β -Lactamase-Mediated Resistance in MDR-Pseudomonas Aeruginosa from Qatar

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Abstract

Background: Anti-pseudomonal β -lactam antibiotics are of paramount importance in the management of *Pseudomonas aeruginosa* infections. The distribution of β -lactam resistance genes in *P. aeruginosa* is often closely related to the distribution of certain high-risk international clones. In the present study whole-genome sequencing (WGS) was used to identify the predominant sequence types (STs) and β -lactamase genes in clinical isolates of multidrug-resistant (MDR)-*P. aeruginosa* from Qatar

Methods: Microbiological identification and susceptibility tests were performed by automated BD PhoenixTM system and manual Liofilchem MIC Test Strips. Seventy-five MDR-*P. aeruginosa* isolates were subsequently processed for WGS.

Results: Among 75 MDR-*P. aeruginosa* isolates; the largest proportions of susceptibility were to ceftazidime-avibactam (n=36, 48%), followed by ceftolozane-tazobactam (30, 40%), ceftazidime (n=21, 28%) and aztreonam (n=16, 21.33%). Almost all isolates possessed Class C and Class D β -lactamases (n=72, 96% each), while metallo- β -lactamases were detected in 20 (26.67%) isolates. Eight (40%) metallo- β -lactamases producers were susceptible to aztreonam and did not produce any concomitant extended-spectrum β -lactamases. The most frequent sequence types identified were ST235 (n=16, 21.33%), ST357 (n=8, 10.67%), ST389 and ST1284 (6, 8% each). Nearly all ST235 (15/16; 93.75%) were resistant to all tested β -lactams.

Conclusion: MDR-*P. aeruginosa* isolates from Qatar are highly resistant to antipseudomonal β -lactams. Global high-risk STs are predominant in Qatar and their associated multi-resistant phenotypes are a cause for considerable concern. The molecular epidemiology trends of *P. aeruginosa* should be surveillance at national levels to track their trends and institute the appropriate control strategies.

Background

Pseudomonas aeruginosa is a frequent cause of hospital-acquired infections.(1) Older patients and those who are critically ill or immunocompromised are at a particularly increased risk of severe *P. aeruginosa* infections.(2) Nosocomial *P. aeruginosa* infections are frequently associated with the presence of indwelling medical devices (e.g. catheter-associated urinary tract infections, ventilator-associated pneumonia and vascular-line associated bacteremia) and invasive procedures (e.g. surgical site infections).(3) Moreover, *P. aeruginosa* is also an important cause of chronic colonization and infective exacerbations in patients with chronic airway diseases such as bronchiectasis and cystic fibrosis.(4, 5)

Due to their established efficacy and safety, anti-pseudomonal β -lactam antibiotics play a vital role in the clinical management of *P. aeruginosa* infections.(6–8) Optimized clinical outcomes with β -lactam-based therapy for *P. aeruginosa* require *in-vitro* activity, early initiation, and appropriate dosing.(9) Poor clinical outcomes are often the result of the combined effects of vulnerable hosts, virulent pathogens and limited or delayed effective therapy.(10, 11)

In addition to a remarkable range of intrinsic antimicrobial resistance mechanisms, *P. aeruginosa* possesses a formidable ability to develop further resistance through chromosomal mutations and horizontal transfer of genetic determinants.(12) Key antimicrobial resistance mechanisms in *P. aeruginosa* include over-expression of efflux pumps, impermeability through porin modification or loss, target modification, and enzyme-mediated antimicrobial inactivation (e.g. β -lactamases).(13) Multiple resistance mechanisms are frequently present in concert resulting in simultaneous resistance to multiple agents.(14)

In many settings, *P. aeruginosa* resistance to β -lactam antibiotics is increasing at alarming rates. In particular, the increasing prevalence of horizontally-acquired β -lactamase genes is especially concerning.(14) In one report, the epidemiology of β -lactamases was closely related to the distribution of certain high-risk international clones.(15) In this study, we used whole-genome sequencing (WGS) to identify the predominant sequence types (STs) and β -lactamase genes in multi-drug resistant (MDR) *P. aeruginosa* clinical isolates from Qatar.

