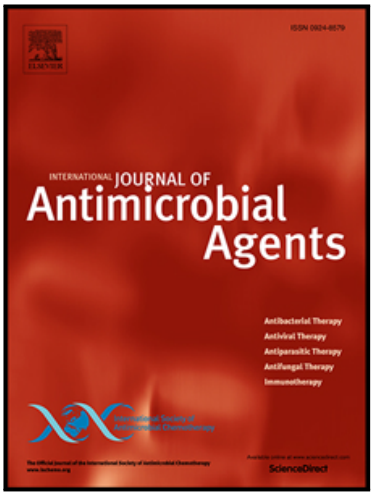
Editor: Dr. Po-Ren Hsueh

Journal Pre-proof



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PII: S0924-8579(23)00061-4
DOI: https://doi.org/10.1016/j.ijantimicag.2023.106776
Reference: ANTAGE 106776

To appear in: *International Journal of Antimicrobial Agents*

Received date: 12 August 2022
Accepted date: 2 March 2023

Please cite this article as: David M LIVERMORE , Shazad MUSHTAQ , Anna VICKERS , Neil WOODFORD , Activity of aztreonam/avibactam against metallo-β-Lactamase-producing Enterobacterales from the UK: impact of penicillin-binding protein-3 inserts and CMY-42 β-lactamase in Escherichia coli, *International Journal of Antimicrobial Agents* (2023), doi: https://doi.org/10.1016/j.ijantimicag.2023.106776

Highlights

- Aztreonam/avibactam had unimodal MICs for *Klebsiella* and *Enterobacter* with MBLs
- Aztreonam/avibactam MICs were multi-modal for NDM-positive *Escherichia coli* with 15% resistance, changing little from 2015 to 2019
- Raised MICs for *E. coli* reflected 4-amino-acid (YRIN or YRIK) PBP3 inserts
- YRIK - or YRIN plus AmpC - was linked to MICs 8-32 mg/L
- YRIN alone was linked to MICs 0.5-4 mg/L

Activity of aztreonam/avibactam against metallo-β-Lactamase-producing Enterobacterales from the UK: impact of penicillin-binding protein-3 inserts and CMY-42 β-lactamase in *Escherichia coli*

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Running head: Aztreonam/avibactam *vs.* MBL Enterobacterales

Abstract

Aztreonam/avibactam is being developed on the rationale that aztreonam evades metallo-β-lactamases (MBLs) whilst avibactam protects against co-produced serine β-lactamases. We measured the activity of aztreonam/avibactam against MBL-producing Enterobacterales referred to the UKHSA in 2015, 2017, 2019. MICs were determined by broth microdilution, genome sequences with Illumina technology. For *Klebsiella* and *Enterobacter* spp. with NDM, IMP or VIM enzymes, the MICs of aztreonam/avibactam were unimodally distributed, with >90% of isolates inhibited at 1+4 mg/L, and all inhibited at 8+4 mg/L. Over 85% of *Escherichia coli* with NDM carbapenemases were inhibited at 8+4 mg/L, but their MIC distribution was multimodal, with major peaks at 0.12 and 8 mg/L. Forty-eight of 50 NDM *E. coli* with 'high' aztreonam/avibactam MICs (defined as ≥8 mg/L) had YRIK inserted after amino-acid 333 of penicillin-binding protein (PBP)3 or had a YRIN insert plus an acquired AmpC β-lactamase, commonly CMY-42. Ten of 15 *E. coli* with 'moderately-raised' aztreonam/avibactam MICs (0.5-4 mg/L) had YRIN inserts without acquired AmpC. Twenty-two of 24 *E. coli* isolates with 'normal' MICs (0.03-0.25 mg/L) lacked PBP3 inserts. YRIK inserts were associated with *E. coli* ST405 and YRIN with ST167; however, many isolates with high or moderately-raised MICs were clonally diverse. No substantive MIC distribution shifts occurred across the 3 survey years; ST405 isolates with YRIK comprised more high-MIC organisms in 2019 *versus* earlier years, but the apparent excess lacked significance (P >0.05).

Keywords: Aztreonam/avibactam; *Escherichia coli*; Penicillin-binding protein (PBP)3; Metallo-β-lactamases; NDM carbapenemase; PBP inserts

1.0 Introduction

Ceftazidime/avibactam, imipenem/relebactam and meropenem/vaborbactam are widely active and increasingly used clinically against Enterobacterales producing KPC carbapenemases. Ceftazidime/avibactam is also active and used against those with OXA-48-related enzymes [1-3]. However, none of these recently licensed β-lactam/β-lactamase inhibitor combinations achieves useful activity against Enterobacterales with metallo-β-lactamases (MBLs), and none of the inhibitors inactivates MBLs.

