Intrinsic Resistance Mechanisms Detected Among Ceftazidime-Avibactam-Susceptible and -Resistant *Pseudomonas aeruginosa* Isolates Collected from United States Hospitals (2015)

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M Castanheira, TB Doyle, AP Davis, RE Mendes, HS Sader

JMI Laboratories, North Liberty, Iowa, USA

Contact Information:
Mariana Castanheira, PhD
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: mariana-castanheira@jmilabs.com

AMENDED ABSTRACT

Background: Avibactam (AVI) is a non-β-lactam β-lactamase inhibitor of class A, C, and some class D enzymes developed for clinical use in combination with ceftazidime (CAZ), a cephalosporin displaying antipseudomonal activity. CAZ-AVI has demonstrated activity against >97.0% of the PSA isolates from the US; however, resistance (R) has been documented. We investigated β-lactam (BL) R mechanisms among CAZ-AVI-R isolates and compared with CAZ-AVI-susceptible (S) isolates displaying R to other antipseudomonal BLs.

Methods: Among 2,548 PSA clinical isolates collected during 2015 from 106 US hospitals and tested by broth microdilution methods, all 47 CAZ-AVI-R and 60 CAZ-AVI-S isolates were further analyzed. CAZ-AVI-R isolates were submitted to whole genome sequencing to analyze genes associated with BL R, and all isolates were evaluated for the expression of chromosomal (c) *ampC*, *mexA*, *mexC*, *mexE* and *mexX, poxB* and *Pa5542,* and OprD loss/ decrease by Western blot analysis.

Results: Only 2 of 47 (4.3%) CAZ-AVI-R isolates carried metallo-β-lactamase genes (bla_{VIM-1} or $_2$). ESBL bla_{OXA6} and 1 bla_{GES-7} (carbapenemase) were also detected among 3 isolates. Alterations on cAmpC Ω loop previously associated with R to CAZ-AVI and/or other antipseudomonal BLs were noted among 10 CAZ-AVI-R isolates. When comparing mutation driven R mechanisms among the CAZ-AVI-R and -S subsets, OprD loss, overexpression of cAmpC, or MexCD-OprJ were equally noted in both groups. Overexpression of MexAB-OprM, MexXY-OprM, Pa5542 (metallo-imipenemase), and PoxB (OXA-50) were more common among CAZ-AVI-S isolates, but these mechanisms varied among isolates nonsusceptible to other BLs (Table). Multiple R mechanisms (2 to 6) were observed among 43 CAZ-AVI-R (80.1%) and 46 CAZ-AVI-S (76.7%) isolates.

Conclusions: The occurrence of mutation driven R mechanisms in CAZ-AVI-R PSA isolates was similar or lower than that of CAZ-AVI-S isolates. CAZ-AVI displayed activity against isolates carrying R mechanisms commonly observed in PSA that cause R to other BL with antipseudomonal activity.

Resistance phenotype (no. of isolates)	No. of isolates displaying positive results for[a]:						
	OprD loss (78)	↑cAmpC (64)	↑MexAB-OprM (30)	↑MexCD-OprJ (48)	↑MexXY-OprM (36)	↑Pa5542 (18)	↑PoxB (11)
CAZ-AVI-R (47)	37	27	8	24	6	1	0
CAZ-AVI-S (60)	41	37	22	24	30	17	11
+ CAZ-NS (25)	12	24	8	9	11	7	3
+ FEP-NS (20)	10	16	9	6	11	6	2
+ MER-NS (44)	39	24	15	17	23	10	9
+ P-T-NS (31)	15	26	11	11	14	10	4

[a]Positive results were: lack of a band for OprD, >10X of PAO1 expression for AmpC, and >5X of PAO1 expression for all other genes tested; ↑, overexpression

INTRODUCTION

- *Pseudomonas aeruginosa* is a common cause of nosocomial infections worldwide
- This organism is intrinsically resistant or less susceptible to several antimicrobial agents, and infections caused by *P. aeruginosa* are challenging to eradicate
- Antipseudomonal β-lactam agents play an important role in empirically treating *P. aeruginosa* infections
- Resistance mechanisms against these antimicrobial agents include production of intrinsic or acquired β-lactamases, hyperexpression of efflux systems, and decrease or loss of the outer membrane protein OprD
- Ceftazidime is a cephalosporin displaying antipseudomonal activity that has been paired with avibactam, a non-β-lactam β-lactamase inhibitor of class A, C, and some class D enzymes
  - This combination displays enhanced activity against *P. aeruginosa* isolates when compared to ceftazidime alone
- In this study, we investigated β-lactam resistance mechanisms among ceftazidime-avibactam-resistant (47 isolates) or -susceptible (60 isolates) *P. aeruginosa* clinical isolates
  - Ceftazidime-avibactam-resistant isolates were submitted to whole genome sequencing analysis
  - All isolates were evaluated for expression of intrinsic resistance genes

