | E258_S259insE         |

ATM/AVI, aztreonam/avibactam; CAZ/AVI, ceftazidime/avibactam.

$^a$Reported expression results are relative to a control isolate.

$^b*K. pneumoniae$ isolates had WT sequences of OmpK37.
Aztreonam/avibactam activity against Enterobacterales

Aztreonam/ and ceftazidime/clavulane MICs (0.25–1 mg/L) were 16- to 32-fold lower than when these drugs were combined with avibactam against a PER-2 producer (1122568). Avibactam seems to inhibit PER-2 to a lesser extent than other ESBLs, which can partially explain the elevated MICs.16 Notably, clavulanate did not bring the aztreonam (0.25 mg/L) and ceftazidime (1 mg/L) MICs down to WT levels (modal MIC, 0.03 mg/L and 0.12 mg/L, respectively; data not shown). The absence of OmpK35 or OmpK36 does not significantly affect susceptibility to ceftazidime.17 However, the absence of both porins or absence of any porin and the presence of an ESBL increases the ceftazidime MIC around 4-fold, which seems to fit the results observed for 1122568.17

The remaining K. pneumoniae isolates 1116221 and 1125511 produced DHA-1. The former isolate had a premature stop codon within OmpK36, whereas the latter isolate had a premature stop codon within OmpK35. Both isolates expressed moderate levels of AcrAB-ToIC. Nicolas-Chanoine et al.18 demonstrated that a DHA-1-producing K. pneumoniae strain exhibited a ceftazidime/avibactam MIC of 2 mg/L, and isogenic strains expressing DHA-1 and additional resistance mechanisms associated with drug influx or efflux had MICs of 4–16 mg/L. These results are consistent with those obtained here (MIC, 4–16 mg/L) and suggest that the aztreonam/avibactam and ceftazidime/avibactam MICs obtained against isolates 1116221 and 1125511 were likely due to the production of DHA-1 in combination with drug efflux and porin deficiencies.9

One possible hypothesis for the elevated aztreonam/avibactam MICs in isolates 1102685 and 1108008 (MIC, 4 mg/L) would be the similar elevated expression of AmpC. It is tempting to speculate that the amount of enzyme produced could overcome the in vitro inhibitory capability of avibactam used at 4 mg/L. However, while isolate 1108008 had a WT PBP3 sequence, isolate 1102685 showed a G306V mutation. This glycine is located within the n3 loop region. Although it is considered a conserved amino acid, it is situated at the opposite side of the active β-lactam binding site and may not affect enzyme–substrate affinities, unless G306V causes conformational changes in the PBP3 structure that affect the active site. E. cloacae 1118254 had a higher aztreonam/avibactam MIC (16 mg/L), but a much lower expression of AmpC compared with isolates 1102685 and 1108008. However, isolate 1118254 had a glutamic acid insertion in the transpeptidase domain (amino acid 237–577) of PBP3. This insertion was previously reported in an E. cloacae that displayed an aztreonam/avibactam MIC of >8 mg/L,19 and it is located adjacent to the conserved alanine at position 257 at the end of the n3 loop, which adjoins the active binding site.20

This study further analysed 17 (17/8787; 0.2%) Enterobacteriales isolates that showed a decreased susceptibility to aztreonam/avibactam to discern their associated resistance mechanisms. In summary, E. coli tended to be carbapenem susceptible and produce an altered PBP3, likely as a relevant aztreonam/avibactam resistance mechanism acting in conjunction with the β-lactamase background.11 The K. pneumoniae showed multiple mechanisms, whereas the E. cloacae did not show clear evidence to explain their elevated MICs, other than an overexpression of AmpC.

Acknowledgements
We would like to thank all participants of the SENTRY Antimicrobial Surveillance Program for providing bacterial isolates. We would also like to thank Amy Chen, Judy Oberholser and Sean DeVries for editorial assistance.

Funding
This study at JMI Laboratories was supported by Pfizer Inc. (New York, NY, USA). Pfizer was involved in the decision to present these results.

Transparency declarations
M.C., T.B.D., J.M.S., H.S.S. and R.E.M. are employees of JMI Laboratories, which was a paid consultant to Pfizer in connection with the development of this study and manuscript. F.F.A. is an employee of Pfizer, Inc.

