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Evaluation of *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against MDR *Pseudomonas aeruginosa* isolates from Qatar

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Objectives: To investigate the *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against clinical isolates of MDR *Pseudomonas aeruginosa* from Qatar, as well as the mechanisms of resistance.

Methods: MDR *P. aeruginosa* isolated between October 2014 and September 2015 from all public hospitals in Qatar were included. The BD Phoenix™ system was used for identification and initial antimicrobial susceptibility testing, while Liofilchem MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) were used for confirmation of ceftazidime/avibactam and ceftolozane/tazobactam susceptibility. Ten ceftazidime/avibactam- and/or ceftolozane/tazobactam-resistant isolates were randomly selected for WGS.

Results: A total of 205 MDR *P. aeruginosa* isolates were included. Of these, 141 (68.8%) were susceptible to ceftazidime/avibactam, 129 (62.9%) were susceptible to ceftolozane/tazobactam, 121 (59.0%) were susceptible to both and 56 (27.3%) were susceptible to neither. Twenty (9.8%) isolates were susceptible to ceftazidime/avibactam but not to ceftolozane/tazobactam and only 8 (3.9%) were susceptible to ceftolozane/tazobactam but not to ceftazidime/avibactam. Less than 50% of XDR isolates were susceptible to ceftazidime/avibactam or ceftolozane/tazobactam. The 10 sequenced isolates belonged to six different STs and all produced AmpC and OXA enzymes; 5 (50%) produced ESBL and 4 (40%) produced VIM enzymes.

Conclusions: MDR *P. aeruginosa* susceptibility rates to ceftazidime/avibactam and ceftolozane/tazobactam were higher than those to all existing antipseudomonal agents, except colistin, but were less than 50% in extremely resistant isolates. Non-susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam was largely due to the production of ESBL and VIM enzymes. Ceftazidime/avibactam and ceftolozane/tazobactam are possible options for some patients with MDR *P. aeruginosa* in Qatar.

Introduction

Pseudomonas aeruginosa remains a leading cause of hospital-acquired infections including those of the bloodstream, respiratory tract, urinary tract and surgical sites.[1–3] In addition to an array of virulence determinants, *P. aeruginosa* possesses and can readily acquire a broad variety of antimicrobial resistance mechanisms.[4,5] These include up-regulation of efflux pumps, loss of outer membrane porins, the production of AmpC, ESBL and carbapenemase enzymes, and modification of antimicrobial target sites.[6,7] Multiple resistance mechanisms are usually expressed simultaneously, resulting in resistance to agents in multiple antimicrobial classes.[6,7] The existence of limited effective treatment options for MDR *P. aeruginosa* infections has been associated with poor clinical outcomes.[8–10] In their 2017 report, the WHO designated research,

discovery and development of new antibiotics for carbapenem-resistant *P. aeruginosa* a critical priority.[11]

Ceftazidime/avibactam and ceftolozane/tazobactam are licensed for the treatment of patients with a variety of clinical infections.[12] Avibactam is a non-β-lactam β-lactamase inhibitor that inhibits class A, class C and most class D β-lactamases.[13] On the other hand, ceftolozane is a novel cephalosporin that is active against *P. aeruginosa* isolates with AmpC hyperproduction and overexpressed efflux mechanisms.[14] The combination of ceftolozane and the β-lactamase inhibitor tazobactam is active against many, but not all, ESBL-producing Gram-negative bacteria.[15] Several studies have reported rates and mechanisms of *P. aeruginosa* resistance to ceftazidime/avibactam and ceftolozane/tazobactam, including MDR isolates, from Europe and North America.[15–20] However, there are limited data on the potential utility of ceftazidime/avibactam and ceftolozane/tazobactam for MDR *P. aeruginosa* from the Arabian Peninsula, a region of extremely diverse demography and close travel links to all corners of the world.[21,22] The aim of this study was to investigate the *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against MDR *P. aeruginosa* from Qatar and to explore the associated genetic diversity and mechanisms of resistance.