Methods

The study was conducted on clinical specimens received at Hamad Medical Corporation Microbiology Department during the period between October 2014 and September 2017. The department provides diagnostic microbiology services to all public hospitals in Qatar. The isolates were recovered from patients who received medical care in Hamad General Hospital (630-bedded acute care hospital), Rumailah Hospital (600 bedded long-term care hospital), and the National Center for Cancer Care and Research (46 bedded hemato-oncology center).

MDR status was defined as *in-vitro* resistance to at least one agent from three or more classes of anti-pseudomonal agents.(16) Bacterial identification and initial antimicrobial susceptibility testing was performed on BD PhoenixTM (Becton, Dickinson and Company, Franklin Lakes, New Jersey, United States). If required, identification was confirmed using Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Bruker Corporation, Billerica, Massachusetts). Liofilchem[®] MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) were used for minimum inhibitory concentration (MIC) determination. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853 were used as controls. Clinical Laboratory Standards Institute (CLSI) breakpoints were used for sensitive and resistant classification.(17) Out of 525 MDR-*P. aeruginosa* isolates which were collected between October 2014 and September 2017, 75 isolates were subsequently processed for WGS performed by Eurofins Genomics using Illumina HiSeq 2000 system (Illumina, San Diego, California).

The clean reads were assembled using SPAdes, Version 3.13.0 (Center for Algorithmic Biotechnology, St Petersburg, Russia).(18) Multi-locus sequence type (MLST) of MDR-*P. aeruginosa* isolates was performed using MLST 1.8 (Center for Genomic Epidemiology), based on seven housekeeping genes.(19) Genes associated with antimicrobial resistance were predicted using the Comprehensive Antibiotic Resistance Database (CARD), Version 1.2.0 (McMaster University, Hamilton, Ontario).(20) Clinical data were retrieved from the electronic healthcare system.

The study data are presented as frequency and percentage. Statistical analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, Washington). The study was approved by the Institutional Review Board, Hamad Medical Corporation (IRGC-01-51-033).

Results

Seventy-five MDR-*P. aeruginosa* isolates included were most frequently isolated from the respiratory tract (26, 34.67%) and skin and soft tissue (17, 22.67%) and urine (16, 21.33%) (Table 1). The largest proportions of susceptibility were to ceftazidime-avibactam (36, 48%; MIC_{50/90} 12/256 µg/ml) and ceftolozane-tazobactam (30, 40%; MIC_{50/90} 24/256 µg/ml), followed by ceftazidime (21, 28%; MIC_{50/90} 96/256 µg/ml) and aztreonam (16, 21.33%; MIC_{50/90} 24/256 µg/ml) (Fig. 1).

Table 1
Demographic characteristics and molecular characterization and susceptibility testing results for 75 MDR-*P. aeruginosa* isolates.

Isolate	ST	Collection m-y	Hospital	Location	Sample type	Molecular β -lactamase Class Detected in the Isolate				Antimicrobial susceptibility test					
						Class A	Class B	Class C	Class D	FEP	MEM	TZP	ATM	CAZ	CZA
PA1	ST235	Sep-14	HGH	OPD	SST		IMP-1	PDC-2	OXA-488	R	R	R	R	R	R
PA9	ST235	Oct-14	HGH	IPF	Urine		VIM-2	PDC-2	OXA-488	R	R	R	R	R	R
PA26	ST235	Nov-14	HGH	ICU	SST		VIM-2	PDC-2	OXA-17 OXA-488	R	R	R	R	R	R
PA27	ST235	Nov-14	HGH	OPD	SST		IMP-1	PDC-2	OXA-488	R	R	R	R	R	R
PA37	ST235	Dec-14	HGH	IPF	Other		VIM-2	PDC-2	OXA-17 OXA-488	R	R	R	R	R	R
PA99	ST235	Mar-15	HGH	OPD	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA128	ST235	Apr-15	HH	ICU	RT		VIM-2	PDC-2	OXA-17 OXA-114 OXA-488	R	R	R	R	R	R
PA129	ST235	Apr-15	HGH	OPD	Urine		VIM-2	PDC-2	OXA-114 OXA-488	R	R	R	R	R	R
PA131	ST235	May-15	HGH	OPD	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA134	ST235	May-15	HGH	OPD	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA143	ST235	May-15	HGH	IPF	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA169	ST235	Jun-15	HGH	OPD	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA176	ST235	Jul-15	HGH	IPF	SST		VIM-2	PDC-2	OXA-488	R	R	R	R	R	R

