# AmpC β-lactamase induction by avibactam and relebactam

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**Abstract.**

**Background**. Diazabicyclooctanes, e.g. avibactam and relebactam, are a new class of β-lactamase inhibitors. Their spectrum includes AmpC enzymes, but it is important to understand if they also induce these enzymes. **Methods.** Levels of *ampC* mRNA were measured by RT-PCR during 4h exposure of *Enterobacter cloacae*, *Citrobacter freundii* and *Pseudomonas aeruginosa* (n=5 strains per species) to avibactam, relebactam and cefoxitin at 0, 1, 4 and 32 mg/L. The method had low precision compared with conventional specific-activity-based induction assays, which are impracticable for inhibitors. Accordingly, induction was only considered to be significant if induction ratios >10-fold were found at two consecutive time intervals, with ‘strong induction’ if one of more ratio was $\geq$100. **Results**. Cefoxitin, as expected, gave concentration-dependent induction for all strains, with strong induction for 13/15. At the other extreme, relebactam caused no significant induction for any strain. Avibactam gave strain-variable results, with strong concentration-dependent induction for 2/5 *E. cloacae* and 2/5 *P. aeruginosa* but little or no induction for the other strains, including all the *C. freundii.* **Conclusions.** Avibactam, but not relebactam, had some strain-variable ability to induce AmpC enzymes though at concentrations (32 mg/L) above those reached in the patient.

## Introduction

Diazabicyclooctanes (DBOs) such as avibactam and relebactam inhibit AmpC β-lactamases .[1,2] It is of interest to know if they also induce these enzymes, both to answer the question of whether a non-β-lactam can induce and because induction hypothetically might lead to antagonism if the DBO is combined with a weak-inducer β-lactam and the AmpC enzyme had mutated so as to become resistant to inhibition by DBOs. On this basis we examined the AmpC inducer behaviour of avibactam and relebactam for *Enterobacter cloacae*, *Citrobacter freundii* and *Pseudomonas aeruginosa*, as the species where these enzymes are most important.

Because it is impracticable to measure β-lactamase specific activity when an inducer is also an inhibitor, we adopted an alternative approach, using RT-PCR to measure the levels of AmpC-encoding mRNA.

## Materials and Methods

*Organisms*

The test strains were reference submissions to PHE, collected in 2010-11, or were from an earlier UK survey.3 They comprised five isolates each of *E. cloacae*, *C. freundii* and *P. aeruginosa*. The *E. cloacae* and C*. freundii* strains were confirmed as AmpC inducible, based on being susceptible (MICs $\leq$1 mg/L) to cefotaxime and ceftazidime but resistant to cefoxitin, with antagonism of cefotaxime and ceftazidime by cefoxitin in double disc tests;[4] *P. aeruginosa* isolates were AmpC inducible based on being susceptible to carbenicillin (MIC $\leq$128 mg/L) and ceftazidime (MIC $\leq$2 mg/L), with antagonism of ceftazidime by imipenem in double disc tests. All the strains were susceptible to imipenem at CLSI breakpoints; MICs of avibactam and relebactam ranged from 16->128 mg/L.

*Antibiotics*

Avibactam and ceftaroline were provided by AstraZeneca (Wilmington, Delaware, USA); imipenem and relebactam were supplied by Merck Sharp & Dohme Corp. (Whitehouse Station, NJ, USA); ceftazidime and cefoxitin was purchased from Sigma (Poole, Dorset, UK).

*Susceptibility tests*

MICs were determined by CLSI agar dilution.[5]

*Induction assays*

Isolates were grown overnight in 10-mL volumes of LB broth, with 1-mL amounts of these cultures then used to inoculate 100-mL volumes of fresh LB. The diluted cultures were incubated with shaking to $OD_{600}$ of 0.4-0.5, then inducers (cefoxitin, avibactam or relebactam) were added at 0, 1, 4 or 32 mg/L. Cultures were sampled immediately before this addition and at 30, 60, 120 and 240 minutes thereafter, with 0.5 mL samples transferred to 2-mL tubes containing 1 mL of RNAprotect (Qiagen, Manchester UK). These samples were mixed, centrifuged at 13000 rpm for 10 min, with the pellets retained at -80°C pending RNA extraction.