Methods

Ethics

This study was approved with a waiver for informed consent by the Institutional Review Board, Hamad Medical Corporation, Doha, Qatar (IRGC-01-51-033) and Swedish Research Council Formas (Dn. 219-2014-837).

Materials and setting

This prospective evaluation was conducted on routine clinical specimens received by the Microbiology Laboratory at Hamad Medical Corporation, Doha during the period from 1 October 2014 to 30 September 2015, prior to any clinical use of ceftazidime/avibactam or ceftolozane/tazobactam in Qatar. The facility provides routine and tertiary diagnostic services to all public acute and referral hospitals across Qatar.

The isolates underwent standard diagnostic work-up then were stored at −70°C pending further analysis. MDR *P. aeruginosa* isolates were defined as having *in vitro* resistance to at least one agent from three or more antimicrobial classes.[23]

Identification and susceptibility testing

The BD Phoenix™ automated system was used for bacterial identification and initial antimicrobial susceptibility testing, while Liofilchem® MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) were used for confirmation of ceftazidime/avibactam and ceftolozane/tazobactam susceptibility. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853 were used as controls. Susceptibility reporting was based on current recommendations of the CLSI.[24] No intermediate susceptibility category was available for ceftazidime/avibactam against *P. aeruginosa.* Isolates were therefore described as susceptible to ceftazidime/avibactam if the MIC was ≤8 mg/L and non-susceptible if the MIC was >8 mg/L.[24] For consistency, intermediate and resistant categories were grouped together as non-susceptible for all reported antimicrobial agents.

WGS

Ten MDR *P. aeruginosa* isolates that were resistant to ceftazidime/avibactam and/or ceftolozane/tazobactam were randomly selected to undergo WGS using the Illumina HiSeq 2000 system (Illumina, San Diego, CA, USA). WGS was performed by Eurofins GATC Biotech GmbH, Konstanz, Germany.

Genomic assembly, annotation and identification

The clean reads were assembled using SPAdes, Version 3.13.0 (Center for Algorithmic Biotechnology, St Petersburg, Russia). To determine whether the GC content had a significant effect on sequencing randomness or not, the GC content and average depth of the genomic sequence were calculated without repetition as a unit of 500 bp.[25] The assembled data were subjected to RAST annotation, as previously described.[26] MDR *P. aeruginosa* isolates were subjected to SpeciesFinder 1.2 (Center for Genomic Epidemiology, Lyngby, Denmark) to determine their 16S rRNA-based species identification.[27]

In silico serotyping

In silico serotyping of the *P. aeruginosa* isolates was performed using *P. aeruginosa* serotyper (PAst) Version 1.0 (Center for Genomic Epidemiology, Lyngby, Denmark). The programme utilizes sequencing data and is based on Basic Local Alignment Search Tool (BLAST) analysis of the OSA gene.[28]

MLST

MLST 1.8 (Center for Genomic Epidemiology) was used to perform MLST of MDR *P. aeruginosa* isolates, based on the seven housekeeping genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA* and *trpE*), as previously described.[29]

Antibiotic resistance genes

Antibiotic resistance genes were predicted using the Comprehensive Antibiotic Resistance Database (CARD), Version 1.2.0 (McMaster University, Hamilton, Ontario).[30]

Statistical analysis

Susceptibility patterns of MDR *P. aeruginosa* to the study antibiotics were presented as frequency and percentages. Cohen's Kappa (*k*) was used to measure agreement between ceftazidime/avibactam and ceftolozane/tazobactam susceptibility results and those of other agents. Type I error threshold of 0.05 was used for statistical significance. Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY, USA).

Results

P. aeruginosa was isolated from a total of 2533 clinical samples over the study period, of which 205 (8.1%) fulfilled the MDR definition. Respiratory cultures (92, 44.9%) were the most common source of MDR *P. aeruginosa* isolates, followed by skin and soft tissue (54, 26.3%), urine (48, 23.4%), blood (5, 2.4%), sterile body fluids (4, 2.0%) and vascular line tips (2, 1.0%).