Susceptibility was reported according to Clinical Laboratory Standards Institute (CLSI) breakpoints (17)

m, month; y, year; HGH, Hamad General Hospital; RH, Rumailah Hospital; NCCCR, National Center for Cancer Care and Research; ICU, intensive care unit; IPF, inpatient floors; OPD, outpatient department; RT, respiratory tract; SST, skin and soft tissue; FEP, ceftazidime; MEM, meropenem; TZP, piperacillin-tazobactam; AT, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam. R, resistance; S, susceptible

Isolate	ST	Collection m-y	Hospital	Location	Sample type	Molecular β -lactamase Class Detected in the Isolate				Antimicrobial susceptibility test					
						Class A	Class B	Class C	Class D	FEP	MEM	TZP	ATM	CAZ	CZA
PA203	ST235	Sep-15	HGH	OPD	RT	VEB-9			OXA-10 OXA-114 OXA-488	R	S	S	R	S	S
PA209	ST235	Sep-15	HGH	OPD	Urine	VEB-9		PDC-2	OXA-10 OXA-488	R	R	R	R	R	R
PA250	ST235	Dec-15	HGH	IPF	Urine	VEB-9		PDC-2	OXA-488	R	R	R	R	R	R
PA16	ST357	Oct-14	HGH	IPF	RT	VEB-9		PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
PA17	ST357	Oct-14	HGH	OPD	Urine	VEB-9		PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
PA40	ST357	Dec-14	HGH	IPF	SST	VEB-9		PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
PA41	ST357	Dec-14	HGH	IPF	Urine	TEM-126 VEB-9		PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
PA130	ST357	May-15	HGH	IPF	SST	VEB-9		PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
RPA135	ST357	May-15	HGH	OPD	Urine			PDC-3	OXA-50	R	S	R	R	S	S
PA208	ST357	Sep-15	HGH	ICU	Blood	CTX-M15 VEB-9 SHV-11	VIM-5	PDC-3	OXA-10 OXA-50	R	R	R	R	R	R
PA212	ST357	Oct-15	HGH	IPF	Blood	VEB-9	VIM-5	PDC-3	OXA-10 OXA-50	R	R	R	R	R	R

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Isolate	ST	Collection m-y	Hospital	Location	Sample type	Molecular β -lactamase Class Detected in the Isolate				Antimicrobial susceptibility test					
						Class A	Class B	Class C	Class D	FEP	MEM	TZP	ATM	CAZ	CZA
PA19	ST389	Oct-14	HGH	OPD	RT			PDC-1	OXA-50	R	R	S	R	S	S
PA140	ST389	May-15	HGH	OPD	RT	TEM-116		PDC-1	OXA-50	R	R	R	R	R	R
PA175	ST389	Jul-15	HGH	OPD	RT	TEM-116		PDC-1	OXA-50	R	R	R	R	R	R
PA253	ST389	Dec-15	HGH	OPD	RT			PDC-1	OXA-50	R	R	R	R	R	R
PA311	ST389	Mar-16	HGH	OPD	RT			PDC-1	OXA-50	R	R	R	R	R	R
PA350	ST389	Jun-16	HGH	IPF	RT			PDC-1	OXA-50	R	R	R	R	R	R
PA6	ST1284	Sep-14	HGH	ICU	SST			PDC-3	OXA-488	S	R	R	R	R	S
PA20	ST1284	Oct-14	HGH	OPD	Urine	TEM-116		PDC-3	OXA-488	R	R	R	R	S	S
PA148	ST1284	May-15	HGH	IPF	Blood			PDC-3	OXA-488	R	R	R	S	S	S
PA166	ST1284	Jun-15	HGH	IPF	SST			PDC-3	OXA-488	R	R	R	R	S	S
PA187	ST1284	Aug-15	RH	IPF	SST			PDC-3	OXA-488	R	R	R	R	S	S
PA196	ST1284	Aug-15	HGH	OPD	Urine	CTX-M15 SHV-11			OXA-488	R	R	R	R	R	S
PA51	ST233	Dec-14	HGH	ICU	SST		VIM-2	PDC-3	OXA-4 OXA-486	R	R	R	S	R	R
PA199	ST233	Sep-15	RH	IPF	RT		VIM-2	PDC-3	OXA-4 OXA-486	R	R	R	S	R	R
PA220	ST233	Oct-15	HGH	IPF	Blood		VIM-2	PDC-3	OXA-4 OXA-114 OXA-486	R	R	R	R	R	R
PA508	ST233	Jul-17	HGH	IPF	Blood		VIM-2	PDC-3	OXA-4 OXA-486	R	R	R	S	R	R
PA527	ST233	Aug-17	HGH	IPF	Blood		VIM-2	PDC-3	OXA-4 OXA-486	R	R	R	S	R	R
PA5	ST274	Oct-14	HGH	ICU	RT	TEM-116		PDC-10	OXA-486	R	R	R	R	S	S

