Evaluation of in vitro activity of ceftolozane/tazobactam and comparators against recent clinical bacterial isolates, and genomics of Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli isolates that demonstrated resistance to ceftolozane/tazobactam: data from Kuwait and Oman

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Background: The treatment options for infections caused by MDR Gram-negative bacteria have been limited, especially for infections caused by bacteria that produce carbapenemases and/or ESBLs. Ceftolozane/tazobactam is a cephalosporin/β-lactamase inhibitor developed to treat Gram-negative bacteria.

Methods: Ceftolozane/tazobactam and 14 comparators (amikacin, aztreonam, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, colistin, ertapenem, imipenem, levofloxacin, meropenem and piperacillin/tazobactam) were evaluated against Pseudomonas aeruginosa and Enterobacterales isolates collected from Kuwait and Oman (n = 606) during 2016–17. In addition, further analysis of resistance mechanisms to ceftolozane/tazobactam was done utilizing WGS. Non-susceptible isolates from ceftolozane/tazobactam surveillance were selected for analysis. Overall, 35 strains underwent WGS.

Results: Among isolates from Kuwait, susceptibility of P. aeruginosa, Escherichia coli and Klebsiella pneumoniae to ceftolozane/tazobactam was 79.8%, 95.7% and 87.5%, respectively, and from Oman was 92.3%, 93.1% and 88.5%, respectively. No P. aeruginosa with a ceftolozane/tazobactam MIC, 32 mg/L encoded β-lactamases besides normal chromosomal enzymes (PDC variants or OXA-50-like) whereas all but one P. aeruginosa isolate with MIC > 32 mg/L encoded either MBLs (60%), VEB-1 (19%) or additional OXAs (3.7%).

Conclusions: Colistin followed by ceftolozane/tazobactam showed the greatest activity against P. aeruginosa. Enterobacterales showed more susceptibility to ceftolozane/tazobactam than to piperacillin/tazobactam, but meropenem and colistin showed better activity.

Introduction

The worldwide increase of MDR Gram-negative bacteria (GNB) is causing a global concern and an economic burden.1–4 It is, therefore, important to understand the epidemiology of this multifaceted problem in order to prevent the spread of MDR GNB. As treatment choices for infections caused by these organisms remain limited, patients will continue to present with difficult-to-treat infections caused by MDR GNB. Serious infections, including bloodstream infections, hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP), complicated urinary-tract infections (cUTIs), and complicated intra-abdominal infections (cIAIs) are commonly caused by MDR GNB, often resulting in high morbidity and mortality.

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Among these organisms, Enterobacterales (especially Escherichia coli and Klebsiella pneumoniae) and Pseudomonas aeruginosa have emerged as major causes of nosocomial infections.\(^5\-\^7\) Newer antibiotic drugs with anti-GNB activity are being developed, albeit at a slower pace, to combat such infections and among them ceftolozane/tazobactam was recently approved for treatment of cIAIs, cUTIs including acute pyelonephritis, HAP and VAP by the US FDA\(^8\-\^10\) and EMA. Ceftolozane/tazobactam is a cephalosporin/β-lactamase inhibitor developed for use against infections caused by GNB, including MDR P. aeruginosa isolates that harbour chromosomally encoded resistance mechanisms such as Pseudomonas-derived cephalosporinase (PDC), porin defects and upregulated efflux transport systems and ESBL-producing Enterobacterales. The chemical structure of ceftolozane, though similar to that of ceftazidime, possesses a modified side-chain at the 3-position of the cepham nucleus that confers potent antipseudomonal activity. Ceftolozane/tazobactam displays reduced activity against P. aeruginosa isolates carrying carbapenemases and against Enterobacterales carrying AmpC β-lactamases, serine carbapenemases, metallo-β-lactamases and OXA-type carbapenemases\.\(^11\,\^12\) Therapy should be individualized based on genotypes of resistance, susceptibility profiles, disease severity and patient characteristics. More studies are needed to guide effective treatment for infections caused by MDR GNB. The purpose of this work was to evaluate the antibacterial activity of ceftolozane/tazobactam against P. aeruginosa (n = 150) and Enterobacterales isolates (n = 456) ceftolozane/tazobactam-resistant isolates utilizing a WGS approach.

**Materials and methods**

**Study design**

GNB isolates from the EM200 study collected by International Health Management Associates Inc. (IHMA) from 2016 to 2017 were selected for analysis. The sites each collected up to 250 consecutive Gram-negative pathogens from patients with lower respiratory, intra-abdominal, urinary tract, bloodstream and other infections. The study evaluated the in vitro activity of ceftolozane/tazobactam and 14 comparator compounds against GNB isolates (P. aeruginosa, K. pneumoniae and E. coli) collected from 10 countries: in Europe (Turkey, Ukraine), Middle East (Israel, Jordan, Kuwait, Oman, Saudi Arabia, United Arab Emirates) and Africa (Morocco, South Africa). For selected ceftolozane/tazobactam non-susceptible strains from Kuwait and Oman, demographics, susceptibilities and WGS data were retrieved from the IHMA database. We evaluated the antibacterial activity of ceftolozane/tazobactam and 14 comparator compounds against a collection of these isolates from Kuwait and Oman (n = 606). The secondary purpose of this work was to investigate mechanisms of resistance to ceftolozane/tazobactam utilizing a WGS approach. In Kuwait and Oman 27 P. aeruginosa isolates (25 from Kuwait and 2 from Oman) and 8 Enterobacterales isolates (6 from Kuwait and 2 from Oman) underwent WGS.