Antimicrobial susceptibility patterns of MDR *P. aeruginosa* isolates

Antimicrobial susceptibility results and MIC distributions for 205 MDR *P. aeruginosa* isolates are summarized in Table 1. One hundred and forty-one (68.8%) of the isolates were susceptible to ceftazidime/avibactam, 129 (62.9%) were susceptible to ceftolozane/tazobactam, 121 (59.0%) were susceptible to both and 56 (27.3%) were susceptible to neither agent. Twenty (9.8%) isolates were

susceptible to ceftazidime/avibactam but not to ceftolozane/tazobactam, and only 8 (3.9%) were susceptible to ceftolozane/tazobactam but not to ceftazidime/avibactam. Rates of susceptibility to ceftazidime/avibactam or ceftolozane/tazobactam in the presence of resistance to other antipseudomonal antibiotics is shown in Table 2. There was agreement in susceptibility results between ceftazidime/avibactam and tobramycin ($k = 0.25$, $P < 0.001$), ceftazidime/avibactam and amikacin ($k = 0.27$, $P < 0.001$), ceftolozane/tazobactam and tobramycin ($k = 0.4$, $P < 0.001$) and ceftolozane/tazobactam and amikacin ($k = 0.37$, $P < 0.001$).

Genotypic profile of selected MDR P. aeruginosa isolates that were non-susceptible to ceftazidime/avibactam, ceftolozane/tazobactam or both

The 10 randomly selected isolates of MDR *P. aeruginosa* that were non-susceptible to ceftazidime/avibactam, ceftolozane/tazobactam or both belonged to six different STs (Table 3). Class A ESBLs were identified in five (50%) isolates and Verona integron-encoded MBL (VIM) in four (40%). Genes encoding different types of *Pseudomonas*-derived cephalosporinases (PDCs) and oxacillinases (OXAs) were present in all of the isolates. Each isolate possessed genes for three or four different β-lactamases from three different molecular classes (Table 3). No mutations were detected in genes encoding for efflux pump regulators or efflux pump complexes in any of the 10 isolates. No shared distinctive genotypic pattern was apparent for the three isolates that were susceptible to ceftazidime/avibactam but not to ceftolozane/tazobactam (Table 3). Furthermore, none of the previously described ceftolozane/tazobactam and ceftazidime/avibactam resistance-associated PDC mutations was identified in any of the isolates.[5]

Discussion

The impact of bacterial resistance on clinical outcomes and healthcare expenditure cannot be overstated.[31] We found relatively high levels of non-susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam in a clinical collection of MDR *P. aeruginosa* that pre-dated the introduction of these agents into clinical practice in Qatar. Moreover, ceftazidime/avibactam and ceftolozane/tazobactam activity was not consistent with each other or with other β-lactams. Susceptibility testing of *P. aeruginosa* isolates for ceftazidime/avibactam and ceftolozane/tazobactam is therefore essential for reliable clinical use.

The availability of ceftazidime/avibactam and ceftolozane/tazobactam as additional options for the treatment of MDR *P. aeruginosa* infections is a promising development. However, as noted in previous studies,[17–19] their added value is limited by the observation that less than half of the isolates that were resistant to existing antipseudomonal β-lactam agents, aminoglycosides and quinolones were susceptible to either ceftazidime/avibactam or ceftolozane/tazobactam (Table 2). Unfortunately, the critical need for effective new treatment options for MDR *P. aeruginosa* remains to be met. The case for the importance of judicious clinical use has already been made by reports of rapid *in vivo* emergence of MDR *P. aeruginosa* resistance to ceftazidime/avibactam[32] and ceftolozane/tazobactam[33,34] in patients

Table 1. Cumulative MIC distributions of ceftazidime/avibactam, ceftolozane/tazobactam and comparator agents for clinical MDR *P. aeruginosa* isolates from Qatar