JMI Laboratories contracted to perform services in 2020 for Affinity Biosensors, Allergan, Amicrobe, Inc., Amplyx Pharma, Artugen Therapeutics USA, Inc., Astellas, Basileea, Beth Israel Deaconess Medical Center, BiDMC, bioMerieux, Inc., BioVersys Ag, Bugworks, Cidara, Cipla, Contrafect, Cordymex, Crestone, Inc., Curza, CXC7, Entasis, Federa Pharmaceutical, Fimbrius Therapeutics, Fox Chase, GlaxoSmithKline, Guardian Therapeutics, Hardy Diagnostics, IHMA, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, KPB Biosciences, Luminex, Matrivix, Mayo Clinic, Medpace, Meiji Seika Pharma Co., Ltd., Melinta, Menarini, Merck, Meridian Bioscience Inc., Micromyx, MicuRx, N8 Medical, Narbiva, National Institutes of Health, National University of Singapore, North Bristol NHS Trust, Novome Biotechnologies, Paratek, Pfizer, Prokaryotics Inc., QPEX Biopharma, Rhode Island Hospital, RHIML, Roche, Roivant, Salvat, Scynexis, Selux Diagnostics, Shionogi, Specific Diagnostics, Spero, SuperTrans Medical LT, T2 Biosystems, The University of Queensland, Thermo Fisher Scientific, Tufts Medical Center, Universite de Sherbrooke, University of Iowa, University of Iowa Hospitals and Clinics, University of Wisconsin, UNT System College of Pharmacy, URMC, UT Southwestern, VenatoRx, Viosera Therapeutics and Wayne State University. There are no speakers’ bureaus or stock options to declare.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online.

References
1 CDC. Antibiotic resistance threats in the United States. 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf.
2 ECDC. Surveillance of antimicrobial resistance in Europe (EARS-Net) 2018. 2019. https://www.ecdc.europa.eu/sites/default/files/documents/surveil lance-antimicrobial-resistance-Europe-2018.pdf.
3 WHO. Global action plan on antimicrobial resistance. http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf.
4 Cornely OA, Cisneros JM, Torre-Cisneros J et al. Pharmacokinetics and safety of aztreonam/avibactam for the treatment of complicated intra-abdominal infections in hospitalized adults: results from the REJUVENATE study. J Antimicrob Chemother 2020; 75: 618–27.
5 Ehmann DE, Johic H, Ross Pt et al. Kinetics of avibactam inhibition against Class A, C, and D β-lactamases. J Biol Chem 2013; 288: 27960–71.
6 Marshall S, Hujer AM, Rajas LJ et al. Can ceftazidime-avibactam and aztreonam overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae? Antimicrob Agents Chemother 2017; 61: e02243-16.
7 Sader HS, Carvalhaes CG, Arends SJR et al. Aztreonam/avibactam activity against clinical isolates of Enterobacteriales collected in Europe, Asia and Latin America in 2019. J Antimicrob Chemother 2021; 76: 659–66.
8 CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Eleventh Edition: M07. 2018.

9 CLSI. Performance Standards for Antimicrobial Susceptibility Testing—Thirtieth Edition: M100. 2020.

10 Mendes RE, Jones RN, Woosley LN et al. Application of next-generation sequencing for characterization of surveillance and clinical trial isolates: analysis of the distribution of \( \beta \)-lactamase resistance genes and lineage background in the United States. Open Forum Infect Dis 2019; 6: S69–78.

11 Castanheira M, Costello SE, Woosley LN et al. Evaluation of clonality and carbapenem resistance mechanisms among Acinetobacter baumannii-Acinetobacter calcoaceticus complex and Enterobacteriaceae isolates collected in European and Mediterranean countries and detection of two novel \( \beta \)-lactamases, GES-22 and VIM-35. Antimicrob Agents Chemother 2014; 58: 7358–66.

12 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: 1420–8.

13 Sadek M, Juhas M, Poirel L et al. Genetic features leading to reduced susceptibility to aztreonam-avibactam among metallo-\( \beta \)-lactamase-producing Escherichia coli isolates. Antimicrob Agents Chemother 2020; 64: e01659-20.

14 Periasamy H, Joshi P, Palwe S et al. High prevalence of Escherichia coli clinical isolates in India harbouring four amino acid inserts in PBP3 adversely impacting activity of aztreonam/avibactam. J Antimicrob Chemother 2020; 75: 1650–1.

15 Karlowsky JA, Kazmierczak KM, de Jonge BLM et al. In vitro activity of aztreonam-avibactam against Enterobacteriaceae and Pseudomonas aeruginosa isolated by clinical laboratories in 40 countries from 2012 to 2015. Antimicrob Agents Chemother 2017; 61: e00472-17.

16 Ruggiero M, Papp-Wallace KM, Taracila MA et al. Exploring the landscape of diazabicyclooctane (DBO) inhibition: avibactam inactivation of PER-2 \( \beta \)-lactamase. Antimicrob Agents Chemother 2017; 61: e02476-16.

17 Tsai YK, Fung CP, Lin JC et al. Klebsiella pneumoniae outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother 2011; 55: 1485–93.

18 Nicolas-Chanoine MH, Mayer N, Guyot K et al. Interplay between membrane permeability and enzymatic barrier leads to antibiotic-dependent resistance in Klebsiella pneumoniae. Front Microbiol 2018; 9: 1422.

19 Mushtaq S, Vickers A, Doumith M et al. Activity of \( \beta \)-lactam/toniborbac- tam (VNRX-5133) combinations against carbapenem-resistant Gram-negative bacteria. J Antimicrob Chemother 2021; 76: 160–70.

20 Sun S, Selmer M, Andersson DI. Resistance to \( \beta \)-lactam antibiotics conferred by point mutations in penicillin-binding proteins PBP3, PBP4 and PBP6 in Salmonella enterica. PLoS One 2014; 9: e97202.