One answer to this limitation is to combine aztreonam, which is stable to MBLs, with avibactam, which should protect against co-produced aztreonam-hydrolysing enzymes, including ESBLs and AmpC types [4]. Numerous case reports, and one 100-patient series, describe the successful coadministration of ceftazidime/avibactam together with aztreonam on this rationale [5,6], and an aztreonam/avibactam (1.5+0.5g, q6h) combination has successfully progressed through Phase II development, [7] with a Phase III trial underway.

Most MBL-producing Enterobacterales are susceptible to low concentrations of aztreonam/avibactam *in vitro*, but activity is not universal [6]. In particular, some *Escherichia coli* have inserts to penicillin-binding protein (PBP)3 that reduce its affinity for aztreonam and other β-lactams, raising the MICs of aztreonam/avibactam [6,8-10]. This mechanism appears particularly widespread in India [11], but has been reported across Asia, Europe and Africa.

In a recent study of 124 sequenced Enterobacterales with NDM MBLs, dating from 2015-16, we found 13/29 *Escherichia coli* were not inhibited by aztreonam/avibactam 8+4 mg/L compared with only 2/82 *Klebsiella* and 1/13 *Enterobacter* spp. [12]. A strong association existed between raised MICs for

aztreonam/avibactam and raised MICs for cefepime/taniborbactam. Several of the 'resistant' *E. coli* had PBP3 inserts and there was some association to sequence type, notably ST167, as well as to production of NDM-5 or -7 rather than NDM-1 β-lactamase. These findings led to the present study which aimed, first, to ascertain whether the proportion of *E. coli* and other Enterobacterales with raised aztreonam/avibactam MICs was changing over time and, secondly, to fully investigate possible mechanisms associated with raised aztreonam/avibactam MICs.

2.0 Materials and Methods

2.1 Bacteria

The test panel comprised a representative sample of MBL producers (n=464) referred to the UK Health Security Agency's Antimicrobial Resistance and Healthcare Associated Infection (AMRHAI) Reference Unit, largely from UK hospitals, in 2015, 2017 and 2019. In the case of *E. coli,* we tested 40-41 isolates from each year, all with NDM carbapenemases; for *Klebsiella* spp. we tested 61-66 isolates per year, with an approximate 4:1:1 ratio between those with NDM, VIM and IMP carbapenemases; for *Enterobacter* spp. we tested 50-52 isolates per year, with an approximate 3:1:1 ratio between those with NDM, VIM and IMP carbapenemases. These proportions mirror those seen among the totality of MBL-producing Enterobacterales referred to AMRHAI (data on file). *E. coli* with MBLs other than NDM types are extremely rare and were not represented. Individual isolates of the tested species and carbapenemase type were randomly selected from among a year's submissions, with obvious duplicates from the same patient avoided. Where a quota of isolates could not be met from submissions in the stated year, it was

supplemented from those in the subsequent year: thus, for example 2015 VIM-MBL-positive *Enterobacter* spp. include some isolates referred in 2016.

2.2 MIC determinations

MICs were determined using broth microdilution in Mueller-Hinton broth, following EUCAST/CLSI-compatible methodology, with pre-prepared plates sourced from IHMA (Schaumburg, Il, USA). These contained: aztreonam, 0.03-128 mg/L; aztreonam/avibactam 4 mg/L, 0.03-128 mg/L; meropenem 0.25-128 mg/L; ceftazidime, 0.25-128 mg/L; ceftazidime/avibactam 4 mg/L, 0.06-128 mg/L; amikacin, 0.25-64 mg/L; ciprofloxacin, 0.03-64 mg/L; colistin, 0.25-8 mg/L and tigecycline 0.03-8 mg/L. Results were related to EUCAST 2022 breakpoints [13], with prospective values of S $\leq$8+4 mg/L, R >8+4 mg/L adopted for aztreonam/avibactam, based upon similar dosage (1.5+0.5g q6h vs 2+1g q8h) and pharmacokinetics to ceftazidime/avibactam.

Synergy studies between aztreonam/avibactam 4 mg/L and EDTA 320 mg/L were performed by CLSI agar dilution [14].

2.3 Molecular characterisation

Isolates with aztreonam/avibactam MICs $\geq$8+4 mg/L were subjected to WGS on an Illumina HiSeq instrument. Antimicrobial resistance genes, plasmid types, and sequence types were identified and designated using ResFinder4.1 [15], PlasmidFinder2.1 [16] and MLST2.0 [17], respectively, accessed via the Center for Genomic Epidemiology website [18]. The PBP3-encoding gene, *ftsI*, was extracted and examined for insertions using UKHSA's in-house GeneFinder pipeline described previously [19].