Antibiotic	Number (cumulative %) of isolates inhibited at an MIC (mg/L) of:[a]																	MIC_{50} (mg/L)	MIC_{90} (mg/L)
	≤0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192	256	
CZA	9 (4.4)	6 (7.3)	11 (12.7)	24 (24.4)	27 (37.6)	29 (51.7)	24 (63.4)	11 (68.8)	10 (73.7)	8 (77.6)	7 (81)	6 (83.9)	7 (87.3)	4 (89.3)	4 (91.2)	3 (92.7)	0 (92.7)	15 (100)	4	64
C/T	40 (19.5)	39 (38.5)	18 (47.3)	13 (53.7)	7 (57.1)	12 (62.9)	5 (65.4)	3 (66.8)	3 (68.3)	5 (70.7)	5 (73.2)	2 (74.1)	1 (74.6)	2 (75.6)	0 (75.6)	0 (75.6)	0 (75.6)	50 (100)	2	256
FEP	0 (0)	0 (0)	1 (0.5)	1 (1)	0 (1)	1 (1.5)	0 (1.5)	4 (3.4)	13 (9.8)	16 (17.6)	19 (26.8)	18 (35.6)	17 (44)	13 (50.2)	8 (54.1)	8 (58)	8 (62)	78 (100)	64	256
GEN	7 (3.4)	7 (6.8)	8 (10.7)	10 (15.6)	13 (22)	9 (26.3)	12 (32.2)	17 (40.5)	1 (41)	6 (44)	6 (46.8)	4 (48.8)	5 (51.2)	2 (52.2)	0 (52.2)	0 (52.2)	3 (53.7)	95 (100)	48	256
TZP	0 (0)	2 (1)	0 (1)	1 (1.5)	0 (1.5)	1 (2)	3 (3.4)	3 (4.9)	3 (6.3)	7 (9.8)	10 (14.6)	12 (20.4)	12 (26.3)	12 (32.2)	9 (36.6)	4 (38.5)	8 (42.4)	118 (100)	256	256
AMK	1 (0.5)	1 (1)	2 (2)	2 (2.9)	8 (6.8)	12 (12.7)	8 (16.6)	19 (25.9)	15 (33.2)	16 (41)	13 (47.3)	8 (51.2)	9 (55.6)	6 (58.5)	8 (62.4)	8 (66.3)	2 (67.3)	67 (100)	32	256
TOB	13 (6.3)	20 (16.1)	27 (29.3)	18 (38)	7 (41.4)	7 (44.9)	2 (45.9)	0 (45.9)	4 (47.8)	1 (48.3)	12 (54.1)	13 (60.5)	9 (64.9)	5 (67.3)	4 (69.3)	5 (71.7)	1 (72.2)	57 (100)	24	256
CST	43 (21)	38 (39)	82 (79.5)	35 (96.6)	3 (98)	2 (99)	1 (99.5)	1 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	1.5	2
MEM	8 (3.9)	1 (4.4)	5 (6.8)	6 (9.8)	4 (11.7)	4 (13.7)	4 (15.6)	9 (20)	4 (22)	3 (23.4)	1 (23.9)	156 (100)	not applicable						32	32
CIP	12 (5.9)	5 (8.3)	5 (10.7)	7 (14.1)	9 (18.5)	14 (25.4)	6 (28.3)	6 (31.2)	6 (34.1)	2 (35.1)	3 (36.6)	130 (100)	not applicable						32	32

AMK, amikacin; CIP, ciprofloxacin; CST, colistin; C/T, ceftolozane/tazobactam; CZA, ceftazidime/avibactam; FEP, cefepime; GEN, gentamicin; MEM, meropenem; TOB, tobramycin; TZP, piperacillin/tazobactam.

[a]White, susceptible; grey, non-susceptible.

Table 2. MDR *P. aeruginosa* susceptibility to ceftazidime/avibactam or ceftolozane/tazobactam in the presence of resistance to other antipseudomonal antimicrobial agents