**Antimicrobial susceptibility testing**

MICs of ceftolozane/tazobactam, amikacin, aztreonam, cefepime, cefotaxime, cefoxitin, cefazidime, ceftriaxone, ciprofloxacin, colistin, ertapenem, imipenem, levofloxacin, meropenem and piperacillin/tazobactam were determined by broth micro-dilution following the CLSI reference method.\(^13\,\^14\) Interpretive criteria followed CLSI 2020 guidelines\(^14\) for all compounds except colistin for which the EUCAST 2017 breakpoint was used for Enterobacterales.\(^15\) Quality control (QC) testing was performed each day of testing as specified by CLSI using E. coli ATCC 25922, P. aeruginosa ATCC-27853 and K. pneumoniae ATCC-700603. All QC data were within CLSI approved ranges.\(^14\)

**DNA extraction and WGS analysis**

Isolates were cultured overnight at 37°C on tryptic soy agar with 5% sheep blood. A single colony from each plate was inoculated into 5 mL of brain heart infusion broth and incubated at 37°C with 200 rpm shaking overnight. A broth sterility control was incubated concurrently. Genomic DNA was purified using DNeasy UltraClean kits. Extracted DNA was sent to an external sequencing provider for library preparation using Nextera kits and sequencing using a HiSeq sequencing instrument with 2 × 150 bp pair-end reads with a target coverage depth of approximately 150x. All analyses were carried out using Qiagen's CLCBio Genomics Workbench version 11.

**Analysis of β-lactamase variants**

For β-lactam resistance gene identification, de novo assemblies of each genome were queried. To detect better highly divergent ompC genes for which there are few variants defined, the threshold for minimum nucleotide sequence identity and minimum sequence length were set to 72% and 80%, respectively. However, results that were less than 100% identical or did not contain the full-length sequence were appended as such for clarity. Only β-lactam resistance genes were identified this way. Nucleotide sequences for all PDC variants assigned in GenBank were collected from NCBI (BioProject 313047) and added to the database used to identify resistance genes. K. pneumoniae is known to frequently encode a chromosomal copy of bla\_SHV. Due to the difficulties associated with differentiating multiple copies of the same gene by Illumina WGS, all reads from K. pneumoniae isolates were aligned directly to the bla\_SHV-1 gene and the sites associated with an ESBL phenotype (G238S and E240K by Ambler numbering) were reviewed manually to ensure the presence of a chromosomally encoded SHV did not mask the presence of a horizontally transferred (plasmid-encoded) ESBL copy of SHV.

**Analysis of porins**

For porin gene identification, ompC and ompF in E. coli, ompK35 and ompK36 in K. pneumoniae and oprD in P. aeruginosa were searched by tblastn in the de novo assemblies of the genomes. The minimum threshold for E-values was 10E-75, however only the hit with the lowest E-value in each genome was assessed. Lesions were defined as changes in the coding sequence of a gene that would result in a premature stop codon. For PBP gene analysis, reference sequences for the protein products of ftsI were searched on a species-specific basis in de novo assemblies.
of each genome. In brief, tblastn was used to find the gene with the lowest E value to the reference sequence, for which mutations encoding amino acid changes were identified.

**Molecular typing: MLST**

For MLST, the best matching complete prokaryotic genome from GenBank was identified computationally for each set of genomic reads and used for guided assembly. The appropriate MLST scheme and allelic profile of each of the guided assemblies was determined. A minimum coverage depth of 30× for each of the seven loci was exceeded in every genome. Due to the large number of sequence types identified, phylogenetic trees were used to supplement MLST.

**Ethical approval**

Ethical approval was not required.

**Results**

**Antimicrobial susceptibility testing**

A total of 510 and 96 clinical GNB isolates from Kuwait and Oman, respectively, were tested for antimicrobial activity against a set of 15 antimicrobial agents including ceftolozane/tazobactam, amikacin, aztreonam, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, colistin, ertapenem, imipenem, levofloxacin, meropenem and piperacillin/tazobactam. Colistin was the most active compound tested, with a 96.8% susceptibility rate (Table 1). Ceftolozane/tazobactam inhibited 88.8% of 89 other Enterobacterales isolates. All these isolates were susceptible to meropenem and amikacin, and 93.3% of them were susceptible to piperacillin/tazobactam (Table 2).

**Organism selection and demographics**

Among the 510 GNB isolates from Kuwait, 31 strains (6%) tested non-susceptible, and among the 96 isolates from Oman, 4 strains (4.1%) tested non-susceptible (MIC values ≥8 mg/L) to ceftolozane/tazobactam by antimicrobial susceptibility testing (Table 3). These isolates were selected for further analysis. The various sources of these isolates (number/percentage of total) from Kuwait were: respiratory (13/41.9%), UTI (11/35.5%), SSTI (3/9.7%), IAI (2/6.5%) and body aspirate (cerebrospinal fluid, ascites, bone biopsy, tissue biopsy from sterile anatomical sites such as brain, liver, spleen, and lymph nodes, bone marrow aspirate, synovial biopsy and synovial fluid samples) (2/6.5%) whereas of four strains from Oman two were isolated from extra-abdominal samples, one from urine and one from respiratory samples. The demographic information for the isolates that were selected for WGS is as follows:

- For *P. aeruginosa* isolates (*n* = 25) from Kuwait, the age of patients ranged from 6 to 87 years, 11 patients were male while 14 were female and culture-positive samples were 9 from urine, 11 from lower respiratory tract, 3 from wound cultures and 2 from pus samples.
- For two *P. aeruginosa* isolates from Oman, the age of patients ranged from 18 to 34 years and positive cultures were one isolate from respiratory origin and the other from intra-abdominal source.