3.0 Results

3.1 Overall resistance of the test collection

As is typical of MBL-producing Enterobacterales [20,21], those in the test panel mostly were highly resistant to standard antibiotics (Table 1). Fewer than 40% of any species/MBL group were susceptible to unprotected aztreonam, even based upon EUCAST's high-dose criteria (R, MIC >4 mg/L); virtually all were resistant to ceftazidime and its avibactam combination; and, except in the case of *Enterobacter* spp. with IMP and VIM enzymes, fewer than 25% counted as susceptible to high-dose ciprofloxacin. Meropenem MICs were above EUCAST's high dose breakpoint (MIC >8 mg/L) for over 75% of isolates with NDM MBLs, regardless of species. Although 51-71% of *Klebsiella* and *Enterobacter* spp. with IMP and VIM enzymes counted as high dose meropenem susceptible, the clinical utility of any carbapenem against carbapenemase producers is doubtful [20]. Amikacin remained active against over 80% of *Klebsiella* and *Enterobacter* spp. isolates with IMP or VIM MBLs but against fewer than 60% of those with NDM MBLs, regardless of species, with only 18.2% susceptible in the case of *Klebsiella* spp with NDM enzymes. Colistin alone remained active against over 90% of isolates in all species/MBL groups; tigecycline also did so in the case of *E. coli* with NDM enzymes but lacks EUCAST breakpoints for other Enterobacterales species; based upon the FDA breakpoint of S $\leq$2 mg/L, 304 of the 344 (88.4%) MBL-producing *Klebsiella* and *Enterobacter* spp. would count as susceptible.

Based on a prospective breakpoint of 8+4 mg/L aztreonam/avibactam would count as active against all MBL-producing *Klebsiella* (n=191) and *Enterobacter* (n=153) regardless of MBL type, and against 104 of the 122 (85.2%) *E. coli* with

NDM enzymes. Further detail is shown in Table 2: for all species/enzyme groups except *E. coli* with NDM MBLs, the MIC distributions were unimodal with little skew, and with modes, for all years combined, of 0.12 to 0.25 mg/L, or slightly higher (0.25-1 mg/L) in the case of *Enterobacter* spp with VIM MBLs. In contrast, the MIC distribution was bimodal for *E. coli* with NDM MBLs, with peaks around 0.13 mg/L and 8 mg/L. These patterns, including the complex MIC distribution for *E. coli* persisted in each of the 3 years considered, without obvious shifts in MIC modes or ranges.

3.2 Basis of bimodal MIC distribution for NDM E. coli

We prospectively sequenced 50 NDM-positive *E. coli* with aztreonam/avibactam MICs of 8-16 mg/L and 19 with aztreonam/avibactam MICs ≤1 mg/L; in addition, prior WGS data were available for 20 isolates with MICs from 0.03-4 mg/L. In practice, it proved useful to divide the isolates as three groups: those with MICs of 0.03-0.25 mg/L (hereafter 'normal MIC'), essentially corresponding to the distribution around the lower modal MIC, those with 'substantially raised' MICs of 8-32 mg/L (hereafter 'high MIC') encompassing the upper mode and an intermediate group, with MICs of 0.5-4 mg/L (hereafter 'moderate-raised MIC'), (Table 3).

WGS indicated that the moderately-raised and high MICs were strongly associated with four-amino acid inserts comprising either a Tyr-Arg-Ile-Asn (YRIN) duplication or Tyr-Arg-Ile-Lys (YRIK) after tyrosine 333 of the mature PBP3, but also with the carriage of CMY-42 or other plasmid AmpC β-lactamases. Coding sequences for YRIN and YRIK were TATCGAATTAAC and TATCGAATTAAA respectively, thus differing by only a single base.

Among the 50 isolates with high aztreonam/avibactam MICs (≥8 mg/L), 35 had YRIK and 14 had YRIN. Among the 35 with YRIK, 25 belonged to ST405 or, in

one case, to its single locus variant, whereas the remaining 10 belonged to eight different STs. Only eight of these 35 had genes for plasmid AmpC enzymes, with *bla*$_{CMY-42}$ present in five. By contrast, among the 14 'high-MIC' isolates with YRIN, 10 had *bla*$_{CMY-42}$ and three had other acquired AmpC types (variously CMY-6, -59 and -148 enzymes); only one lacked any acquired AmpC enzyme. Four of the 14 high-MIC isolates with YRIN belonged to ST167 and none to ST405. Only one isolate with an aztreonam/avibactam MIC $\geq$8 mg/L lacked a PBP3 insert. Perhaps of note, this isolate belonged to the internationally prevalent ST131 lineage and carried *bla*$_{OXA-10}$, encoding classical OXA-10 β-lactamase (see Discussion).