MDR resistance phenotype that included resistance to:	Isolates with resistance, *n* (%)	Isolates susceptible to CZA, *n* (%)	Isolates susceptible to C/T, *n* (%)
FEP	198 (96.6)	134 (67.7)	122 (61.6)
TZP	186 (90.7)	126 (67.7)	119 (63.9)
MEM	185 (90.2)	126 (68.1)	140 (75.7)
CIP	187 (91.2)	142 (75.9)	131 (70.1)
AMK	119 (58.0)	67 (56.3)	55 (46.2)
GEN	150 (73.2)	98 (65.3)	82 (54.7)
CAZ, FEP, TZP and MEM	165 (80.5)	106 (64.2)	103 (62.4)
CAZ, FEP, TZP, MEM and CIP	150 (73.2)	91 (60.7)	91 (60.7)
CAZ, FEP, TZP, MEM, CIP, GEN and AMK	86 (42.0)	38 (44.2)	36 (41.9)

AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; C/T, ceftolozane/tazobactam; CZA, ceftazidime/avibactam; FEP, cefepime; GEN, gentamicin; MEM, meropenem; TZP, piperacillin/tazobactam.

Table 3. Genotypic and phenotypic profiles of 10 MDR *P. aeruginosa* isolates that were found to be non-susceptible to ceftazidime/avibactam, ceftolozane/tazobactam or both

	Sample number									
	PA9	PA37	PA99	PA11	PA12	PA98	PA123	PA125	PA199	PA154
ST (serogroup)	235 (O11)	235 (O11)	235 (O11)	308 (O11)	308 (O11)	308 (O11)	292 (O12)	823 (O11)	233 (O6)	27 (O1)
β-Lactamase gene (molecular class), gene presence (% identitiy)										
TEM-116 (class A)	–	–	–	–	–	–	–	–	–	yes (100)
VEB-1a (class A)	–	–	yes (100)	yes (100)	yes (100)	yes (100)	–	–	–	–
CARB-3 (class A)	–	–	–	–	–	–	yes (99.67)	–	–	–
VIM-2 (class B)	yes (100)	yes (100)	–	–	–	–	–	yes (100)	yes (100)	–
PDC-2 (class C)	yes (99.75)	yes (99.75)	yes (99.75)	–	–	–	–	–	–	–
PDC-3 (class C)	–	–	–	–	–	–	–	–	yes (100)	–
PDC-5 (class C)	–	–	–	–	–	–	yes (99.75)	–	–	yes (99.75)
PDC-7 (class C)	–	–	–	yes (99.75)	yes (99.75)	yes (99.75)	–	yes (98.99)	–	–
OXA-4 (class D)	–	–	–	–	–	–	–	–	yes (100)	–
OXA-10 (class D)	–	yes (99.62)	yes (100)	–	–	–	–	–	–	–
OXA-50 (class D)	yes (99.24)	yes (99.24)	yes (99.24)	yes (99.24)	yes (99.24)	yes (99.24)	yes (98.09)	yes (98.33)	yes (99.24)	yes (99.24)
Efflux pump regulators										
MexR	+	+	+	+	+	+	+	+	+	+
NalC	+	+	+	+	+	+	+	+	+	+
NalD	+	+	+	+	+	+	+	+	+	+
CpxR	+	+	+	–	+	+	–	+	+	+
SoxR	+	+	+	–	+	+	–	–	+	+
type B NfxB	+	+	+	–	+	+	–	–	+	+
Efflux pump complex										
MexAB-OprM	+	+	+	–	+	+	+	+	+	+
MexCD-OprJ	+	+	+	+	+	+	+	+	+	+
MexPQ-OpmE	+	+	+	+	+	+	+	+	+	+
MuxABC-OpmB	+	+	+	+	+	+	+	+	+	+
Antimicrobial susceptibility result (MIC in mg/L)										
CZA	NS (128)	NS (96)	NS (12)	NS (24)	NS (16)	S (2)	S (1.5)	NS (24)	NS (32)	S (8)
C/T	NS (256)	NS (256)	NS (256)	NS (256)	NS (256)	NS (256)	NS (50)	NS (256)	NS (256)	NS (12)
MEM	NS (32)	NS (32)	NS (32)	NS (32)	S (2)	S (1.5)	S (0.75)	NS (32)	NS (32)	NS (32)
Mucoidity	non-mucoid	non-mucoid	non-mucoid	mucoid	non-mucoid	mucoid	non-mucoid	non-mucoid	mucoid	non-mucoid

CZA, ceftazidime/avibactam; C/T, ceftolozane/tazobactam; MEM, meropenem; NS, non-susceptible; S, susceptible; +, present; –, absent.