For *K. pneumoniae*, the demographics for the isolates that underwent WGS were as follows:

- Three isolates from Kuwait were selected, for which the age of patients ranged from 52 to 62 years, one isolate was from a male and two from females and the positive cultures were obtained from urine, respiratory and extra-abdominal samples.
- For two isolates from Oman, the age of patients ranged from 58 to 72 years, one isolate was from a male and the other from a female and the positive cultures were obtained from urine and intra-abdominal samples.

For *E. coli* the demographics for the isolates that underwent WGS were as follows:

- For three isolates from Kuwait that were selected, the age of patients ranged from 20 to 72 years, one isolate was from a male and two were from females and the positive cultures were obtained from urine, respiratory and intra-abdominal samples.
- No *E. coli* isolate from Oman underwent WGS.

**WGS**

Overall, 35 isolates underwent WGS and analysis. The genomes of 27 *P. aeruginosa* strains (25 isolates from Kuwait and 2 isolates from Oman) (Table 4) that were completely sequenced showed the presence of *bla*VIM-2 in 15 (55.6%), *bla*OXA-4 in 10 (37%), *bla*OXA-10 in 6 (22%), *bla*VEB-1 in 5 (18.5%), *bla*LAC in 4 (14.8%),

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*In vitro activity of C/T and comparators*
OXA-2 in 3 (11.1%) and blaVIM-6 in 1 (3.7%). The blaOXA-50-like gene was found in 24 (88.9%) of the strains; however, this chromosomally encoded class D enzyme is not associated with β-lactam resistance in *P. aeruginosa*.16 Most *P. aeruginosa* isolates presented a combination of genes, e.g. blaVIM-2 + blaOXA-2 + blaPDC. Independently, class D β-lactamases were found in 14.8% of ceftolozane/tazobactam non-susceptible *P. aeruginosa* isolates (Table 4). Sequencing of the blaPDC gene revealed the occurrence of 10 PDC variants (8 from Kuwait and 2 from Oman), different PDC variants with PDC-119-like (40%) and PDC-252-like (40%) being produced by most of the ceftolozane/tazobactam non-susceptible strains from Kuwait. No PDC allele observed contains amino acid substitutions previously identified to be associated with ceftolozane/tazobactam resistance. The oprD gene sequence revealed mutations in 17 (68%) of 25 non-susceptible *Pseudomonas* isolates from Kuwait. There was no relationship between the disruption of the oprD gene and ceftolozane/tazobactam MIC, since isolates with lower MICs also had lesions in oprD. Genotyping by MLST showed that these *P. aeruginosa* strains from Kuwait belonged to ST233 (44%), followed by ST357 (20%) and ST2613 (16%), and all others (20%) were detected as single isolates and included ST272, ST244, ST499, ST3582 and ST671 while the two strains from Oman belonged to ST664 and ST207, respectively.

WGS of *K. pneumoniae* strains with MIC ≥8 mg/L identified five unique STs among the three (ST831, ST336 and ST985) *K. pneumoniae* isolates from Kuwait and two (ST1658, ST231) *K. pneumoniae* isolates from Oman. Though each ST was only seen in one isolate, all five isolates carried the ESBL gene blaCTX-M-15, which was present in combination with blaTEM-1 (n=2) and blaSHV genes (n=5). Among carbapenemase genes, while no blaKPC genes were found, blaNDM-1 and blaOXA-48 were detected

| Antimicrobial agent       | Antimicrobial susceptibility (%) | MIC (mg/L)          |
|----------------------------|---------------------------------|---------------------|
|                            | susceptible | intermediate | resistant | MIC_{50} | MIC_{90} | range    |
| Ceftolozane/tazobactam    |            |             |           |          |          |          |
| Oman                      | 92.3       | 0           | 7.7       | 1        | 4        | 0.5 to >32 |
| Kuwait                     | 79.8       | 2.4         | 17.7      | 1        | >32      | 0.25 to >32 |
| Amikacin                  |            |             |           |          |          |          |
| Oman                      | 92.3       | 0           | 7.7       | ≤4       | 16       | ≤4 to >32 |
| Kuwait                     | 78.2       | 3.2         | 18.6      | ≤4       | >32      | ≤4 to >32 |
| Aztreonam                 |            |             |           |          |          |          |
| Oman                      | 61.5       | 19.2        | 19.2      | 8        | >16      | ≤1 to >16 |
| Kuwait                     | 58.1       | 12.9        | 29        | 8        | >16      | ≤1 to >16 |
| Ceftepime                 |            |             |           |          |          |          |
| Oman                      | 80.8       | 3.9         | 15.4      | 4        | >32      | 2 to >32  |
| Kuwait                     | 66.1       | 9.7         | 24.2      | 4        | >32      | ≤1 to >32 |
| Ceftazidime               |            |             |           |          |          |          |
| Oman                      | 80.8       | 0           | 19.2      | 4        | >32      | ≤1 to >32 |
| Kuwait                     | 73.4       | 1.6         | 25        | 4        | >32      | ≤1 to >32 |
| Ciprofloxacin             |            |             |           |          |          |          |
| Oman                      | 84.6       | 3.9         | 11.5      | ≤0.25    | >2       | ≤0.25 to >2 |
| Kuwait                     | 59.7       | 4.8         | 35.5      | 0.5      | >2       | ≤0.25 to >2 |
| Colistin                  |            |             |           |          |          |          |
| Oman                      | 100        | —           | 0         | ≤1       | ≤1       | ≤1 to ≤1 |
| Kuwait                     | 96.8       | —           | 3.2       | ≤1       | 2        | ≤1 to >4  |
| Imipenem                  |            |             |           |          |          |          |
| Oman                      | 80.8       | 0           | 19.2      | 1        | 16       | ≤0.5 to 32 |
| Kuwait                     | 55.7       | 8.1         | 36.3      | 2        | >32      | ≤0.5 to >32 |
| Levofloxacin              |            |             |           |          |          |          |
| Oman                      | 80.8       | 3.9         | 15.4      | ≤1       | >4       | ≤1 to >4  |
| Kuwait                     | 56.5       | 8.1         | 35.5      | 2        | >4       | ≤1 to >4  |
| Meropenem                 |            |             |           |          |          |          |
| Oman                      | 76.9       | 3.9         | 19.2      | 0.5      | >16      | ≤0.12 to >16 |
| Kuwait                     | 59.7       | 4.8         | 35.5      | 1        | >16      | ≤0.12 to >16 |
| Piperacillin/tazobactam   |            |             |           |          |          |          |
| Oman                      | 76.9       | 3.9         | 19.2      | 8        | >64      | ≤2 to >64 |
| Kuwait                     | 63.7       | 16.1        | 20.2      | 8        | >64      | ≤2 to >64 |