Susceptibility was reported according to Clinical Laboratory Standards Institute (CLSI) breakpoints (17)

m, month; y, year; HGH, Hamad General Hospital; RH, Rumailah Hospital; NCCCR, National Center for Cancer Care and Research; ICU, intensive care unit; IPF, inpatient floors; OPD, outpatient department; RT, respiratory tract; SST, skin and soft tissue; FEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam; AT, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam. R; resistance, S; susceptible

Isolate	ST	Collection m-y	Hospital	Location	Sample type	Molecular β -lactamase Class Detected in the Isolate				Antimicrobial susceptibility test					
						Class A	Class B	Class C	Class D	FEP	MEM	TZP	ATM	CAZ	CZA
PA32	ST274	Nov-14	HGH	ICU	RT			PDC-10	OXA-486	R	R	R	R	S	S
PA110	ST274	Mar-15	HGH	IPF	RT	TEM-116		PDC-10	OXA-486	R	R	R	R	S	S
NS PA161	ST274	Jun-15	HGH	ICU	RT			PDC-10	OXA-486	R	R	R	R	S	S
PA36	ST244	Dec-14	HGH	OPD	SST	TEM-1	VIM-2 IMP-1	PDC-1	OXA-4	R	R	R	R	R	R
									OXA-10						
									OXA-129						
									OXA-50 LCR-1						
NS NS NS PA126	ST244	Apr-15	HGH	ICU	SST			PDC-1	OXA-10 OXA-50	R	R	R	R	S	S
PA11	ST308	Oct-14	HGH	IPF	RT	VEB-9		PDC-7	OXA-488	R	R	R	R	R	R
PA12	ST308	Oct-14	HGH	OPD	Urine	VEB-9		PDC-7	OXA-488	R	R	R	R	R	R
PA98	ST308	Mar-15	HGH	OPD	Urine	VEB-9		PDC-7	OXA-488	R	R	R	R	R	S
PA125	ST823	Apr-15	HGH	IPF	RT		VIM-2	PDC-7		R	R	R	S	R	R
PA183	ST823	Jul-15	NCCCR	IPF	Blood		VIM-2	PDC-7		R	R	R	S	R	R
PA200	ST823	Sep-15	HGH	IPF	RT	SHV-11	VIM-2	PDC-7		R	R	R	S	R	R
PA207	ST2819	Sep-15	HGH	ICU	RT			PDC-5	OXA-50	R	R	R	R	R	S
PA232	ST2819	Nov-15	HGH	IPF	Blood			PDC-5	OXA-50	R	R	R	S	R	S
RRPA241	ST1076	Dec-15	HGH	IPF	Blood	CTX-M15		PDC-7	OXA-50	R	R	R	R	S	S
PA323	ST446	Apr-16	HGH	IPF	RT			PDC-7	OXA-50	R	R	R	R	S	S
PA142	ST560	May-15	HGH	IPF	SST			PDC-10	OXA-488	R	R	R	R	R	S
PA447	ST598	Feb-17	RH	IPF	Blood			PDC-3	OXA-50	R	R	R	R	R	S
PA180	ST639	Jul-15	HGH	ICU	RT			PDC-3	OXA-486	R	R	R	S	S	S