Table 4. Summary of studies comparing ceftazidime/avibactam and ceftolozane/tazobactam *in vitro* activity against MDR *P. aeruginosa*

Study	Geographical location	Susceptibility testing method	Inclusion criteria	Collection years	Number included	Number (%) susceptible to MEM	Number (%) susceptible to CZA	Number (%) susceptible to C/T
Humphries *et al.*,[17] 2017	Los Angeles, CA, USA	broth microdilution, except C/T by Etest	resistant to at least one antipseudomonal β-lactam antibiotic	2015–16	309	49 (15.9)	191 (61.8)	224 (72.5)
Buehrle *et al.*,[18] 2016	Pittsburgh, PA, USA	broth microdilution	MEM NS	not reported	38	0 (0)	35 (92.1)	35 (92.1)
Grupper *et al.*,[19] 2017	USA	broth microdilution	MEM NS	not reported	290	0 (0)	235 (81.0)	264 (91.0)
Gonzalez et al.,[20] 2017	St Louis, MO, USA	Etest	MEM NS	2014	45	13 (28.9)	37 (82.2)	39 (86.7)
Alatoom *et al.*,[21] 2017	Abu Dhabi, United Arab Emirates	Etest	resistant to at least one agent from at least three antimicrobial classes	2015–16	31	15 (48.4)	29 (93.5)	30 (96.8)

CZA, ceftazidime/avibactam; C/T, ceftolozane/tazobactam; MEM, meropenem; NS, non-susceptible.
All studies reported the isolates as susceptible if the MIC was ≤8 mg/L for ceftazidime/avibactam and ≤4 mg/L for ceftolozane/tazobactam.

who received 10 days or less of treatment with the respective agent.

There are a few notable differences in our results compared with previous studies that compared ceftazidime/avibactam and ceftolozane/tazobactam activity against MDR *P. aeruginosa* recovered from patients without prior exposure to either agent (Table 4). The proportion of MDR *P. aeruginosa* isolates that were susceptible to ceftazidime/avibactam and/or ceftolozane/tazobactam was comparable to results reported in one previous study, but was considerably lower than in other reports (Table 4). This could be explained by the fact that the isolates included in those studies were generally less resistant.[18–21] Moreover, unlike in this study, the majority of *P. aeruginosa* isolates in previous reports did not produce ESBLs or carbapenemases.[18,19] Another possible explanation for this discrepancy is that different susceptibility testing methods were used. Whereas we used Liofilchem® MIC Test Strips, previous reports had used either broth microdilution[17–19] or Etest.[20,21] Previous investigators expressed concern that considerable proportions of their *P. aeruginosa* isolates had ceftazidime/avibactam MICs at the current CLSI breakpoint of 8 mg/L.[17–19] In our study, a total of 82 (40.0%) of the isolates had ceftazidime/avibactam MICs within one doubling dilution of the breakpoint. Similarly, 40 (19.5%) isolates had ceftolozane/tazobactam MICs within one doubling dilution of the CLSI breakpoint of 4 mg/L.

In this study, 10 MDR *P. aeruginosa* isolates were subjected to WGS. Four isolates produced the class B carbapenemase VIM, the most common type of carbapenemase identified in *P. aeruginosa* isolates from the region.[35,36] Half of the sequenced isolates produced ESBL enzymes (Table 3). Vietnamese ESBLs (VEB enzymes) are class A ESBLs that were originally described in *P. aeruginosa* isolates from South-East Asia.[37] They are widely disseminated in *P. aeruginosa* from the Middle East,[38–40] and have been associated with MDR *P. aeruginosa* outbreaks in Eastern Europe[41,42] and in China.[43] VEB enzymes are inhibited *in vitro* by avibactam, but result in resistance to ceftolozane/tazobactam.[44,45]