*RNA extraction*

Cellular RNA was extracted with an RNA Purification 96-Well Kit (Norgen, Thorold, Canada), used according to manufacturer's instructions. Briefly, the bacterial pellet was resuspended in 75 µL of TE buffer containing 1 mg/mL lysozyme and incubated at room temperature for 5 min. Afterwards, 225 µL of Lysis Solution was added followed, after mixing, by 120 µL of 95-100% ethanol. The resulting lysate was transferred to a 96-well filter plate and the RNA binding, wash, and elution steps were followed. On-filter genomic DNA digestion was performed using the RNase-free DNase I Kit (Norgen), used in accordance with the manufacturer's instructions.

*RT-PCR assay*.

Primers (Sigma) and probes (Applied Biosystems, Life Technologies, Paisley, UK) were as detailed in Table 1. Probes were labelled with either 6-FAM (6-carboxy-fluorescein) or VIC® at the 5' end, and with TAMRA (6-carboxy-tetramethyl-rhodamine) at the 3' end. RT-PCR was performed using the TaqMan RNA-to-$C_T$ 1-Step kit (Applied Biosystems). Each reaction was prepared in a 20-µL volume and contained: 1 x TaqMan RT-PCR mix, 0.5 µL of RT enzyme mix, 500 nM of each primer, 250 nM of each probe and 1 µL of RNA template. The RT-PCR consisted of a reverse transcription step for 15 min at 48°C, followed by an activation step of 10 min at 95°C and 40 cycles of denaturation for 15 sec at 95°C and anneal/extension for 1 min at 60°C. The absence of genomic DNA contamination was verified for each RNA preparation by running RT-PCR without reverse transcriptase. The reactions and data analyses were conducted using the Fast Real-Time PCR System 7500 (Applied Biosystems). Reactions were performed in triplicate. cDNA derived from expression of *ampC* was measured relative to that arising from housekeeping genes, namely *guaA* in *P. aeruginosa*, *rpoB* in *C. freundii* and *rspL* in *E. cloacae*, thereby correcting for differences in the amount of starting material. These standardised estimates of *ampC* transcript-derived cDNA were then re-standardised against *ampC* transcript-derived cDNA in the non-induced culture at the same time point. Relative quantification was carried out by using the $2^{-\Delta\Delta Ct}$ method, where the Ct value is defined as the first PCR cycle at which the fluorescence is above the threshold value of 0.2, as recommended by the thermal cycler instrument manufacturer.[6] An induction ratio was thus defined as: (time t *ampC* signal ÷ time t housekeeping signal) / (time 0 *ampC* signal ÷ time 0 housekeeping signal), with results averaged across the three replicate mixtures.

## Results and Discussion

*Susceptibility*

The test strains – which were confirmed as AmpC-inducible – all were susceptible to ceftazidime and imipenem in the absence of DBOs (Table 2). *C. freundii* H121940571 was narrowly resistant to ceftaroline (MIC 1 mg/L *versus* a breakpoint of 0.5 mg/L); all the *P. aeruginosa* strains tested (5/5) also had inherent resistance to ceftaroline, as is typical of the species. Addition of DBOs caused small reductions in the MICs of the partner β-lactams (Table 2), typically 2- to 4- fold. No antagonism was seen.

