SHORT REPORT

β-lactamase-mediated resistance in MDR-*Pseudomonas aeruginosa* from Qatar

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Abstract

Background: The distribution of β-lactam resistance genes in *P. aeruginosa* is often closely related to the distribution of certain high-risk international clones. We used whole-genome sequencing (WGS) to identify the predominant sequence types (ST) 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 Phoenix™ system and manual Liofilchem MIC Test Strips.

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, 21.3%). All isolates possessed Class C and/or Class D β-lactamases (n = 72, 96% each), while metallo-β-lactamases were detected in 20 (26.7%) isolates. Eight (40%) metallo-β-lactamase producers were susceptible to aztreonam and did not produce any concomitant extended-spectrum β-lactamases. High risk ST235 (n = 16, 21.3%), ST357 (n = 8, 10.7%), ST389 and ST1284 (6, 8% each) were most frequent. Nearly all ST235 isolates (15/16; 93.8%) were resistant to all tested β-lactams.

Conclusion: MDR-*P. aeruginosa* isolates from Qatar are highly resistant to antipseudomonal β-lactams. High-risk STs are predominant in Qatar and their associated MDR phenotypes are a cause for considerable concern.

Keywords: Beta-lactamases, MDR, *P. aeruginosa*, ST235

Background

Due to their established efficacy and safety, anti-pseudomonal β-lactam antibiotics play a vital role in the clinical management of *P. aeruginosa* infections [1]. 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). Multiple resistance mechanisms are frequently present in concert resulting in simultaneous resistance to multiple agents [1]. The epidemiology of β-lactamases is often closely related to the distribution of certain high-risk international clones [2]. 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 setting, bacterial identification, antimicrobial susceptibility testing, whole genome sequencing, and statistical analysis details are provided in Additional File 1. MDR status was defined as *in-vitro* resistance to at least one agent from three or more classes of anti-pseudomonal agents [3]. β-lactamases were classified according to their molecular groups [4]. Clinical data were retrieved from the electronic healthcare system.

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Results

Seventy-five MDR-*P. aeruginosa* isolates were included (Additional file 1: Table S1). The largest proportions of susceptibility were to ceftazidime-avibactam [36, 48%; minimum inhibitory concentration (MIC)$_{50/90}$ 12/256 μg/ml] and ceftolozane-tazobactam (30, 40%; MIC$_{50/90}$ 24/256 μg/ml) (Fig. 1). Four (5.3%) isolates were resistant to all tested β-lactams except ceftazidime-avibactam, while only one (1.3%) isolate was only susceptible to ceftolozane-tazobactam (Additional file 1: Table S1).