Susceptibility was reported according to Clinical Laboratory Standards Institute (CLSI) breakpoints (17)

m, month; y, year; HGH, Hamad General Hospital; RH, Rumailah Hospital; NCCCR, National Center for Cancer Care and Research; ICU, intensive care unit; IPF, inpatient floors; OPD, outpatient department; RT, respiratory tract; SST, skin and soft tissue; FEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam; AT, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam. R; resistance, S; susceptible

Isolate	ST	Collection m-y	Hospital	Location	Sample type	Molecular β -lactamase Class Detected in the Isolate				Antimicrobial susceptibility test					
						Class A	Class B	Class C	Class D	FEP	MEM	TZP	ATM	CAZ	CZA
PA66	ST664	Jan-15	HGH	IPF	SST			PDC-8	OXA-4 OXA-10 OXA-50	R	R	R	R	R	R
PA420	ST699	Dec-16	RH	IPF	Blood			PDC-1	OXA-50	R	R	R	R	R	R
PA498	ST773	Jun-17	HGH	IPF	Blood		VIM-2	PDC-7	OXA-50	R	R	R	S	R	R
PA10	ST381	Oct-14	HGH	IPF	RT			PDC-3	OXA-50	S	R	R	R	S	S
RRRRPA4	ST17	Sep-14	HGH	OPD	SST			PDC-8	OXA-50	R	S	R	R	S	S
PA154	ST27	Jun-15	HGH	ICU	RT	TEM-116		PDC-5	OXA-50	R	R	R	R	R	S
PA428	ST179	Dec-16	HGH	IPF	SST			PDC-8	OXA-14 OXA-50	R	R	R	R	R	R
PA86	ST252	Feb-15	HGH	ICU	RT			PDC-3	OXA-486	R	R	R	R	R	S
PA190	ST253	Sep-15	HGH	IPF	SST			PDC-9	OXA-488	R	R	R	S	S	S
PA123	ST292	Apr-15	HGH	IPF	Blood	CARB-3		PDC-5	OXA-50	R	R	R	S	R	S
PA349	ST310	Jun-16	HGH	IPF	RT			PDC-3	OXA-488	R	R	R	S	S	S
PA78	ST313	Jan-15	HGH	OPD	RT			PDC-7	OXA-488	S	R	S	S	S	S
PA263	ST348	Jan-16	RH	IPF	Blood			PDC-5	OXA-50	R	R	R	R	R	S
PA119	ST3022	Apr-15	HGH	ICU	RT	VEB-9			OXA-10 OXA-50	R	R	R	R	R	S
PA457	ST3043	Mar-17	HGH	IPF	Blood				OXA-50	R	S	S	R	S	S
Susceptibility was reported according to Clinical Laboratory Standards Institute (CLSI) breakpoints (17)															
m, month; y, year; HGH, Hamad General Hospital; RH, Rumailah Hospital; NCCCR, National Center for Cancer Care and Research; ICU, intensive care unit; IPF, inpatient floors; OPD, outpatient department; RT, respiratory tract; SST, skin and soft tissue; FEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam; AT, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam. R; resistance, S; susceptible															

Seven (9.33%) isolates were resistant to ceftolozane-tazobactam but susceptible to ceftazidime-avibactam, while one (1.33%) isolate was resistant to ceftazidime-avibactam but susceptible to ceftolozane-tazobactam. Furthermore, 4 (5.33%) were resistant to all tested β -lactams except ceftazidime-avibactam, while only one (1.33%) isolate was only susceptible to ceftolozane-tazobactam (Table 1).

Almost all isolates possessed Class C and Class D β -lactamases (72, 96% each). All 4 β -lactamase classes were present in three (4%) isolates. Metallo- β -lactamases (MBL) were detected in 20 (26.67%) isolates. Eight (40%) MBL producers were susceptible to aztreonam and did not produce any concomitant extended-spectrum β -lactamases (ESBL) (Table 1).

