

The resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa* isolates to in vitro Ceftazidime/Avibactam and Ceftolozane/Tazobactam, in Qatar

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BACKGROUND

Antimicrobial resistance (AMR) is one of the top health priorities facing humanity in the 21st century (1). Healthcare associated infections (HAIs) cause significant morbidity and mortality as well as being associated with substantial management costs. *Pseudomonas aeruginosa* is one of the leading causes of HAIs with rising trend of resistance rates. Infections with Multidrug-resistant *P. aeruginosa* (MDR-PA) are associated with serious clinical challenges due to their resistance to most available antibiotics hence significantly limiting available treatment options (2). Recently, ceftazidime/avibactam (CZA) and ceftolozane/tazobactam (C/T) have been approved for clinical use and these combinations have demonstrated excellent activity against Gram-negative bacteria, including MDR-PA (3). Despite the reported success resistance to these new antimicrobial combinations has already been reported; the mechanisms of which has not been fully explored (4). In this study, we aimed to characterize the genetic mechanisms driving resistance to the novel antibiotics against MDR-PA.

Study background and Objectives

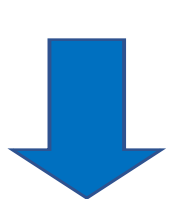
CZA and C/T have demonstrated proven efficacy in the management of MDR Gram-negative infections. The Prevalence of MDR-PA resistance to CZA and C/T is currently between 11-30% worldwide. Understanding the underlying molecular mechanisms driving MDR-PA bacterial resistance to the new antimicrobial is not well-established.

Our study aims to determine antimicrobial susceptibility patterns of CZA and C/T against MDR-PA isolates, to compare CZA and C/T resistant isolates with their comparator antibiotics and to characterize the genetic mechanisms driving CZA & C/T resistance in MDR-PA isolates in Qatar

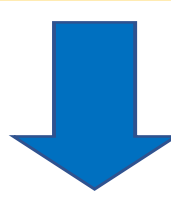
MATERIALS AND METHODS

A total of 205 MDR-PA isolates were collected from different routine clinical specimens between 2014 and 2015. Antimicrobial susceptibility was determined using the Etest according to standard recommendations. MDR-PA were defined as: resistant to at least 1 antibiotic agent from ≥ 3 antibiotic classes (5). From a total of 205 MDR-PA, 94 isolates were found to be resistant to one or both CZA and C/T antibiotics, of which 9 isolates (10%) were randomly selected for this study for detailed analysis. The 9 isolates were subjected to whole genome sequencing (WGS) and bioinformatics analysis.

205 MDR-PA isolates



In vitro susceptibility for CZA & C/T:
92 isolates were found to be resistant to CZA and/or C/T



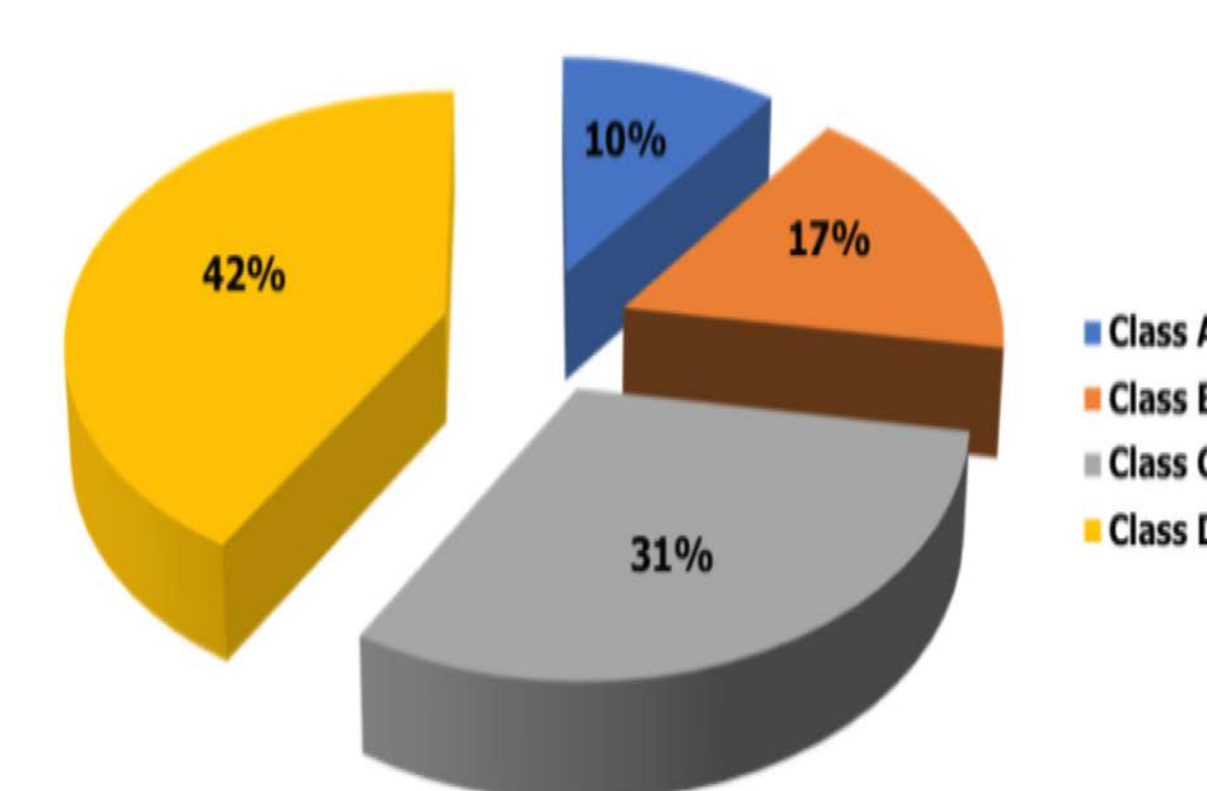
9 isolates (10%) were randomly selected for WGS