PDC enzymes, also known as AmpC, were identified in all of our sequenced isolates. Mutations leading to AmpC hyperproduction are amongst the most common mechanisms for β-lactam resistance in *P. aeruginosa,* including *de novo* and emergent resistance to ceftazidime/avibactam and ceftolozane/tazobactam.[5,34,46–48] However, no such mutations were identified in any of the sequenced isolates in this study. Additionally, oxacillinases were detected in all sequenced isolates in this study. OXA-4, OXA-10 and OXA-50 are all narrow-spectrum β-lactamases.[49,50] OXA-50, a naturally occurring β-lactamase, was present in all of the sequenced MDR *P. aeruginosa* included is this study. It was also described in previous reports of ceftazidime/avibactam- and/or ceftolozane/tazobactam-resistant MDR *P. aeruginosa,* without any evidence of mutation or overproduction.[19,34,48] Interestingly, OXA-14, which is the product of a single point mutation in the OXA-10 gene, generates high-level resistance to ceftolozane/tazobactam in *P. aeruginosa*,[33] while a 3 bp deletion in bla_{OXA-2} produced a novel enzyme, designated OXA-539, conferring high-level resistance to ceftazidime/avibactam.[51]

Unlike previous studies that compared the activity of ceftazidime/avibactam and ceftolozane/tazobactam against MDR *P. aeruginosa*, ceftazidime/avibactam was more active than ceftolozane/tazobactam in this study (Table 4). Focusing on the sequenced isolates offers a possible explanation for this observation. Isolates that were susceptible to ceftazidime/avibactam but not to ceftolozane/tazobactam (PA98, PA123 and PA154) produced β-lactamases belonging to class A, class C and class D, all of which are inhibited by avibactam, but not tazobactam. The VIM-producing isolates (PA9, PA37, PA125 and PA199) were, as expected, resistant to both ceftazidime/avibactam and ceftolozane/tazobactam. However, no immediate explanation is

available for the remaining sequenced isolates (PA11 and PA12), which were non-susceptible to both agents without MBL production or detectable AmpC mutations.

The 10 sequenced MDR *P. aeruginosa* isolates belonged to six different STs. Three belonged to ST235, an international high-risk clone that has been associated with innumerable horizontally transferred resistance determinants.[52,53] Others included the global ST233 and the Asian ST308 clones.[53] These clones were previously reported from the Arabian Peninsula region, including from Qatar.[36] This finding raises alarming concern of the potential for clonal dissemination of these high-risk multiresistant isolates and emphasizes the need to ensure the effective application of infection prevention and control measures.

To the best of our knowledge, this is the largest study from the Middle East comparing in parallel the *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against MDR *P. aeruginosa* and investigating the possible underlying molecular mechanisms. A limitation of the current study is the use of Liofilchem® MIC Test Strips for ceftazidime/avibactam and ceftolozane/tazobactam susceptibility testing. Recent reports suggest that when compared to broth microdilution, this method can result in misclassification of some *P. aeruginosa* isolates as resistant to ceftazidime/avibactam and ceftolozane/tazobactam.[54,55] Thus confirmation of our results using the broth microdilution reference method would have been ideal.

In conclusion, MDR *P. aeruginosa* susceptibility rates to ceftazidime/avibactam and ceftolozane/tazobactam were higher than those to all existing antipseudomonal agents, except colistin, but were less than 50% in extremely resistant isolates. Worryingly, MDR *P. aeruginosa* isolates from Qatar belonged to international high-risk clones and non-susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam was largely driven by the production of β-lactamases, including ESBL and VIM enzymes. Though ceftazidime/avibactam and ceftolozane/tazobactam offer opportunities to treat some patients with MDR *P. aeruginosa*, their extensive cross-resistance with other β-lactam agents implies that the need to continue to develop new agents, preferably with novel targets and mechanisms of action, remains as critical as ever.

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Author contributions

M. A. S. A., A. A. I. H. and S. A. J. conceived and designed the study and performed the experimental work. M. A. S. A., J. J. and A. S. O. analysed and interpreted the data. M. A. S. A., H. A. H., A. A. I. H. and A. S. O. prepared the manuscript. All authors critically reviewed the manuscript. All authors read and approved the final manuscript. All authors agreed on submission.

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