Almost all isolates possessed Class C and Class D β-lactamases (72, 96% each). All 4 β-lactamase classes were present in 3 (4%) isolates. Metallo-β-lactamases (MBL) were detected in 20 (26.7%) isolates. Eight (40%) MBL producers were susceptible to aztreonam and did not produce any concomitant extended-spectrum β-lactamases (ESBL) (Additional file 1: Table S1).
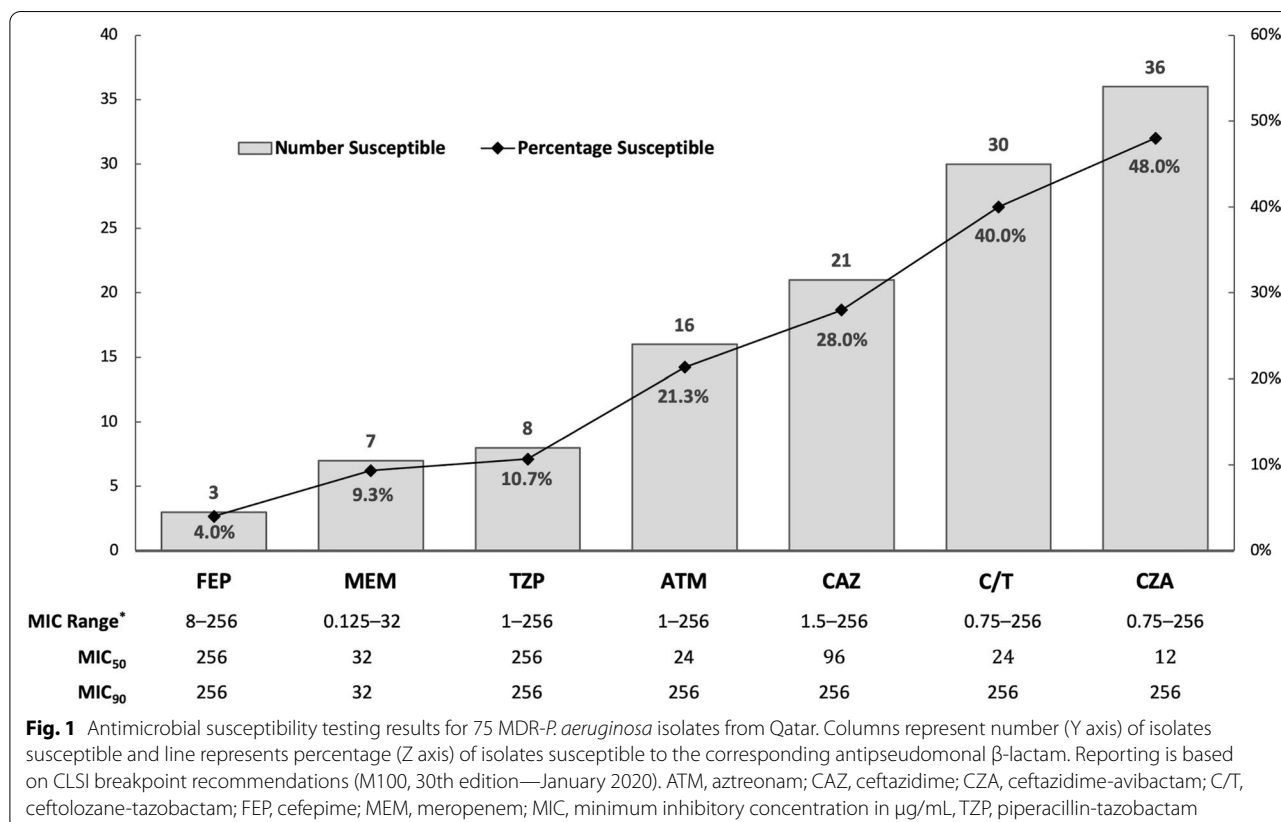
The most frequent STs identified were ST235 (16, 21.3%) and ST357 (8, 10.7%) (Fig. 2). All but one ST235 isolate were resistant to all tested β-lactam agents. Furthermore, amongst the 16 ST235 MDR-*P. aeruginosa* isolates included in this study, MBL were detected in nine (56.3%), *bla*$_{VEB-9}$ in 8 (50%), *bla*$_{PDC-2}$ in 15 (93.8%), and *bla*$_{OXA-10}$ and *bla*$_{OXA-50}$ in all 16 (100%). There were five ST233 MDR-*P. aeruginosa* isolates; all possessed *bla*$_{VIM-2}$,
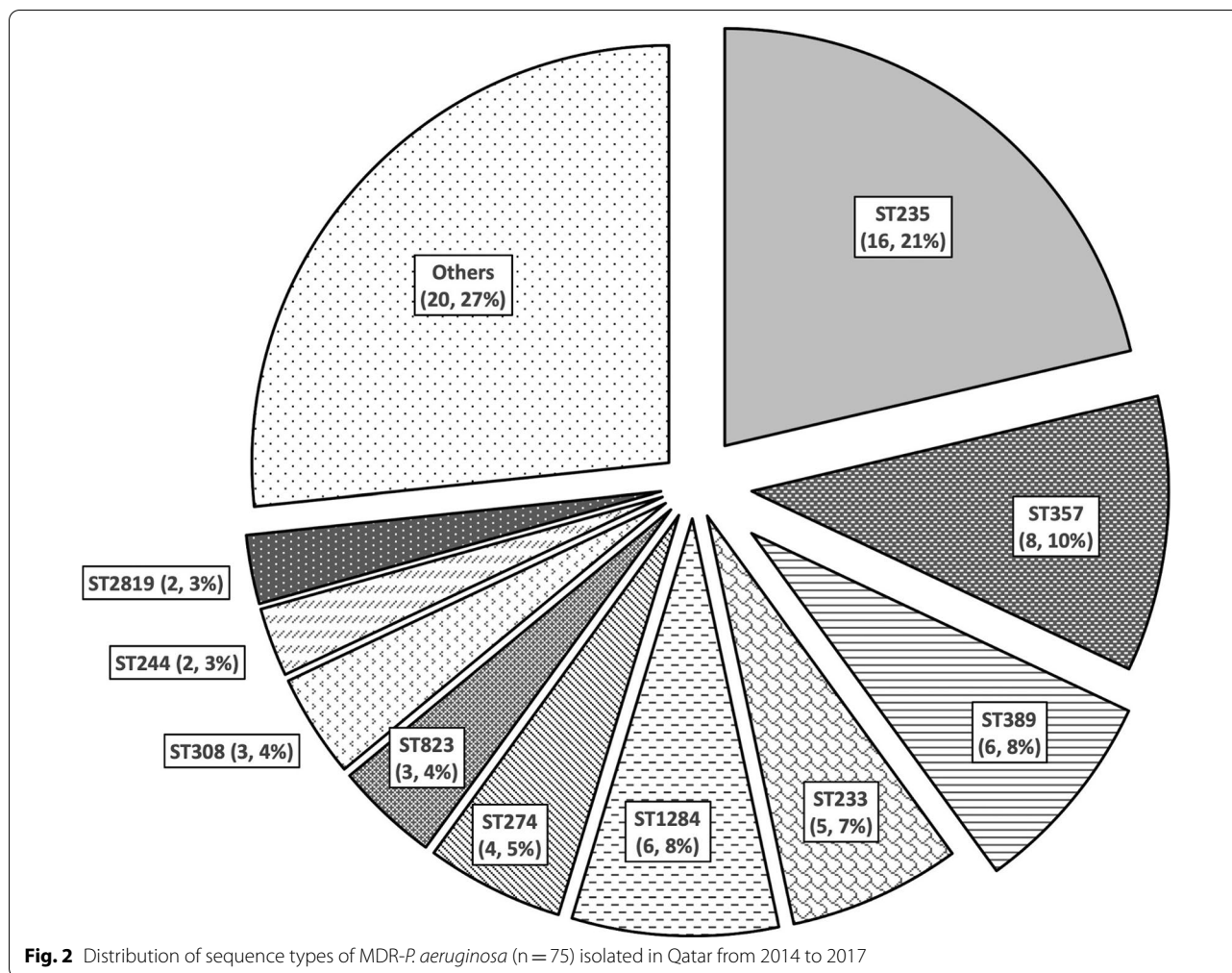
bla$_{PDC-3}$, *bla*$_{OXA-4}$ and *bla*$_{OXA-486}$, and four (80%) of them were resistant to all tested β-lactams except aztreonam. Different patterns of β-lactamase genes and β-lactam susceptibility were observed in other STs (Additional file 1: Table S2).