RESULTS

The 9 isolates belonged to 5 different sequence types; ST-292 (n=1), ST-233 (n=1), ST-308 (n=3), and ST-823 (n=1) ST-2613 (n=3). The isolates' genomes contained 2 – 4 different β -lactamase genes from various classes. Four isolates had VEB-1a (Class A), 1 isolate had CARB-3, 4 isolates had VIM-2 (Class B), PDC-2, 3, 5, and 7 (Class C) were found in 3, 1, 1, and 4, respectively and OXA-4, 10, and 50 (class D) were found in 1, 2, and 9, respectively. The genes encoding the efflux pump regulators *mexR*, *nalC*, and *nalD*, as well as the efflux pump complexes, MexAB-OprM, MexCD-OprJ, MexPQ-OpmE and MuxABC-OpmB were found in all the isolates, however, the efflux pump regulator *cpxR*, *soxR*, and type B *NfxB* were detected in 7, 6, and 6 isolates.

Isolates	P1	P2	P3	P4	P5	P6	P7	P8	P9
β -Lactamase gene	β -Lactamase Class								
VEB-1a	Class A								
CARB-3	Class B								
VIM-2	Class B								
PDC-2	Class C								
PDC-3	Class C								
PDC-5	Class C								
PDC-7	Class C								
OXA-4	Class D								
OXA-10 (PSE2)	Class D								
OXA-50	Class D								

The prevalence of β -lactamase classes



Efflux & Pump Genes Present in isolates

Efflux pump gene	P1	P2	P3	P4	P5	P6	P7	P8	P9
The Efflux Pump Regulators									
MexR	+	+	+	+	+	+	+	+	+
nalC	+	+	+	+	+	+	+	+	+
nalD	+	+	+	+	+	+	+	+	+
CpxR	+	+	+	+	+	+	+	+	+
soxR	+	+	+	+	+	+	+	+	+
Type B NfxB	+	+	+	+	+	+	+	+	+
Efflux Pump Complex									
MexAB-OprM	+	+	+	+	+	+	+	+	+
MexCD-OprJ	+	+	+	+	+	+	+	+	+
MexPQ-OpmE	+	+	+	+	+	+	+	+	+
MuxABC-OpmB	+	+	+	+	+	+	+	+	+

CONCLUSIONS

The resistance mechanisms of MDRA-PA to CZA and CT are probably complex. Two or more β -lactamase resistance genes were present in all isolates, class D being predominant. Since class B and D are poorly inhibited by β -lactamase inhibitors that's might explain the resistance of these isolates to CZA and C/T. Likewise, the strains also contained four different efflux pump complexes together with the two-component regulatory systems that control their expression pointing towards their role in the resistance mechanism.

The exact mechanism that mainly drives MDRA-PA resistance to CZA and C/T remains not fully explored and need further evaluation.

References

1. Jim O'Neill, et al., 2014
2. Humphries et al., 2017; Winkler et al 2015; Cabot et al 2014
3. Dee Shortridge et al, AAC July 2017
4. Sader et al, AAC June 2015
5. Magiorakos et al. 2012

Acknowledgement

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