*aStratified by country.*
Table 2. *In vitro* activity of ceftolozane/tazobactam and comparators against 386 and 70 Enterobacteriales isolates from Kuwait and Oman, respectively

| Antimicrobial agent | Antimicrobial susceptibility<sup>a</sup> | Other Enterobacteriales: Kuwait (n=89), Oman (n=15) |
|---------------------|-----------------------------------------|--------------------------------------------------|
|                     | E. coli: Kuwait (n=164), Oman (n=29) | K. pneumoniae: Kuwait (n=133), Oman (n=26) | |
|                     | % | mg/L | % | mg/L | % | mg/L | % | mg/L |
|                     | S | I | R | MIC<sub>50</sub> | MIC<sub>90</sub> | range | S | I | R | MIC<sub>50</sub> | MIC<sub>90</sub> | range |
| Ceftolozane/ | | | | | | | | | | | | |
| Tazobactam | | | | | | | | | | | | |
| Kuwait | 95.7 | 0.6 | 3.7 | 0.25 | 1 | ≤0.06 to >32 | 85.7 | 0.8 | 13.5 | 0.5 | 32 | 0.12 to >32 | 88.8 | 2.3 | 9 | 0.5 | 4 | 0.12 to >32 |
| Oman | 93.1 | 0 | 6.9 | 0.25 | 2 | 0.12 to >32 | 88.5 | 3.9 | 7.7 | 0.5 | 4 | 0.25 to >32 | 86.7 | 6.7 | 6.7 | 0.5 | 4 | 0.25 to 16 |
| Amikacin | | | | | | | | | | | | | |
| Kuwait | 100 | 0 | 0 | ≤4 | 8 | ≤4 to >32 | 95.5 | 0 | 4.5 | ≤4 | 8 | ≤4 to >32 | 98.9 | 1.1 | 0 | ≤4 | 8 | ≤4 to >32 |
| Oman | 93.1 | 3.5 | 3.5 | ≤4 | 8 | ≤4 to >32 | 92.3 | 0 | 7.7 | ≤4 | 8 | ≤4 to >32 | 100 | 0 | 0 | ≤4 | 8 | ≤4 to 16 |
| Astreacin | | | | | | | | | | | | | |
| Kuwait | 56.1 | 6.7 | 37.2 | 4 | >16 | ≤1 to >16 | 53.4 | 3 | 43.6 | 2 | >16 | ≤1 to >16 | 71.9 | 2.3 | 25.8 | ≤1 | >16 | ≤1 to >16 |
| Oman | 55.2 | 3.5 | 41.4 | 2 | >16 | ≤1 to >16 | 53.9 | 0 | 46.2 | ≤1 | >16 | ≤1 to >16 | 73.3 | 6.7 | 20 | ≤1 | >16 | ≤1 to >16 |
| Cefepime | | | | | | | | | | | | | |
| Kuwait | 58.5 | 11 | 30.5 | ≤1 | >32 | ≤1 to >32 | 55.6 | 9 | 35.3 | 2 | >32 | ≤1 to >32 | 74.2 | 5.6 | 20.2 | ≤1 | >32 | ≤1 to >32 |
| Oman | 55.2 | 6.9 | 37.9 | ≤1 | >32 | ≤1 to >32 | 53.9 | 7.7 | 38.5 | ≤1 | >32 | ≤1 to >32 | 73.3 | 13.3 | 13.3 | ≤1 | >16 | ≤1 to >16 |
| Cefotaxime | | | | | | | | | | | | | |
| Kuwait | 50 | 0.6 | 49.4 | ≤1 | >32 | ≤1 to >32 | 48.9 | 0.8 | 50.4 | 4 | >32 | ≤1 to >32 | 61.8 | 3.4 | 34.8 | ≤1 | >32 | ≤1 to >32 |
| Oman | 44.8 | 10.3 | 44.8 | 2 | >32 | ≤1 to >32 | 53.9 | 0 | 46.2 | ≤1 | >32 | ≤1 to >32 | 73.3 | 0 | 26.7 | ≤1 | >32 | ≤1 to >32 |
| Cefoxitin | | | | | | | | | | | | | |
| Kuwait | 70.7 | 15.2 | 14 | 8 | >16 | ≤2 to >16 | 71.4 | 9 | 19.6 | 4 | >16 | ≤2 to >16 | 60.7 | 0 | 39.3 | 4 | >16 | ≤2 to >16 |
| Oman | 79.3 | 6.9 | 13.8 | 8 | >16 | 4 to >16 | 88.5 | 3.9 | 7.7 | 4 | >16 | ≤2 to >16 | 33.3 | 6.7 | 60 | >16 | >16 | ≤2 to >16 |
| Ceftazidime | | | | | | | | | | | | | |
| Kuwait | 60.4 | 12.2 | 27.4 | 2 | 32 | ≤1 to >32 | 54.1 | 5.3 | 40.6 | 2 | >32 | ≤1 to >32 | 71.9 | 5.6 | 22.5 | ≤1 | 32 | ≤1 to >32 |
| Oman | 62.1 | 6.9 | 31 | ≤1 | >32 | ≤1 to >32 | 53.9 | 3.9 | 42.3 | ≤1 | >32 | ≤1 to >32 | 73.3 | 6.7 | 20 | ≤1 | >32 | ≤1 to >32 |
| Ceftriaxone | | | | | | | | | | | | | |
| Kuwait | 48.8 | 1.8 | 49.4 | 2 | >32 | ≤1 to >32 | 49.6 | 2.3 | 48.1 | 2 | >32 | ≤1 to >32 | 62.9 | 2.3 | 34.8 | ≤1 | >32 | ≤1 to >32 |
| Oman | 55.2 | 0 | 44.8 | ≤1 | >32 | ≤1 to >32 | 50 | 0 | 50 | ≤1 | >32 | ≤1 to >32 | 73.3 | 0 | 26.7 | ≤1 | >32 | ≤1 to >32 |
| Ciprofloxacin | | | | | | | | | | | | | |
| Kuwait | 41.5 | 1.2 | 57.3 | >2 | ≤0.25 to >2 | 60.2 | 9 | 30.8 | 0.5 | >2 | ≤0.25 to >2 | 46.1 | 10.1 | 43.8 | 2 | >2 | ≤0.25 to >2 |
| Oman | 65.5 | 3.5 | 31 | 0.5 | >2 | ≤0.25 to >2 | 65.4 | 11.5 | 23.1 | ≤0.25 | >2 | ≤0.25 to >2 | 80 | 0 | 20 | ≤0.25 | >2 | ≤0.25 to >2 |