Discussion

This study included data representative of the whole country, as it analyzed isolates from a national diagnostic laboratory. 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 [5].

This report included 20 (26.67%) isolates that possessed 21 MBL-encoding genes (16 *bla*$_{VIM-2}$, 2 *bla*$_{VIM-5}$, and 3 *bla*$_{IMP-2}$) (Additional file 1: Tables S1 and S2). This is consistent with the known predominance of Verona integron-encoded metallo-β-lactamases (VIM), and to a lesser extent imipenemases (IMP), in *P. aeruginosa* from

Fig. 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 μg/mL, TZP, piperacillin-tazobactam

	FEP	MEM	TZP	ATM	CAZ	C/T	CZA
MIC Range*	8–256	0.125–32	1–256	1–256	1.5–256	0.75–256	0.75–256
MIC$_{50}$	256	32	256	24	96	24	12
MIC$_{90}$	256	32	256	256	256	256	256

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Fig. 2 Distribution of sequence types of MDR-*P. aeruginosa* (n = 75) isolated in Qatar from 2014 to 2017

the Middle East [6–8]. Unlike other geographic settings, New Delhi metallo-β-lactamases (NDM) have not been detected in *P. aeruginosa* from the Arabian Peninsula [7, 9].

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 [1]. 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 [10, 11]. 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 Guiana-Extended-Spectrum (GES)-5 (46%) [12]. 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 (Additional file 1: Table S1).

The β-lactamase bla_{VEB-9} (19, 25.33%), formerly known as bla_{VEB-1a}, was the most frequent ESBL gene identified in the present study [11]. bla_{VEB-1} is one of the most frequently reported ESBLs in *P. aeruginosa* from the Middle East including Kuwait, Saudi Arabia and Iran [13–15]. 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 [11, 16]. In this study, MDR-*P. aeruginosa* producing Vietnamese extended-spectrum beta-lactamase-9 (VEB-9) belonged to ST235 (8/16), ST357 (7/8), ST308 (1/3) and ST3022 (1/1) (Additional file 1: Tables S1 and S2). 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

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antipseudomonal β-lactams tested (Additional file 1: Table S1). Aztreonam is a weak inducer of Class C enzymes and is not a substrate for Class B and narrow-spectrum Class D β-lactamases [17]. 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 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 (Additional file 1: Table S2). ST235, ST233, and ST357 are already known as high-risk clones in Qatar, Saudi Arabia, Bahrain, and the United Arab Emirates [7]. These three STs are globally disseminated MDR-*P. aeruginosa* clones [2]. Often, these strains cause regional or nation-wide outbreaks, express MDR phenotypes, and are associated with high mortality [12, 18, 19]. VIM-producing ST1284 *P. aeruginosa* have been described from Brazil, and ST389 from cystic fibrosis patients in Italy [20, 21]. Both sequence types have otherwise limited geographic distribution.

Conclusion

MDR-*P. aeruginosa* isolates from Qatar are highly resistant to antipseudomonal β-lactams. Global high-risk STs predominate in Qatar and their associated multi-resistant phenotype is a cause for considerable concern.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13756-020-00838-y.

Additional file 1. Supplementary data file.

Abbreviations

CLSI: Clinical Laboratory Standards Institute; ESBL: Extended-spectrum β-lactamase; GES: Guiana-Extended-Spectrum; IMP: Imipenemase; MBL: Metallo-β-lactamase; MIC: Minimum inhibitory concentration; MDR: Multi-drug resistant; NDM: New Delhi metallo-β-lactamase; ST: Sequence type; VEB: Vietnamese extended-spectrum β-lactamase; VIM: Verona integron-encoded metallo-β-lactamase; WGS: Whole-genome sequencing.

Authors' contributions

Conception and design of study: M.A.S., E.B.I.; Acquisition of data: M.A.S., F.A.K.; Data analysis and interpretation: M.A.S., A.S.O.; Drafting of manuscript: M.A.S., A.S.O. All authors critically reviewed the manuscript and agreed to its publication. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

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

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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