*Induction assays*

RT-PCR-based induction assays (Table 3) proved less precise than those based on measurement of β-lactamase specific activity (see e.g. ref 7), no doubt owing to the much more complex multi-step method needed for estimation, and perhaps also because mRNA persists more briefly than induced AmpC enzyme. This variability is reflected in the scatter of induction ratios, from 0.1-58, for the $T_0$ estimates, where values around unity would be expected. Moreover, assays for avibactam and relebactam were run several months apart, each time with cefoxitin as a control, and, whilst both sets of experiments showed that cefoxitin induced strongly, there was considerable inter-run scatter for results with this cephamycin, without clear systematic bias (not shown). On this basis we only considered induction significant if induction ratios >10 were obtained for at least two successive time points, whilst 'Strong' induction was taken as one ratio $\geq$100, with a ratio >10 at the preceding or subsequent time point. Based upon these criteria, cefoxitin counted as an inducer for all 15 strains and a strong inducer for all except one *C. freundii* and one *P. aeruginosa*. The rises in AmpC mRNA were greatest and most prolonged at the highest cefoxitin concentration (32 mg/L), but induction was often also apparent with the drug at 4 mg/L, confirming a dose-response relationship. These data are in keeping with a considerable body of data from conventional induction assays.[7]

Relebactam, at the other extreme, gave no convincing evidence of induction for any strain, with only two isolated instances of ratios >10, neither of them supported by raised ratios at adjacent time points nor with any relation to concentration. Avibactam had more variable behaviour, meeting our definitions of a strong inducer for 2/5 *E. cloacae* and 2/5 *P. aeruginosa* at highest avibactam concentration (32 mg/L). However there was no significant induction for the other 11/15 strains, including all the *C. freundii*, nor at lower avibactam concentrations. Miossec *et al.*[8] studied a further three *E. cloacae* by similar methodology and found no AmpC induction by avibactam at up to 64 mg/L.

Strain-to-strain differences in inducer response to avibactam may be a thresholding effect, with the top concentration tested being on the border of that needed for induction, whilst the differences in inducer power between avibactam and relebactam may reflect difference in the strength of PBP interactions. By itself avibactam has greater activity and lower MICs than relebactam, albeit with values significantly above the clinical range, and has been shown by several researchers to bind to PBP2 of Enterobacteriaceae.[9-11] One group also found binding to PBP4.[10] Linking these observations to inducer power is however speculative. The higher MICs of relebactam may relate to uptake rather than PBP affinity; moreover the precise links between PBP inhibition and the perturbation of the peptidoglycan fragment recycling that regulates AmpC induction[12] remain elusive, perhaps because PBP assays only detect the formation of covalent adducts, not other interactions. Clavulanic acid, which likewise binds PBP2[13] is an inducer for some strains,[14] but mecillinam, which also binds this target, has little inducer power.[15] PBP4 interactions, as found for avibactam by one group[10] have been suggested to be a correlate of AmpC induction in *P. aeruginosa.*[16]

Any practical significance of AmpC induction by avibactam is doubtful. Significant induction with avibactam, where it occurred, was only seen with 32 mg/L avibactam, a concentration around the $C_{max}$ following a standard 500 mg dosage and therefore far above the mean inter-dose level.[17,18,19] Moreover induced enzyme should be inhibited, and ceftazidime-avibactam is active against strains with derepressed AmpC, producing more enzyme than is ever likely to be induced.[1,2] The only circumstances in which this induction might become clinically significant would be if the AmpC enzyme (i) mutated to lose affinity for avibactam and (ii) remained inducible. Avibactam-induced enzyme might then attack its partner cephalosporin. Protein sequence changes within AmpC, arising via mutation, can engender resistance to ceftaroline/avibactam and ceftazidime/avibactam[20] (also PHE, data on file), however these seem more likely to be selected, if at all, once the enzyme expression is already derepressed, not when it remains inducible. We therefore consider the present data largely of academic interest, in showing that a non-β-lactam can act as an AmpC inducer as well as inhibiting β-lactamases and targeting PBP2.