| Colistin | | | | | | | | | | | | | |
| Kuwait | 100 | — | 0 | ≤1 | ≤1 | ≤1 to 2 | 94 | — | 6 | ≤1 | ≤1 | ≤1 to 4 | 41.6 | — | 58.4 | >4 | >4 | ≤1 to 4 |
| Oman | 96.6 | — | 3.5 | ≤1 | ≤1 | ≤1 to 4 | 100 | — | 0 | ≤1 | ≤1 | ≤1 to 2 | 40 | — | 60 | >4 | >4 | ≤1 to 4 |
| Ertapenem | | | | | | | | | | | | | |
| Kuwait | 97.6 | 1.2 | 1.2 | ≤0.06 | ≤0.06 | ≤0.06 to 4 | 86.5 | 0.8 | 12.8 | ≤0.06 | 2 | ≤0.06 to >4 | 92.1 | 2.3 | 5.6 | ≤0.06 | 0.5 | ≤0.06 to >4 |

Continued
| Antimicrobial         | E. coli: Kuwait (n = 164), Oman (n = 29) | K. pneumoniae: Kuwait (n = 133), Oman (n = 26) | other Enterobacterales: Kuwait (n = 89), Oman (n = 15) |
|----------------------|------------------------------------------|--------------------------------------------------|--------------------------------------------------------|
|                      | % S | I | R | MIC<sub>50</sub> | MIC<sub>90</sub> | range | % S | I | R | MIC<sub>50</sub> | MIC<sub>90</sub> | range | % S | I | R | MIC<sub>50</sub> | MIC<sub>90</sub> | range |
| Imipenem             | 100 |   |   | ≤ 0.5 | ≤ 0.5 | 0.5 to 1  | 93.3 | 67.0 | 3.9 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 96.2 | 6.3 | 3.9 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
|                      | 94.5 | 4.5 | 1 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 96.2 | 6.3 | 3.9 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
|                      | 68.5 | 30.3 | 1.1 | 2 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 68.5 | 30.3 | 1.1 | 2 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
| Levofoxacin          | 94.2 | 4.5 | 1 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 60.7 | 11.2 | 28.1 | 2 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
|                      | 80.8 | 7.1 | 11.5 | 1 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 80.8 | 7.1 | 11.5 | 1 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
|                      | 67.6 | 6.7 | 26.3 | 2 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 67.6 | 6.7 | 26.3 | 2 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
|                      | 50.2 | 6.7 | 32.4 | 32 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 | 50.2 | 6.7 | 32.4 | 32 | ≤ 0.06 | ≤ 0.06 | ≤ 0.06 to 0.25 |
| Meropenem            | 100 | 0 | 0 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 97.8 | 11.1 | 1.1 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 94.2 | 4.5 | 1 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 94.2 | 4.5 | 1 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 60.7 | 11.2 | 28.1 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 60.7 | 11.2 | 28.1 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 40.3 | 6.7 | 32.4 | 32 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 40.3 | 6.7 | 32.4 | 32 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 20.0 | 6.7 | 32.4 | 32 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 20.0 | 6.7 | 32.4 | 32 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
| Piperacillin/ tazobactam | 100 | 0 | 0 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 100 | 0 | 0 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 90.9 | 4.3 | 4.9 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 90.9 | 4.3 | 4.9 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |
|                      | 85.2 | 6.9 | 6.9 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 | 85.2 | 6.9 | 6.9 | 2 | ≤ 0.12 | ≤ 0.12 | ≤ 0.12 to 0.5 |