The most frequent STs identified were ST235 (16, 21.33%), ST357 (8, 10.67%), ST389 (6, 8%) and ST1284 (6, 8%) (Fig. 2). All but one ST235 isolate were resistant to all tested β -lactam agents. Furthermore, amongst the 16 ST235 MDR-*P. aeruginosa* isolates included in this study, MBL were detected in nine (56.25%), *bla*_{VEB-9} in 8 (50%), *bla*_{PDC-2} in 15 (93.75%) and *bla*_{OXA-10} and *bla*_{OXA-50} in all 16 (100%). There were five ST233 MDR-*P. aeruginosa* isolates; all

possessed *bla*_{VIM-2}, *bla*_{ADC-3}, *bla*_{OXA-4} and *bla*_{OXA-486}, and four (80%) of them were resistant to all tested β-lactams except aztreonam. Different patterns of β-lactamase genes and β-lactam susceptibility were observed in other STs (Table 2).

Table 2
Most frequent sequence types and associated β -lactamase genes and susceptibility to β -lactam antipseudomonal agents.

Sequence Type	β -lactamase Genes Detected	Number (%) susceptible						
		FEP	MEM	TZP	ATM	CAZ	CZA	C/T
ST235 (n = 16)	<i>bla</i> _{VEB-9} (8, 50%)	0	1	1	0	1	1	0
	<i>bla</i> _{VIM-2} (6, 38%)		(6%)	(6%)		(6%)	(6%)	
	<i>bla</i> _{IMP-1} (2, 13%)							
	<i>bla</i> _{PDC-2} (15, 94%)							
	<i>bla</i> _{OXA-10} (16, 100%)							
	<i>bla</i> _{OXA-488} (16, 100%)							
ST357 (n = 8)	<i>bla</i> _{VEB-9} (7, 88%)	0	1	0	0	1	1	0
	<i>bla</i> _{VIM-5} (2, 25%)		(13%)			(13%)	(13%)	
	<i>bla</i> _{PDC-3} (8, 100%)							
	<i>bla</i> _{OXA-10} (8, 100%)							
	<i>bla</i> _{OXA-50} (8, 100%)							
ST389 (n = 6)	<i>bla</i> _{TEM-116} (2, 33%)	0	0	1	0	1	5	3
	<i>bla</i> _{PDC-1} (6, 100%)			(17%)		(17%)	(83%)	(50%)
	<i>bla</i> _{OXA-50} (6, 100%)							
ST1284 (n = 6)	<i>bla</i> _{TEM-116} (1, 17%)	1	0	0	1	4	6	5
	<i>bla</i> _{CTX-M15} (1, 17%)				(17%)	(67%)	(100%)	(83%)
	<i>bla</i> _{PDC-3} (5, 83%)							
	<i>bla</i> _{OXA-488} (6, 100%)							
ST233 (n = 5)	<i>bla</i> _{VIM-2} (5, 100%)	0	0	0	4	0	0	0
	<i>bla</i> _{PDC-3} (5, 100%)				(80%)			
	<i>bla</i> _{OXA-4} (5, 100%)							
	<i>bla</i> _{OXA-486} (5, 100%)							
ST274 (n = 4)	<i>bla</i> _{TEM-116} (2, 50%)	0	0	0	3	2	2	1
	<i>bla</i> _{PDC-10} (4, 100%)				(75%)	(50%)	(50%)	(25%)
	<i>bla</i> _{OXA-486} (4, 100%)							
ST823 (n = 3)	<i>bla</i> _{VIM-2} (3, 100%)	0	0	0	3	2	2	1
	<i>bla</i> _{PDC-7} (3, 100%)				(100%)	(67%)	(67%)	(33%)
ST308 (n = 3)	<i>bla</i> _{VEB-9} (3, 100%)	0	2	2	0	0	1	0
	<i>bla</i> _{PDC-7} (3, 100%)		(67%)	(67%)			(33%)	

ATM, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam; FEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam