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**Transparency declaration**

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**Table 1.** Primers and probes used in RT-PCR

| Species | Primer/probe | Sequence (5' – 3') |
|---|---|---|
| *P. aeruginosa* | pse_guaA_F | CTGACCTGCGTGTTCGTC |
| | pse_guaA_R | GAACATGGCCATCACCTG |
| | pse_ampC_F | ATGAAGGCCAATGACATTCC |
| | pse_ampC_R | CCATAGCTGAAGTAATGCGG |
| | pse_guaA | VIC-CTGCTGCGCCTGCACGAAG-TAMRA |
| | pse_ampC | 6-FAM-TCTCCTTTCAGGCTGATGGCTACGG-TAMRA |
| *E. cloacae* | ent_rspL_F | ACGTACAGCACCACGACG |
| | ent_rspL_R | AGCGTGTCTTCCAGACTCAC |
| | ent_ampC_F | CGGATGAGGTCACGGATAAC |
| | ent_ampC_R | TGGCGTTGGCGTAAAGA |
| | ent_rspL | VIC-CACTCTCCGGTAGTTGACAGCATTGCT-TAMRA |
| | ent_ampC | 6-FAM-ACTGCGGCTGCCAGTTTTGATAAAAG-TAMRA |
| *C. freundii* | cit_rpoB_F | CGTACACCCGACTCACTACG |
| | cit_rpoB_R | AGACCGATGTTCGGACCTT |
| | cit_apmC_F | GTGATATGTACCAGGGATTAGGC |
| | cit_ampC_R | AATGCCACTTTGCTGTCG |
| | cit_rpoB | VIC-CGCGTATGTCCAATCGAAACGC-TAMRA |
| | cit_ampC | 6-FAM-ATCGAATCAGCTTTCAGCGGCC-TAMRA |

**Table 2.** MICs (mg/L) for test strains, determined by BSAC agar dilution

| | Ceftazidime | | Ceftaroline | | Imipenem | | DBOs alone | |
|---|---|---|---|---|---|---|---|---|
| | Alone | +AVI, 4 mg/L | Alone | +AVI, 4 mg/L | Alone | +REL 4 mg/L | AVI | REL |
| *E. cloacae* | | | | | | | | |
| H101440920 | 0.5 | 0.5 | 0.5 | 0.25 | 0.5 | 0.125 | 16 | 128 |
| H111900378 | 0.25 | 0.25 | 0.25 | 0.125 | 0.5 | 0.25 | 32 | 128 |
| SE04013 | 0.5 | 0.25 | 0.5 | 0.5 | 0.5 | 0.25 | 32 | 128 |
| SE04027 | 0.5 | 0.25 | 0.25 | 0.125 | 0.25 | 0.125 | 32 | 128 |
| SE06012 | 0.25 | 0.25 | 0.25 | 0.125 | 0.5 | 0.25 | 32 | 128 |
| *C. freundii* | | | | | | | | |
| H103540377 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 | 0.125 | 128 | >128 |
| H121940571 | 0.5 | 0.125 | 1 | 0.06 | 0.25 | 0.25 | 128 | >128 |
| LN10083 | 0.5 | 0.125 | 0.25 | 0.06 | 0.25 | NT | NT | NT |
| SE02016 | 0.5 | 0.125 | 0.25 | 0.06 | 0.25 | 0.25 | >128 | >128 |
| SE02071 | 0.5 | 0.125 | 0.5 | 0.12 | 0.5 | 0.25 | >128 | >128 |
| *P. aeruginosa* | | | | | | | | |
| H111840682 | 2 | 1 | 8 | 1 | 0.25 | 0.25 | >128 | >128 |
| H112220257 | 2 | 1 | 32 | 8 | 0.5 | 0.25 | >128 | >128 |
| H114900202 | 2 | 2 | 8 | 8 | 2 | 0.5 | >128 | >128 |
| H114980582 | 2 | 2 | N/T | N/T | 2 | 0.25 | >128 | >128 |
| H115280631 | 2 | 2 | 16 | 2 | 0.5 | 0.5 | >128 | >128 |

Cefoxitin MICs were >128 mg/L for all isolates

**Notes to Table 2**. Isolates with numbers starting LN or SE were collected in a London and Southeast England survey of resistance in 2004;[3] those with numbers starting H10, H11 and H12 were submissions to PHE's Antimicrobial Resistance and Healthcare Associated Infection Reference Unit in 2010, 2011 and 2012 respectively. Abbreviations: AVI, avibactam; NT, not tested; REL, relebactam.