Among the 15 sequenced isolates with 'moderately-raised' aztreonam/avibactam MICs (0.5 to 4 mg/L), 13 had YRIN. Of the remaining two, one had YRIK, and one had no insert or other plausible resistance mechanism found; both these latter two belonged to ST405. In contrast to isolates with YRIN and high MICs ($\geq$8 mg/L), only three of the 15 isolates with YRIN and moderately-raised MICs also carried genes for acquired AmpC enzymes (*bla*$_{CMY-2}$ in two cases and *bla*$_{CMY-42}$ in one). Six of the 15 belonged to ST167; the remainder belonged to diverse types, with ST405 not represented.

Lastly, among 24 sequenced isolates with 'normal' aztreonam/avibactam MICs (0.03-0.25 mg/L) none had YRIK and only two had YRIN, with neither of these two having acquired AmpC. Among the entire 24, just four had acquired AmpC; two of these having DHA enzymes, which are atypical among AmpC types, e.g., in being inhibited by tazobactam [22]. One normal isolate (aztreonam/avibactam MIC 0.06 mg/L) had *bla*$_{OXA-10}$ (see Discussion). Only single isolates among the 24 belonged to each of STs405 and ST167.

Asides from STs 167 and 405, no ST had more than five representatives. The third-most-frequent type, ST410, had five representatives, three with YRIK and high

aztreonam/avibactam MICs and two had YRIN with no AmpC, and moderately-raised MICs.

NDM-5 was the predominant NDM type across all three groups of isolates, though its dominance was greater among those with high MICs, where it was present in 42/50, and those with moderately-raised MICs (present in 12/15) than among those with normal MICs (present in 14/24). We do not, however, believe that carriage of NDM-5 rather than NDM-1 was pertinent to aztreonam/avibactam MICs, for two reasons. First, NDM-5 was strongly represented among isolates with normal MICs. Secondly, experimentation showed that addition of EDTA at 320 mg/L had an equally minimal effect on aztreonam/avibactam MICs irrespective of the NDM variant carried (Supplementary Table S1).

Additional β-lactamases, besides NDM and AmpC types, were widespread across the collection. ESBLs – principally CTX-M-15 enzymes – were frequent across all three *E. coli* groups; however, all groups also included sizeable numbers of isolates lacking ESBLs. None of the ESBLs found, including SHV-12, CTX-M-3, -14, -24 and -55 as well as CTX-M-15, is noted for avibactam resistance and none carried unusual mutations. The only secondary carbapenemase found was OXA-181, present in just two of the NDM-positive *E. coli* isolates, one with an aztreonam/avibactam MIC of 1 mg/L and a YRIN insert and one with a YRIK insert and an aztreonam/avibactam MIC of 16 mg/L, both lacked acquired AmpC. Turning, last, to enzymes conventionally counted as penicillinases, TEM-1 was frequent in all groups (not shown) whereas OXA-10 – which some authors assert to have weak activity against some oxyimino cephalosporins and aztreonam (see Discussion) – was found in two NDM *E. coli* isolates, one ST131 isolate with an aztreonam/avibactam MIC of 8 mg/L and no PBP3 insert, and one ST95 isolate with no insert and an aztreonam/avibactam MIC of 0.06 mg/L. In 14 of the total 16

isolates where *bla*$_{CMY-42}$ was found it was associated with IncI(γ) plasmids; none had the *inc* mutations associated with increased copy number and resistance by Ma *et al* [23].

The 50 sequenced isolates with high MICs comprised 15-19 from each of the 3 collection years. The temporal distributions of the predominant resistance types recognised – i.e. (i) ST405 with YRIK, (ii) non-ST405 with YRIK and (iii) YRIN-positive with acquired AmpC, irrespective of type – is shown in Table 4. Inspection suggested that ST405 isolates with YRIK had become more prominent in 2019, accounting for 11/16 isolates versus 7/19 in 2017 and 6/15 in 2015; however, this apparent increase failed to achieve statistical significance ($p > 0.05$, Chi-square test).