Sequence Type	β -lactamase Genes Detected	Number (%) susceptible						
		FEP	MEM	TZP	ATM	CAZ	CZA	C/T
ST244 (n = 2)	<i>bla</i> _{TEM-1} (1, 50%)	0	0	0	0	1	1	1
	<i>bla</i> _{VIM-2} (1, 50%)					(50%)	(50%)	(50%)
	<i>bla</i> _{IMP-1} (1, 50%)							
	<i>bla</i> _{PDC-1} (2, 100%)							
	<i>bla</i> _{OXA-4} (1, 50%)							
	<i>bla</i> _{OXA-10} (2, 100%)							
	<i>bla</i> _{OXA-50} (2, 100%)							
	<i>bla</i> _{OXA-129} (1, 50%)							
	<i>bla</i> _{LCR-1} (1, 50%)							
ST2819 (n = 2)	<i>bla</i> _{PDC-5} (2, 100%)	0	0	0	1	0	2	2
	<i>bla</i> _{OXA-50} (2, 100%)				(50%)		(100%)	(100%)
ATM, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam; FEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam								

Discussion

The present study included MDR-*P. aeruginosa* isolates from a national diagnostic microbiology laboratory in Qatar and are therefore representative of the whole country. Notably, MDR-*P. aeruginosa* in Qatar are highly resistant to β -lactam agents. The most active β -lactam antibiotics in this study were those in combination with β -lactamase inhibitors, ceftazidime-avibactam and ceftolozane-tazobactam, were not available for clinical use at the time of the study. Yet, less than half of the isolates were susceptible. Given their recent availability for patients in Qatar, the results reported demonstrate the importance of their appropriate clinical use to minimize further loss of activity.(21)

Both ceftazidime-avibactam and ceftolozane-tazobactam are potent anti-pseudomonal agents.(22) In contrast to this study, previous reports have shown that more than 90% of MDR-*P. aeruginosa* isolates from different parts of the world were susceptible to both agents.(23, 24) To a large extent this could be explained by underlying β -lactamases of the isolates investigated in the present study.

With exception of monobactam, MBL result in phenotypic resistance to all other antipseudomonal agents tested in this study.(14) This report included 20 (26.67%) isolates that possessed 21 MBL-encoding genes (16 *bla*_{VIM-2}, 2 *bla*_{VIM-5}, and 3 *bla*_{IMP-2}) (Table 1 and Table 2). This is consistent with the known predominance of VIM enzymes, and to a lesser extent IMP enzymes, in *P. aeruginosa* from the Middle East.(25–27) Unlike other geographic settings such as in Indian subcontinent, NDM enzymes have not been detected in *P. aeruginosa* from the Arabian Peninsula.(26, 28)

Apart from areas with a high prevalence of MBL in *P. aeruginosa*, the presence of Class A ESBL β -lactamases can result in resistance to ceftolozane-tazobactam.(14) Avibactam is an efficient inhibitor of Class A β -lactamases and hence ceftazidime-avibactam combination retains its activity in this situation but not ceftolozane-tazobactam.(29, 30) In a report from Spain of 24 extremely-drug resistant ST235 *P. aeruginosa* isolates, 13% were susceptible to ceftolozane-tazobactam and 58% to ceftazidime-avibactam and the predominant β -lactamases identified were VIM-2 (42%) and the Class A ESBL GES-5 (46%).(31) Consistent with this, five out of seven ceftolozane-tazobactam-resistant, ceftazidime-avibactam-susceptible MDR-*P. aeruginosa* isolates in our study possessed class A *bla*_{SHV-11} and ESBL-encoding genes such as *bla*_{VEB-9} and *bla*_{TEM-116}. Interestingly, those 7 isolates belonged to seven different STs (Table 1).

The β -lactamase *bla*_{VEB-9} (19, 25.33%), formerly known as *bla*_{VEB-1a}, was the most frequent ESBL identified in the present study.(30) *bla*_{VEB-1} is one of the most frequently reported ESBLs in *P. aeruginosa* from the Middle East including Kuwait, Saudi Arabia and Iran.(32–34) Though *bla*_{VEB-9} was reported from Thailand and Eastern Europe, to the best of our knowledge, it has not been previously reported from the Middle East.(30, 35) In this study, VEB-9-producing MDR-*P. aeruginosa* belonged to ST235 (8/16), ST357 (7/8), ST308 (1/3) and ST3022 (1/1) (Table 1 and Table 2). This pattern suggests dissemination within specific *P. aeruginosa* STs in Qatar that may be different from neighboring countries.