**Table 3.** AmpC induction ratios for isolates exposed to cefoxitin and DBOs

| Strain | Inducer | Induction period (minutes) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 120 | 240 |
| *E. cloacae H101440920* | Cefoxitin 1 mg/L | 1.5 | 50 | 23 | 0.85 | 0.95 |
| | Cefoxitin 4 mg/L | 1.7 | 2600 | 2700 | 8.7 | 0.75 |
| | Cefoxitin 32 mg/L | 1.6 | 840 | 65 | 25 | 73 |
| | Avibactam 1 mg/L | 2.3 | 1.4 | 0.7 | 1.1 | 0.8 |
| | Avibactam 4 mg/L | 1.8 | 1.1 | 26 | 3.5 | 0.7 |
| | Avibactam 32 mg/L | 1.4 | 8900 | 6900 | 3600 | 270 |
| | Relebactam 1 mg/L | 1.3 | 0.8 | 1.0 | 0.7 | 0.9 |
| | Relebactam 4 mg/L | 0.8 | 0.9 | 1.4 | 1.5 | 0.7 |
| | Relebactam 32 mg/L | 1.2 | 1.4 | 1.4 | 1.5 | 0.8 |
| *E. cloacae H111900378* | Cefoxitin 1 mg/L | 1.2 | 0.85 | 0.75 | 1.2 | 1.1 |
| | Cefoxitin 4 mg/L | 0.35 | 30 | 0.8 | 1.3 | 0.85 |
| | Cefoxitin 32 mg/L | 0.3 | 110 | 35 | 29 | 20 |
| | Avibactam 1 mg/L | 0.1 | 1.6 | 0.8 | 1.4 | 1.2 |
| | Avibactam 4 mg/L | 0.1 | 4.6 | 0.6 | 1.1 | 1.0 |
| | Avibactam 32 mg/L | 0.1 | 4600 | 7400 | 1.7 | 1.0 |
| | Relebactam 1 mg/L | 0.5 | 0.8 | 1.5 | 2.1 | 1.3 |
| | Relebactam 4 mg/L | 0.5 | 0.8 | 1.8 | 2.2 | 1.5 |
| | Relebactam 32 mg/L | 1.3 | 1.1 | 1.6 | 1.4 | 1.7 |
| *E. cloacae SE04013* | Cefoxitin 1 mg/L | 1.3 | 1.2 | 1.1 | 1.4 | 1.4 |
| | Cefoxitin 4 mg/L | 1.1 | 67 | 2.7 | 1.1 | 1.0 |
| | Cefoxitin 32 mg/L | 1.3 | 2900 | 580 | 750 | 1700 |
| | Avibactam 1 mg/L | 0.8 | 0.7 | 1.2 | 0.5 | 2.1 |
| | Avibactam 4 mg/L | 2.0 | 1.0 | 0.6 | 0.4 | 2.2 |
| | Avibactam 32 mg/L | 1.3 | 0.8 | 0.6 | 0.4 | 2.2 |
| | Relebactam 1 mg/L | 2.0 | 1.7 | 1.7 | 1.8 | 1.2 |
| | Relebactam 4 mg/L | 2.7 | 1.5 | 2.2 | 2.1 | 1.8 |
| | Relebactam 32 mg/L | 2.6 | 1.4 | 2.4 | 2.1 | 1.0 |
| *E. cloacae SE04027* | Cefoxitin 1 mg/L | 1.2 | 2 | 0.65 | 1.05 | 1 |
| | Cefoxitin 4 mg/L | 1.6 | 480 | 1.1 | 1.4 | 0.8 |
| | Cefoxitin 32 mg/L | 1.8 | 4600 | 420 | 1300 | 360 |
| | Avibactam 1 mg/L | 1.2 | 2.0 | 1.0 | 2.8 | 0.9 |