4.0 Discussion

The rationale of aztreonam/avibactam is that aztreonam is not hydrolysed by MBLs (nor by OXA-48-like carbapenemases) whilst avibactam inhibits most serine β-lactamases, importantly including ESBLs, KPC and AmpC types [4-6]. The result is that aztreonam/avibactam should evade almost all β-lactamase-mediated resistance in Enterobacterales. Activity beyond Enterobacterales is restricted by the facts that aztreonam does not bind the PBPs of Gram-positive bacteria and is less active than ceftazidime and ceftolozane against *Pseudomonas aeruginosa*.

Numerous studies [4-6, 24,25], including the present, confirm that this rationale is sound, with aztreonam/avibactam widely active against MBL-producing Enterobacterales. Here, MICs for 187/191 MBL-producing *Klebsiella* spp. and for 140/153 MBL-producing *Enterobacter* spp. were ≤1 mg/L, with all values for these species below 8+4 mg/L (Table 2); moreover, all distributions for these species were unimodal, without temporal change. By contrast we found the MIC distributions of aztreonam/avibactam for *E. coli* with NDM carbapenemases were bimodal, with

peaks at 0.125 and 8 mg/L. Similar bimodality for aztreonam/avibactam against NDM-positive *E. coli* is evident in the data of Sadek *et al.* [9] and raised aztreonam/avibactam MICs are widely reported for subsets of *E. coli* with NDM carbapenemases by other authors [6].

This phenomenon was first associated with PBP3 inserts by Alm *et al.* [8] examining *E. coli* isolates from China, India, Kuwait, Lebanon, Thailand and Turkey. This association subsequently has been confirmed by others using collections of isolates from elsewhere in Europe, Asia and Africa [6,9-11,26,27]. The inserts comprise a duplication of the YRIN motif at amino acids 330-333 of the mature PBP3, with the terminal asparagine (N) residue sometimes substituted by lysine (K) or proline (P). An asparagine to lysine substitution requires only a single base change, whereas an asparagine to proline substitution requires at least two base changes to the terminal codon. It is further reported [9,23,27] that isolates with raised aztreonam/avibactam MICs and PBP3 inserts commonly carry the gene for CMY-42, a plasmid-mediated AmpC variant which some authors report to have increased, though still slight, activity against aztreonam and oxyimino cephalosporins [28]. Also of note, CMY-42 β-lactamase has greater affinity for aztreonam than for avibactam, meaning that substrate binding may protect the enzyme from inactivation [9].

The present data support and extend these observations. Succinctly, and as illustrated in fig 1, we found that high aztreonam/avibactam MICs, ≥8 mg/L, were almost always (48 out of 50 cases) associated with either (i) the combination of a YRIN insert together with CMY-42 or another AmpC variant (13/50 cases), or (ii) with a YRIK insert irrespective of acquired AmpC (35/50 cases). By contrast, a YRIN insert without acquired AmpC was most typical for isolates with moderately-raised aztreonam/avibactam resistance, being recorded for 10/15 representatives with

MICs of 0.5-4 mg/L. YRIN inserts were found in only two of 24 isolates with normal aztreonam/avibactam MICs (0.03-0.25 mg/L), with YRIK never seen.

A plausible interpretation is that the first step to raised MICs or resistance is duplication of a 12-base sequence in the PBP3 gene, leading to the YRIN insert. Two routes can then generate a further rise in MIC. Either (i) the organism acquires (or already carries) an AmpC-β-lactamase-encoding plasmid, most often an IncI type determining CMY-42 enzyme or, alternatively, (ii) the terminal cytosine of the insert is mutated to an adenine, leading to a YRIK insert, rather than YRIN. The greater resistance associated with YRIK rather than YRIN may reflect the increased positive charge from the lysine but investigating this aspect would require protein modelling beyond the scope of this study. Patino-Navarette *et al* [29] likewise suggests that the duplication event typically precedes acquisition of bla_{NDM} genes, and this view is further supported by the fact that these inserts are also found (though much less studied) in carbapenemase-negative *E. coli* [11, also UKHSA data on file].

Notably, 25/36 *E. coli* isolates with YRIK proved to belong to ST405, whereas this ST had only two further, less resistant, representatives in the entire 89-*E. coli* test panel. Over the 5-year collection window, *E. coli* ST405 isolates with YRIK and high aztreonam/avibactam MICs (≥8 mg/L) were collected at 23 hospitals. Although numbers were insufficient to establish statistical significance, they comprised a greater proportion of the high-MIC isolates in 2019 than in 2015 or 2017 (Table 4). This aspect deserves further prospective monitoring. Like ST131 [30], *E. coli* ST405 is a successful uropathogenic lineage, frequently and internationally found to carry bla_{NDM-5} [31,32]. It has, moreover, been reported as a host of YRIK by others, including Sadek *et al.* [26], who collected representatives with the insert from Switzerland and Pakistan.