*Stratified by country.*
in one isolate each. The porin gene profiles of these five isolates showed that while mutations in the \textit{ampF}-like porin (OmpK35) were detected in both strains from Oman, mutation in the \textit{ampC}-like porin (OmpK36) was detected in only one \textit{K. pneumoniae} isolate from Kuwait (Table 5).

Among three \textit{E. coli} isolates from Kuwait that were examined by WGS, three unique STs (ST7395, ST361 and ST131) were identified. Horizontally transferred $\beta$-lactamases were identified in three of three (100\%) isolates with ceftolozane/tazobactam MIC values $\geq$8 mg/L. All these strains carried genes for one or more of the following $\beta$-lactamases: EC-like (Class C $\beta$-lactamase intrinsic to \textit{E. coli}), CTX-M, CMY-7-like, OXA-1-like, TEM-1B and TEM-34-like. Four amino acid insertions (‘YRIK’ or ‘YRIK’) at position 333 of PBP3 were identified in two of three (66.6\%) isolates. No mutations were observed in the gene encoding \textit{OmpC} or \textit{OmpF} in any of the \textit{E. coli} strains (Table 5).

### Discussion

The dissemination of MDR GNB and XDR GNB strains compromises selection of appropriate antimicrobial treatments resulting in significant morbidity and mortality.\textsuperscript{17-19} It has been shown that these pathogens develop resistance to most available antibiotics by selection of mutations on chromosomal genes and from the increasing prevalence of transferable resistant determinants, especially those encoding class B carbapenemases (metallo-$\beta$-lactamases) or ESBLs, frequently co-transferred with genes encoding resistance to other antibiotics.\textsuperscript{20} The recent introduction of ceftolozane/tazobactam, which is stable against \textit{P. aeruginosa} AmpC hydrolysis (PDC), comes as a respite to the dilemma of selecting an effective antibiotic against MDR/XDR GNB, including carbapenem resistant \textit{P. aeruginosa} (not producing carbapenemases).

The \textit{K. pneumoniae} isolates from Kuwait and Oman exhibited susceptibility to ceftolozane/tazobactam of 85.7\% and 88.5\%, respectively, with MICs ranging from 0.12 to $\geq$32 mg/L. \textit{E. coli} isolates from Kuwait and Oman in our study demonstrated similar susceptibility rates to ceftolozane/tazobactam (95.7\% and 93.1\%, respectively, with MIC range of $\leq$0.06 to $\geq$32 mg/L). Concerning data from the region, a study from Lebanon showed similar ceftolozane/tazobactam activity against ESBL-producing \textit{E. coli} and \textit{K. pneumoniae} strains (MIC\textsubscript{90} = 1–1.5 mg/L) with susceptibility rates of 100\% and 96\%, respectively.\textsuperscript{21} In a recent study from Qatar, an overall susceptibility of \textit{P. aeruginosa} isolates against ceftolozane/tazobactam was found to be 62.9\% whereas only $<50\%$ of XDR strains were found to be susceptible to ceftolozane/tazobactam.\textsuperscript{22} In contrast, in a large study from the USA, ceftolozane/tazobactam was found to be one of the most active agents against \textit{P. aeruginosa}, retaining activity against MDR and XDR strains with susceptibility rates varying from 95.1\% to 98.2\%.\textsuperscript{23} In comparison, lower activity of ceftolozane/tazobactam was reported for ESBL-producing Enterobacterales, with a susceptibility range of 85\%–93.3\%.\textsuperscript{24} However, while ceftolozane/tazobactam shows potent in vitro activity against \textit{Pseudomonas} spp. and Enterobacterales, baseline genes encoding resistance including \textit{bla}\textsubscript{OXA-5}, \textit{bla}\textsubscript{OXA-24} and additional \textit{bla}\textsubscript{OXA} genes were detectable. The emergence of resistance to ceftolozane/tazobactam and some other newer antibiotics is of particular concern and needs to be monitored closely.\textsuperscript{25,26}