| | Avibactam 4 mg/L | 1.9 | 4.6 | 1.7 | 1.3 | 1.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Avibactam 32 mg/L | 2.2 | 3.8 | 850 | 0.9 | 0.6 |
| | Relebactam 1 mg/L | 1.0 | 1.2 | 1.6 | 1.0 | 0.9 |
| | Relebactam 4 mg/L | 1.2 | 1.4 | 1.3 | 2.4 | 1.1 |
| | Relebactam 32 mg/L | 1.4 | 1.4 | 1.2 | 1.9 | 1.6 |
| *E. cloacae* SE06012 | Cefoxitin 1 mg/L | 0.8 | 1.2 | 13 | 1.1 | 1.0 |
| | Cefoxitin 4 mg/L | 1.6 | 26 | 220 | 1.8 | 1.0 |
| | Cefoxitin 32 mg/L | 1.5 | 1300 | 250 | 660 | 600 |
| | Avibactam 1 mg/L | 0.7 | 1.4 | 0.7 | 1.0 | 0.4 |
| | Avibactam 4 mg/L | 0.9 | 1.5 | 0.7 | 1.7 | 0.6 |
| | Avibactam 32 mg/L | 0.6 | 1.3 | 0.7 | 0.8 | 0.4 |
| | Relebactam 1 mg/L | 2.3 | 0.6 | 1.0 | 0.7 | 1.1 |
| | Relebactam 4 mg/L | 3.0 | 0.5 | 1.7 | 1.4 | 1.6 |
| | Relebactam 32 mg/L | 3.9 | 0.8 | 1.5 | 1.2 | 1.6 |
| *C. freundii H103540377* | Cefoxitin 1 mg/L | 1.3 | 11 | 13 | 1.7 | 1.6 |
| | Cefoxitin 4 mg/L | 1.2 | 100 | 31 | 12 | 4.5 |
| | Cefoxitin 32 mg/L | 1.4[a] | 180 | 64 | 89 | 22 |
| | Avibactam 1 mg/L | 1.3 | 1.0 | 0.9 | 1.3 | 0.9 |
| | Avibactam 4 mg/L | 1.2 | 0.6 | 0.9 | 1.0 | 1.0 |
| | Avibactam 32 mg/L | 1.6 | 0.8 | 1.3 | 1.0 | 0.8 |
| | Relebactam 1 mg/L | (32)[b] | 1.2 | 0.9 | 0.6 | 1.0 |
| | Relebactam 4 mg/L | 1.2 | 1.2 | 1.2 | 0.9 | 1.2 |
| | Relebactam 32 mg/L | 0.9 | 1.3 | 1.2 | 0.8 | 0.9 |
| *C. freundii H121940571* | Cefoxitin 1 mg/L | 0.8 | 6.3 | 9.7 | 5.0 | 1.1 |
| | Cefoxitin 4 mg/L | 1.0 | 30 | 20 | 42 | 9.6 |
| | Cefoxitin 32 mg/L | 1.9 | 75 | 43 | 120 | 41 |
| | Avibactam 1 mg/L | 1.0 | 1.1 | 1.3 | 1.6 | 3.2 |
| | Avibactam 4 mg/L | 0.5 | 1.4 | 0.8 | 1.6 | 2.2 |
| | Avibactam 32 mg/L | 1.1 | 0.8 | 1.0 | 0.8 | 1.1 |
| | Relebactam 1 mg/L | 1.9 | 0.7 | 0.7 | 0.8 | 1.0 |
| | Relebactam 4 mg/L | 2.0 | 0.8 | 0.8 | 0.8 | 1.1 |
| | Relebactam 32 mg/L | 2.4 | 1.2 | 0.8 | 0.7 | 1.0 |
| *C. freundii LN10083* | Cefoxitin 1 mg/L | 0.8 | 10 | 11 | 7.8 | 2.25 |
| | Cefoxitin 4 mg/L | 0.6 | 61 | 21 | 10 | 6.1 |
| | Cefoxitin 32 mg/L | 0.6 | 130 | 30 | 160 | 260 |
| | Avibactam 1 mg/L | 0.8 | 1.7 | 1.0 | 1.2 | 0.5 |