The second repeatedly-encountered Sequence Type – ST167 – also is a successful international clone and a frequent host of NDM-5 carbapenemases [33]. ST167 isolates with YRIN and CMY-42 β-lactamases – as found here – were reported previously from Pakistan and Switzerland by Sadek *at al.* and in China by Wang *et al.* [26,34].

Only one of the 50 isolates with high aztreonam/avibactam MICs lacked either a YRIK or YRIN insert. This was a member of the global ST131 clone with an OXA-10 β-lactamase. Although OXA-10 β-lactamase has slight activity against oxyimino-β-lactams, including aztreonam [35], and is only slowly acylated by avibactam [36] we are sceptical as to whether its hydrolytic activity made any significant contribution to the raised aztreonam/avibactam MIC, not least because bla_{OXA-10} was also present in one of the low aztreonam/avibactam MIC isolates (MIC, 0.06 mg/L).

Several study limitations should be acknowledged. First, we predominantly sequenced isolates with MICs $\leq$1 mg/L or $\geq$8 mg/L and have WGS data for few isolates with aztreonam/avibactam MICs of 2-4 mg/L, at the upper edge of our 'moderately-raised MIC' group. Accordingly, patterns of insert and enzyme may be more diverse in this group than suggested by the data in Table 3 and fig 1. Secondly, porin loss and (possibly) upregulated efflux may further modulate aztreonam/avibactam MICs, as indicated by Patino-Navarette *et al.* [29]. We did not examine these aspects. Our experience is that multiple *ompC* and *ompF* gene variants are commonly present in *E. coli* populations with variable modifications, complicating analysis. Moreover, porin expression can be modulated by mutations to remote genes, e.g., *envZ* and *ompR* complicating analysis [37]. Thirdly, whilst we detected bla_{AmpC} genes, we did not measure expression, which may be critical. Moreover, whilst there is a consensus that CMY-42 is linked with raised aztreonam/avibactam MICs, even rudimentary kinetic details are lacking for some of

the other AmpC variants found in association with YRIN inserts, notably CMY-59 and -148. Lastly, as noted, the sample size was insufficient to establish whether the apparent rise in ST405 isolates with YRIK in the final survey year (2019) was significant.

5.0 Conclusion

These data support the view that aztreonam/avibactam is a promising combination against MBL-producing *Klebsiella* spp., which are the most frequent hosts of these carbapenemases [20], and against MBL-producing *Enterobacter* spp. All members of these genera appeared susceptible, with modal MICs below 1 mg/L. The situation is more complex and concerning in the case of *E. coli* where a sizeable, but stable, minority of isolates have PBP3 inserts associated with reduced susceptibility. Specifically, YRIK inserts alone, and the combination of a YRIN insert plus acquired AmpC (generally CMY-42) enzymes were associated with 'high' aztreonam/avibactam MICs around 8-16 mg/L, as compared with a norm of 0.03 – 0.25 mg/L for *E. coli* with bla_{NDM} but no insert. The high values straddle the likely breakpoint for aztreonam/avibactam, though only time and experience will establish whether they are associated with clinical resistance. Disturbingly, not only do these inserts affect the activity of aztreonam/avibactam but, if combined with further mutations in *ftsI,* coding PBP3, they are also associated with MICs of up to 128 mg/L for cefiderocol [34] and 256 mg/L for cefepime/taniborbactam [38,39], thus compromising several further promising anti-MBL agents as well as aztreonam/avibactam.

Declarations

Funding: This work was supported by Pfizer.

Competing Interests: DML: Advisory Boards or ad hoc consultancy Accelerate, Antabio, AstraZeneca, Centauri, GenPax, Meiji, Menarini, Mutabilis, Nordic, Paion, ParaGraf, ParaPharm, Pfizer, QPEX, Shionogi, Sumitovant, Summit, T.A.Z., VenatoRx, Wockhardt, Zambon, Paid lectures – bioMérieux, GSK, Hikma, Merck/MSD, Menarini, Nordic, Pfizer, and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck and PerkinElmer, amounting to less than 10% of portfolio value. He also has nominated holdings in Arecor, Avacta, Creo Medical, Destiny, Diaceutics, Evgen, Genedrive, Poolbeg, Renalytics AI and Trellus (all with research/products pertinent to medicines or diagnostics) through Enterprise Investment Schemes but has no authority to trade these shares directly. All other authors are employees of the UKHSA's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, which has received financial support for conference attendance, lectures, research projects, or contracted evaluations from numerous sources, including Accelerate Diagnostics, Achaogen Inc., Allecra Therapeutics, Amplex, AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Integra holdings, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp, Meiji Seika Pharma Co. Ltd, Mobidiag, Momentum Biosciences Ltd, Neem Biotech, Nordic Pharma Ltd, Norgine Pharmaceuticals, Paratek Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Shionogi & Co. Ltd, Trius Therapeutics, T.A.Z., VenatoRx Pharmaceuticals and Wockhardt Ltd.