Bacteria demonstrate diverse mechanisms for developing resistance such as degrading antibiotics, modifying the antibiotic target site or modulating the influx/efflux of antibiotic into or out of the bacterial cell.\textsuperscript{27} WGS is an emerging tool used for advanced molecular epidemiological investigations such as accurate detection and characterization of existing or emergent resistance determinants, especially involving MDR organisms. Adoption of genotyping can help in better understanding the mechanism(s) of resistance among virulent genotypes and elucidation of transmission routes,\textsuperscript{28-30} which forms an essential aspect of public health surveillance to combat the spread of antimicrobial-resistant bacteria.\textsuperscript{31} The molecular epidemiology and resistance mechanisms of ceftolozane/tazobactam-resistant strains of \textit{P. aeruginosa}, \textit{K. pneumoniae} and \textit{E. coli} from Kuwait and Oman were determined by WGS. Diverse STs of \textit{P. aeruginosa} isolates from Kuwait and Oman were observed. Since carbapenemase production is considered to be one of the resistance mechanisms to ceftolozane/tazobactam, resistance to carbapenems is being used as a marker of potential carbapenemase production.\textsuperscript{20} Similar to an earlier study where the VIM-2 type carbapenemase was the most common followed by the IMP type, our study showed that VIM-2 was the most common carbapenemase. However; independently, OXA-type $\beta$-lactamases were found in only 4 of 27 non-susceptible \textit{P. aeruginosa} isolates (14.8\%). In most cases this enzyme was associated with other carbapenemases and ESBLs. Recent studies have revealed that besides PDC overexpression, $\beta$-lactam resistance development may result from mutations leading to the structural modification of PDC or other $\beta$-lactamases. Previously published studies have identified certain amino acid substitutions, as well as deletions, in the omega-loop region of the intrinsic \textit{P. aeruginosa} ampC gene (also known as \textit{bla}\textsubscript{OXA-5}) that are associated with decreased susceptibility to ceftolozane/tazobactam.\textsuperscript{26,32-33} PDC analysis revealed that all of the strains exhibited the PDC gene whereas no mutations in the intrinsic \textit{ampC} gene (\textit{bla}\textsubscript{OXA-5}), which have been reported previously to be associated with increased ceftolozane/tazobactam MICs, were observed. Genetic lesions that introduced a premature stop codon in \textit{oprD} were identified frequently (68\%); however, this feature is not associated with resistance to ceftolozane/tazobactam, and we also did not observe any relationship between ceftolozane/tazobactam MIC and lesions in \textit{oprD}.\textsuperscript{12} For the \textit{P. aeruginosa} isolates in which no

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**Table 3. Ceftolozane/tazobactam MIC distribution for \textit{P. aeruginosa} and Enterobacterales isolates included in WGS, stratified by country**

| Isolate and country of origin | Ceftolozane/tazobactam MIC (mg/L) |
|-----------------------------|-----------------------------------|
|                             | 8 | 16 | 32 | $>32$ |
| \textit{P. aeruginosa}       |   |    |    |      |
| Kuwait                       | 25 | 3  | 1  | —    | 21 |
| Oman                        | 2  | —  | —  | —    | 2  |
| Enterobacterales            |   |    |    |      |
| Kuwait                       | 6  | 1  | 1  | —    | 4  |
| Oman                        | 2  | —  | 1  | —    | 1  |
horizontally transferred β-lactamase was identified (n = 4), all from Kuwait, four unique sequence types were determined. This indicates that the elevated ceftolozane/tazobactam MIC values observed among these isolates, which included three isolates with intermediate (8 mg/L) and one with resistant (>8 mg/L) MIC values, is not due to the spread of one highly successful clone. Among those isolates that encoded horizontally transferred β-lactamases, an association was observed between

Table 4. WGS data for P. aeruginosa isolates from Kuwait and Oman

| Isolates stratified by country | IHMA number | C/T MIC (mg/L) | OprD | PBP3 (ftsI) | WGS lactamase summary (≥72% identity; ≥80% CDS) | Class C (intrinsic) | MLST |
|-------------------------------|------------|----------------|------|------------|-----------------------------------------------|--------------------|------|
| Kuwait                        |            |                |      |            |                                               |                    |      |
| 1                             | 1562186    | 8              | Frameshift, 195 AA protein | WT | PDC-109 | PDC-109 | 499 |
| 2                             | 1562187    | >32            | No lesion | WT | PDC-119-like; OXA-4; VIM-2 | PDC-119-like | 233 |
| 3                             | 1562188    | >32            | No lesion | WT | PDC-252-like; OXA-4; VIM-2 | PDC-252-like | 233 |
| 4                             | 1562192    | >32            | Q158STOP | WT | PDC-119-like; OXA-4; VIM-2 | PDC-119-like | 233 |
| 5                             | 1562196    | >32            | Frameshift, 359 AA protein | A539T | PDC-252-like; VIM-2; OXA-4 | PDC-252-like | 233 |
| 6                             | 1562198    | >32            | No lesion | WT | PDC-35; LCR-1; VIM-2; OXA-2 | PDC-35 | 2613 |
| 7                             | 1572968    | >32            | No lesion | WT | PDC-252-like; VIM-2 | PDC-252-like | 3482 |
| 8                             | 1572981    | >32            | No lesion | WT | PDC-119-like; VIM-2 | PDC-119-like | 233 |
| 9                             | 1572982    | >32            | W138STOP | WT | PDC-119-like; OXA-4-like; VIM-2 | PDC-119-like | 233 |
| 10                            | 1572988    | >32            | Frameshift, 350 AA protein | WT | PDC-35; OXA-2; VIM-6; OXA-10 | PDC-35 | 2613 |
| 11                            | 1607795    | 8              | No lesion | WT | PDC-30 | PDC-30 | 671 |
| 12                            | 1607809    | >32            | Disrupted by insertion | WT | PDC-119-like; OXA-4; VIM-2 | PDC-119-like | 233 |
| 13                            | 1607996    | >32            | No lesion | WT | PDC-35; LCR-1; VIM-2; OXA-2 | PDC-35 | 2613 |
| 14                            | 1652654    | >32            | Frameshift, 354 AA protein | WT | PDC-252-like; OXA-4; VIM-2 | PDC-252-like | 233 |
| 15                            | 1652655    | >32            | Disrupted by insertion | WT | PDC-252-like; OXA-4; VIM-2 | PDC-252-like | 233 |
| 16                            | 1652663    | >32            | Frameshift, 135 AA protein | WT | PDC-11; OXA-10; VEB-1 | PDC-11 | 357 |
| 17                            | 1723966    | >32            | Frameshift, 135 AA protein | WT | PDC-11; OXA-10; VEB-1 | PDC-11 | 357 |
| 18                            | 1724010    | >32            | Frameshift, 135 AA protein | WT | PDC-11; VEB-1; OXA-10 | PDC-11 | 357 |
| 19                            | 1724024    | >32            | No lesion | WT | PDC-35; OXA-2; VIM-2; LCR-1 | PDC-35 | 2613 |
| 20                            | 1724321    | >32            | Frameshift, 135 AA protein | WT | PDC-11; OXA-10; VEB-1 | PDC-11 | 357 |
| 21                            | 1724327    | >32            | Frameshift, 135 AA protein | WT | PDC-11; OXA-10; VEB-1 | PDC-11 | 357 |
| 22                            | 1734172    | 8              | E176STOP | WT | PDC-212-like | PDC-212-like | 272 |
| 23                            | 1734205    | >32            | Disrupted by insertion | WT | PDC-252-like; OXA-4; VIM-2 | PDC-252-like | 233 |
| 24                            | 1734215    | >32            | Frameshift, 354 AA protein | WT | PDC-119-like; OXA-4; VIM-2 | PDC-119-like | 233 |
| 25                            | 1734223    | 16             | Q424STOP | WT | PDC-279-like | PDC-279-like | 244 |
| Oman                         |            |                |      |            |                                               |                    |      |
| 1                             | 1688133    | >32            | Frameshift, 218 AA protein | L346M; F533L | PDC-98; OXA-10; OXA-1-like | PDC-98 | 664 |
| 2                             | 1688134    | >32            | No lesion | WT | PDC-30 | PDC-30 | 207 |