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|---|---|---|---|---|---|---|
| | Avibactam 4 mg/L | 1.0 | 0.9 | 1.0 | 1.1 | 0.9 |
| | Avibactam 32 mg/L | 0.8 | 1.4 | 1.3 | 0.7 | 0.5 |
| | Relebactam 1 mg/L | 1.1 | 1.1 | 0.6 | 0.4 | 1.6 |
| | Relebactam 4 mg/L | 1.2 | 1.4 | 0.7 | 0.5 | 1.8 |
| | Relebactam 32 mg/L | 1.1 | 1.4 | 0.8 | 0.4 | 1.9 |
| *C. freundii SE02016* | Cefoxitin 1 mg/L | 0.7 | 7.8 | 12 | 9.1 | 1.9 |
| | Cefoxitin 4 mg/L | 0.6 | 54 | 19 | 6.5 | 1.6 |
| | Cefoxitin 32 mg/L | 0.6 | 150 | 81 | 140 | 140 |
| | Avibactam 1 mg/L | 1.1 | 0.5 | 0.7 | 0.8 | 1.0 |
| | Avibactam 4 mg/L | 1.0 | 0.4 | 1.3 | 0.5 | 1.0 |
| | Avibactam 32 mg/L | 0.9 | 0.8 | 0.8 | 0.7 | 1.0 |
| | Relebactam 1 mg/L | 1.2 | 1.3 | 0.9 | 0.6 | 0.8 |
| | Relebactam 4 mg/L | 0.9 | 1.8 | 1.1 | 0.4 | 0.6 |
| | Relebactam 32 mg/L | 1.0 | 1.5 | 1.1 | 0.6 | 0.7 |
| *C. freundii SE02071* | Cefoxitin 1 mg/L | 0.9 | 3.3 | 5.1 | 1.4 | 0.9 |
| | Cefoxitin 4 mg/L | 0.5 | 27 | 26 | 4.9 | 6.7 |
| | Cefoxitin 32 mg/L | 0.8 | 70 | 47 | 75 | 59 |
| | Avibactam 1 mg/L | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| | Avibactam 4 mg/L | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| | Avibactam 32 mg/L | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| | Relebactam 1 mg/L | 1.3 | 1.2 | 1.0 | 0.6 | 1.0 |
| | Relebactam 4 mg/L | 1.4 | 1.1 | 1.0 | 0.5 | 1.1 |
| | Relebactam 32 mg/L | 1.7 | 1.2 | 1.3 | 0.6 | 0.9 |
| *P. aeruginosa* H111840682 | Cefoxitin 1 mg/L | 0.7 | 2.9 | 29 | 2.1 | 0.7 |
| | Cefoxitin 4 mg/L | 0.1 | 460 | 800 | 3.8 | 0.5 |
| | Cefoxitin 32 mg/L | 0.25 | 2700 | 780 | 23 | 9.2 |
| | Avibactam 1 mg/L | 0.3 | 2.2 | 0.8 | 0.0 | 0.0 |
| | Avibactam 4 mg/L | 0.3 | 0.7 | 0.5 | 0.7 | 0.1 |
| | Avibactam 32 mg/L | 0.2 | 170 | 860 | 182 | 19 |
| | Relebactam 1 mg/L | 0.3 | 0.5 | 1.4 | 0.3 | 0.6 |
| | Relebactam 4 mg/L | 0.8 | 1.6 | 2.8 | 1.0 | 0.3 |
| | Relebactam 32 mg/L | 0.3 | 1.6 | 1.4 | 1.5 | 0.7 |
| *P. aeruginosa* H112220257 | Cefoxitin 1 mg/L | 3.8 | 3500 | 39 | 1.6 | 2.1 |
| | Cefoxitin 4 mg/L | 2.3 | 33 | 4.0 | 1.2 | 3.6 |
| | Cefoxitin 32 mg/L | 0.8 | 250 | 13 | 0.6 | 9.5 |