An interesting observation in this study was that 16 (21.33%) MDR-*P. aeruginosa* isolates were susceptible to aztreonam but resistant to several other antipseudomonal β -lactams tested (Table 1). Aztreonam is a weak inducer of Class C enzymes and is not a substrate for Class B and narrow-spectrum Class D β -lactamases.(36) The retained aztreonam activity in these isolates despite resistance to other antipseudomonal β -lactams may be explained by the

absence of Class A ESBL in those isolates. Therefore, aztreonam should ideally be included in routine antimicrobial susceptibility testing of clinical *P. aeruginosa* isolates.

Most MDR-*P. aeruginosa* isolates included in this study belonged to five STs and had consistent β -lactamase genetic profiles and β -lactam susceptibility patterns (Table 2). ST235, ST233, and ST357 are already known as high-risk clones in Qatar, Saudi Arabia, Bahrain, and the United Arab Emirates.(26) These three STs are globally disseminated MDR-*P. aeruginosa* clones.(15) Often, these strains cause regional or nationwide outbreaks, express MDR phenotypes, and are associated with high mortality.(31, 37, 38) VIM-producing ST1284 *P. aeruginosa* have been described from Brazil,(39), and ST389 from cystic fibrosis patients in Italy.(40) Both sequence types have otherwise limited geographic distribution.

Conclusions

In summary, MDR-*P. aeruginosa* isolates from Qatar are highly resistant to antipseudomonal β -lactams. to antipseudomonal β -lactams. Global high-risk STs predominate in Qatar and their associated multi-resistant phenotype is a cause for considerable concern. The molecular epidemiology trends of *P. aeruginosa* should be evaluated regularly at national and regional levels to track their trends and institute the appropriate control strategies.

List Of Abbreviations

Whole-genome sequencing (WGS)

Sequence types (STs)

Multi-drug resistant (MDR)

Minimum inhibitory concentration (MIC)

Clinical Laboratory Standards Institute (CLSI)

Metallo-b-lactamases (MBL)

Extended-spectrum b-lactamases (ESBL)

Declarations

Ethical approval

This study was approved by the Research Ethics Committee (Protocol number IRGC-01-51-033) at Hamad Medical Corporation, Doha, Qatar.

Consent for publication

The findings achieved herein are solely the responsibility of the author[s]. The funders were not involved in the conduct of the study, the preparation of the manuscript or the decision to submit the manuscript for publication. The manuscript does not contain data from any individual person

Availability of data and materials

We are disclosing that strictest confidence was maintained for data collection as well as access and application in the study. Data were never shared at any level with any individuals not authorized to access research material. Data were only available upon request by the authors following permission from the Medical Research Center at HMC. We fully understand that the use of confidential data for personal purposes is prohibited.

Competing of Interest

All authors have any conflict of interest in declaring in relation to this manuscript.

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Author Contribution

Conception and design of study: M. A., Sid Ahmed, E. B., Ibrahim, J., Jass. Acquisition of data (laboratory or clinical): M. A., Sid Ahmed, F. A., Khan.

Data analysis and/or interpretation: M. A., Sid Ahmed, A. S., Omrani. Drafting of manuscript and/or critical revision: M. A., Sid Ahmed, F. A., Khan, A. A., Sultan, B. Söderquist, E. B., Ibrahim, J., Jass, A. S., Omrani. Approval of final version of manuscript: M. A., Sid Ahmed, F. A., Khan, A. A., Sultan, B. Söderquist, E. B., Ibrahim, J., Jass, A. S., Omrani. All authors reviewed the manuscript and agreed to its publication.

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Figures

Figure 1

Antimicrobial susceptibility testing results for 75 MDR-P. aeruginosa isolates from Qatar Columns represent number (Y axis) of isolates susceptible and line represents percentage (Z axis) of isolates susceptible to the corresponding antipseudomonal β -lactam. Reporting is based on CLSI breakpoint recommendations (M100, 30th edition – January 2020). ATM, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; C/T, ceftolozane-tazobactam; FEP, cefepime; MEM, meropenem; MIC, minimum inhibitory concentration in $\mu\text{g/mL}$, TZP, piperacillin-tazobactam

Figure 2

Distribution of sequence types of MDR-P. aeruginosa (n=75) isolated in Qatar from 2014 to 2017.