Ethical Approval: Not required

Sequence Information: Submission pending

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Table 1. Susceptibility and resistance in the test panel

Agents and EUCAST 2022 breakpoints	*E. coli* NDM (n=122)		*Klebsiella* NDM (n=121)		*Klebsiella* IMP/VIM (n=70)		*Enterobacter* NDM (n=91)		*Enterobacter* IMP/VIM (n=62)	
	%S	% S+I	%S	% S+I	%S	% S+I	%S	% S+I	%S	% S+I
Aztreonam ≤1/>4	10.7	14.8	16.5	17.4	38.6	38.6	23.1	36.3	24.2	37.1
Aztreonam/avibactam ≤8+4/>8+4	**85.2**	**85.2**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Meropenem ≤2/>8	1.6	9.0	3.3	15.7	17.1	51.4	4.4	22.0	24.2	71.0
Ceftazidime ≤1/>4	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0
Ceftazidime/avibactam ≤8+4/>8+4	1.6	1.6	0.0	0.0	4.3	4.3	0.0	0.0	0.0	0.0
Amikacin ≤8/>8	45.9	45.9	18.2	18.2	**81.4**	**81.4**	56.0	56.0	**85.5**	**85.5**
Ciprofloxacin ≤0.25/>0.5	6.6	9.0	10.7	12.4	14.3	21.4	17.6	23.1	22.6	40.3
Colistin ≤2/>2	**100.0**	**100.0**	**91.7**	**91.7**	**91.4**	**91.4**	**94.5**	**94.5**	**93.5**	**93.5**
Tigecycline ≤0.5/0.5 (*E. coli* only)	93.4	**93.4**	No bpt	No bpt	No bpt	No bpt	No bpt	No bpt	No bpt	No bpt

Agents achieving >80% activity are shown in **bold** font

Abbreviation: S, susceptible; I, high dose susceptible; R, resistant

Table 2. Aztreonam/avibactam MICs for MBL-producing Enterobacterales, by year

	Number of isolates with indicated MIC, mg/L											Grand
E. coli NDM	≤0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	Total
2015	2	5	5	3	1	1	5	3	10	4	1	40
2017		2	3	1	1	2	8	5	13	5	1	41
2019		2	3		2	5	7	6	9	6	1	41
All years pooled	2	9	11	4	4	8	20	14	32	15	3	122
Klebsiella NDM												
2015		3	11	17	8	2						41
2017		4	13	14	7	1	1					40
2019	2	6	9	12	8	2	1					40
All years pooled	2	13	33	43	23	5	2	0	0	0	0	121
Klebsiella IMP												
2015		5	4	1								10
2017		2	9	1	1							13
2019	2	2	6	1	2							13
All years pooled	2	9	19	3	3	0	0	0	0	0	0	36
Klebsiella VIM												
2015 *(16)			2	6	2							10
2017 *(18)	1	2	1	4	4	1						13
2019 *(20)	1		1	5	2		2					11
All years pooled	2	2	4	15	8	1	2	0	0	0	0	34
Enterobacter NDM												
2015 *(16)		4	7	6	10	1	1	1				30
2017 *(18)	1	2	11	9	3	1	3					30
2019 *(20)	4	7	5	7	7	1						31
All years pooled	5	13	23	22	20	3	4	1	0	0	0	91
Enterobacter IMP												
2015 *(16)			4	1	3		1	1				10
2017 *(18)	3	2	3	2		1						11
2019 *(20)	2	1	2	4	1	1						11
All years pooled	5	3	9	7	4	2	1	1	0	0	0	32
Enterobacter VIM												
2015 *(16)		1	1	1	1	3	2	1				10
2017 *(18)			1	3	2	3			1			10
2019 *(20)			1	3	2	2	1	1				10
All years pooled	0	1	3	7	5	8	3	2	1	0	0	30

Table 3. Combinations of PBP3 insert(s), STs and β-lactamase(s) in relation of aztreonam/avibactam MICs among 89 *E. coli* isolates with NDM carbapenemases