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|---|---|---|---|---|---|---|
| | Avibactam 1 mg/L | 13 | 0.7 | 6.7 | 0.7 | 4.4 |
| | Avibactam 4 mg/L | 3.7 | 0.6 | 8.1 | 1.0 | 9.1 |
| | Avibactam 32 mg/L | 5.2 | 9.8 | 110 | 1.7 | 40 |
| | Relebactam 1 mg/L | 0.9 | 1.3 | 0.5 | 0.2 | 0.4 |
| | Relebactam 4 mg/L | 0.4 | 0.9 | 0.5 | 1.2 | 0.3 |
| | Relebactam 32 mg/L | 1.6 | 1.7 | 4.1 | 3.5 | 7.4 |
| *P. aeruginosa* H114900202 | Cefoxitin 1 mg/L | 1.2 | 84 | 5 | 1.3 | 0.8 |
| | Cefoxitin 4 mg/L | 4.1 | 84 | 12 | 0.7 | 1.4 |
| | Cefoxitin 32 mg/L | 2.1 | 14000 | 250 | 1.3 | 5.8 |
| | Avibactam 1 mg/L | 0.7 | 0.5 | 5.8 | 0.1 | 0.2 |
| | Avibactam 4 mg/L | 0.7 | 0.2 | 6.6 | 1.0 | 0.2 |
| | Avibactam 32 mg/L | 0.7 | 1.1 | 0.7 | 1.9 | 21 |
| | Relebactam 1 mg/L | 0.9 | 0.7 | 2.1 | 3.7 | 14 |
| | Relebactam 4 mg/L | 1.0 | 1.0 | 0.9 | 0.1 | 1.0 |
| | Relebactam 32 mg/L | 0.9 | 0.8 | 4.1 | 0.5 | 1.6 |
| *P. aeruginosa* H114980582 | Cefoxitin 1 mg/L | 3.1 | 53 | 4.9 | 3.0 | 3.35 |
| | Cefoxitin 4 mg/L | 1.9 | 43 | 33 | 11 | 170 |
| | Cefoxitin 32 mg/L | 2.6 | 8500 | 160 | 24 | 680 |
| | Avibactam 1 mg/L | 0.9 | 2.5 | 0.1 | 0.5 | 4.3 |
| | Avibactam 4 mg/L | 1.0 | 6.8 | 0.4 | 2.3 | 40.7 |
| | Avibactam 32 mg/L | 0.5 | 6.2 | 29.7 | 22000 | 1000 |
| | Relebactam 1 mg/L | 2.3 | 1.3 | 3.1 | 0.8 | 0.8 |
| | Relebactam 4 mg/L | 0.7 | 0.7 | 2.9 | 1.1 | 0.8 |
| | Relebactam 32 mg/L | 0.7 | 1.7 | 1.3 | 2.3 | 0.6 |
| *P. aeruginosa* H115280631 | Cefoxitin 1 mg/L | 0.4 | 0.9 | 1.2 | 0.5 | 0.5 |
| | Cefoxitin 4 mg/L | 0.9 | 0.7 | 7.1 | 1.25 | 0.3 |
| | Cefoxitin 32 mg/L | 0.3 | 41 | 36 | 13 | 0.8 |
| | Avibactam 1 mg/L | 0.1 | 0.0 | 0.7 | 0.0 | 3.5 |
| | Avibactam 4 mg/L | 0.0 | 0.1 | 0.2 | 0.4 | 1.3 |
| | Avibactam 32 mg/L | 0.1 | 0.0 | 8.0 | 0.1 | 1.1 |
| | Relebactam 1 mg/L | 0.8 | 0.9 | 1.3 | 1.0 | 0.1 |
| | Relebactam 4 mg/L | 4.9 | 0.8 | 1.4 | 0.9 | 1.8 |
| | Relebactam 32 mg/L | 1.0 | 0.9 | 1.4 | 1.0 | 0.1 |

Results for DBOs are averages of three technical replicates; those for cefoxitin are averages of two sets of three technical replicates, once as a control for each DBO, except:

[a] where one set of three replicates was excluded owing to test failure
[b] test failure