AA, amino acid; C/T, ceftolozane/tazobactam.

*Amino acid changes are greater relative to reference sequences.

*Threshold for β-lactamase gene inclusion was 72% and 80% for minimum nucleotide sequence identity and minimum sequence length, respectively.

*Novel MLSTs were given sequential designations for clarity.
The mechanisms of resistance to carbapenems include production or absence of OmpC or OmpF porin channels. Insertions of AA, amino acid; C/T, ceftolozane/tazobactam.

A rise in ESBL-producing and carbapenem-resistant E. coli allele groups may indicate clonal spread.

During the past decade there has been a worldwide increase in ESBL-producing and carbapenem-resistant E. coli. The mechanisms of resistance to carbapenems include production of carbapenemases, AmpC type enzymes and ESBLs, and membrane impermeability, which can be linked to modification or absence of OmpC or OmpF porin channels. Insertions of four amino acids in PBP3, ‘YRIK/YRIN’, previously reported to decrease susceptibility to select cephalosporins, including ceftazidime. 19 were identified in 66.6% of our isolates. The other isolate also had mutation in the ftsI gene, encoding PBP3. All E. coli isolates from Kuwait and Oman in this study were carbapenem susceptible, with rare resistance to ceftolozane/tazobactam. Non-susceptibility of three E. coli isolates was attributed to one of several factors including alterations of PBPs due to mutations. In a similar study from Lebanon, the most common type of E. coli isolated belonged to ST405, which has been detected in the USA, Japan and Norway. 40 This strain of E. coli has been shown to be associated with the worldwide spread of blaCTX-M-15 and acc-(6’)-Ib-cr. 61 However, among our E. coli strains, none belonged to ST405 and while two strains carried genes for CTX-M-15 and one for CMY-7-like cephalosporinase; none of the strains showed any lesion in ompF-like and ompC-like genes. An ompC-like (ompK36) gene mutation was identified in one strain from Kuwait and ompF-like (ompK35) was identified in the two strains from Oman.

**Conclusions**

In Kuwait and Oman, ceftolozane/tazobactam showed greater activity against P. aeruginosa strains when compared with meropenem and piperacillin/tazobactam. Ceftolozane/tazobactam was more or equally active compared with amikacin. Colistin demonstrated the highest activity against P. aeruginosa. Class D carbapenemases were found in all P. aeruginosa ceftolozane/tazobactam non-susceptible isolates in combination with MBL or VEB. Mutations in a gene encoding an outer membrane protein (oprD) were frequently identified however, no relationship was seen between oprD status and ceftolozane/tazobactam MIC. E. coli and K. pneumoniae showed higher susceptibility to ceftolozane/tazobactam than to piperacillin/tazobactam. It may, therefore, have utility as a carbapenem-sparing antibiotic for the treatment of ESBL-producing Enterobacteriales. Meropenem and colistin showed better activity against these isolates from Kuwait and Oman. Resistance of Enterobacteriales isolates to ceftolozane/tazobactam could be explained by the presence of β-lactamase combinations, lesions in the OmpC-like and OmpF-like porins and mutation in PBP3-ftsI. Reduced susceptibility to ceftolozane/tazobactam in some of the isolates may have been caused by higher levels
of β-lactamases, which could have exceeded the available amount of tazobactam.

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