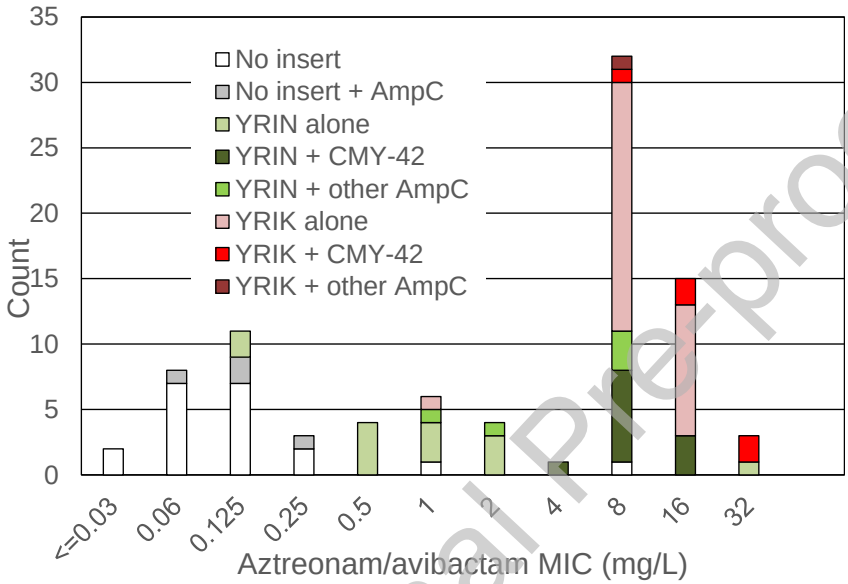
	Number of cases, among isolates with aztreonam/avibactam MIC, mg/L			
	0.03-0.25 'Normal'	0.5-4 'Moderately-raised'	8-32 'High'	Total
All isolates (n=89):	**24**	**15**	**50**	**89**
Ratio: NDM-1: NDM-5: other NDM	10 : 14 : 0	0 : 13: 2	3 : 42 : 5	13 : 69 : 7
Total with acquired AmpC	4	3	21	28
Total with ESBL	12 (6 x CTX-M-15; 6 other)	8 (7 x CTX-M-15; 1 other)	33 (30 x CTX-M-15; 3 other)	53
Total with OXA-10	1	0	1	2
Total with OXA-48 like		1 (OXA-181)	1 (OXA-181)	2
Total belonging to ST167	1	6	4	11
Total belonging to ST405/ST405*	1	2	25	28
Isolates with YRIN (n=29)	**2**	**13**	**14**	**29**
Total with CMY-42	0	1	10	11
Total with other AmpC	0	2 (2 x CMY-2)	3 (1 x CMY-6, 1 x CMY-59; 1 x CMY-148)	5
Total without AmpC	2	10	1	13
Total belonging to ST167	1	6	3	10
Total belonging to ST405	0	0	0	0
Isolates with YRIK (n=36)	**0**	**1**	**35**	**36**
Total with AmpC	0	0	8 (1 x CMY-2; 2 x CMY-6; 5 x CMY-42)	8
Total without AmpC	0	1	27	28
Total belonging to ST167	0	0	1	1
Total belonging to ST405/ST405*	0	1	25	26
Isolates without PBP3 inserts (n=24)	**22**	**1** (ST405)	**1** (ST131, with OXA-10)	**24**
Total with acquired AmpC	2 x DHA variants	0	0	2

	1 x CMY-4 1 x CMY-6			
Total without AmpC	20	1	1	22
Total belonging to ST167	0	0	0	0
Total belonging to ST405/ST405*	1	1	0	1

Greyed rows with bold text show totals for groups by insert type. The rows below each greyed row then provide further detail within that insert type, in respect to the distribution of other β-lactamases and the number of isolates belonging to major sequence types, specifically, ST167 and ST405 and variants.

Table 4. Temporal distribution of major groups of *E. coli* with aztreonam/avibactam MICs of ≥8 mg/L

	2015	2017	2019
Total sequenced with high MICs (≥8 mg/L)	15	19	16
Of which:			
YRIN + AmpC	6 (4 CMY-42)	6 (5 CMY-42)	1 (1 CMY-42)
YRIN lacking AmpC	0	1	0
YRIK ST405/ST405-related	6	7	12
YRIK ST other	3	4	3
Unexplained resistance		1	



Legends

Figure 1: Distribution of inserts and AmpC β-lactamases found in relation to aztreonam/avibactam MICs. 'Other' AmpC means 'other than CMY-42'