MATERIALS AND METHODS

- A total of 2,548 *P. aeruginosa* clinical isolates were collected from 106 US hospitals during 2015 as part of the International Network For Optimal Resistance Monitoring (INFORM) program
- Isolates were susceptibility tested against ceftazidime-avibactam (inhibitor at fixed 4 µg/mL) and comparator agents using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI)
  - Quality control (QC) was performed according to CLSI guidelines (M100-S27), and all QC MIC results were within acceptable ranges as published in CLSI documents
  - Categorical interpretations for all comparator agents were those found in CLSI criteria in M100-S27 (2017), EUCAST breakpoint tables (version 7.0, January 2017), and/or United States Food and Drug Administration (US FDA) package inserts
- All 46 ceftazidime-avibactam-resistant isolates were submitted to whole genome sequencing on a MiSeq (Illumina, San Diego, California, US) instrument targeting a 30X coverage
  - Sequences were *de novo* assembled and searched for the presence of acquired carbapenemases using a curated library and applying criteria of >94% sequence identity and a minimum of 40% length coverage
- A group of 60 ceftazidime-avibactam-susceptible *P. aeruginosa* isolates was selected for comparison purposes
  - These isolates displayed resistance to 1 or more of the following agents: cefepime (20 isolates), meropenem (44), ceftazidime (25), and piperacillin-tazobactam (47)
- Expression levels of intrinsic resistance genes associated with resistance to antipseudomonal β-lactams were determined for 107 isolates by quantitative real-time PCR as previously described
  - Genes tested were the chromosomal *ampC, mexA* (MexAB-OprM), *mexC* (MexCD-OprJ), *mexE* (MexEF-OprN), *mexX* (MexXY-OprM), *poxB* (*oxa*-50-like), and *Pa5542*
  - Transcription levels were considered significantly different if at least a 10-fold difference was noted compared with *P. aeruginosa* PAO1 for AmpC and a 5-fold difference for all other genes
- Outer membrane proteins were purified and Western blot preparations were probed with a polyclonal OprD antibody
  - *P. aeruginosa* PAO1 was used as the positive control for comparative analysis

RESULTS

- Among 47 (1.8%) ceftazidime-avibactam-resistant *P. aeruginosa* isolates collected from US hospitals during 2015, only 2 (4.3%) carried metallo-β-lactamase genes and these were bla_{VIM-1} or bla_{VIM-2}
- The serine-β-lactamases, bla_{GES-7} (carbapenemase), bla_{OXA-2}, and bla_{OXA-4} (isolate also carried bla_{VIM-2}), were detected among 3 isolates
- Most (41/47 isolates) ceftazidime-avibactam-resistant *P. aeruginosa* isolates were resistant to all β-lactam comparators, 5 were susceptible to piperacillin-tazobactam, and 3 were susceptible to cefepime plus meropenem and/or piperacillin-tazobactam
- Alterations on the chromosomal AmpC Ω loop associated with resistance to β-lactamase inhibitors were observed among 10 ceftazidime-avibactam-resistant isolates and included V205L (6 isolates), V205L + G242R/S (2), V239A (1), and a 7 amino acid deletion at positions 235 to 242 (1; Figure 1)
- OprD loss was noted among 37 ceftazidime-avibactam-resistant *P. aeruginosa* isolates
- Of the ceftazidime-avibactam-resistant *P. aeruginosa* isolates, 24 (51.1%) displayed overexpression of chromosomal AmpC, 24 had overexpression of MexCD-OprJ (8 did not amplify), 8 overexpressed MexAB-OprM, and 6 had overexpression of MexXY-OprM
- One isolate overexpressed the chromosomally encoded metallo-imipenemase Pa5542
  - Overexpression of PoxB and MexEF-OprN was not observed among ceftazidime-avibactam-resistant *P. aeruginosa* isolates
- Overexpression of chromosomal AmpC was detected in 32 ceftazidime-avibactam-susceptible *P. aeruginosa* selected isolates, MexXY-OprM in 30, MexCD-OprJ in 24, MexAB-OprM in 22, Pa5542 in 17, and PoxB in 11
  - Overexpression of MexEF-OprN was not observed
- Multiple resistance mechanisms (2 to 6) were observed among 89 (83.2%) isolates, including 43 (80.1%) ceftazidime-avibactam-resistant and 46 ceftazidime-avibactam-susceptible isolates (76.6%)
- When comparing the 2 groups, the relative expression of MexCD-OprJ was greater in the ceftazidime-avibactam-resistant subset when compared to the susceptible subset (Figure 2)
  - The expression of AmpC, MexXY-OprM, Pa5542, and PoxB was greater in the ceftazidime-avibactam-susceptible isolates
  - OprD loss and MexAB-OprM expression was similar in both groups

CONCLUSIONS

- In the US, the production of metallo-β-lactamases in *P. aeruginosa* is uncommon and was detected in only 2 isolates resistant to ceftazidime-avibactam
- Mutation driven resistance mechanisms to β-lactam agents were detected in both ceftazidime-avibactam-susceptible or -resistant subsets of *P. aeruginosa* isolates
  - Ceftazidime-avibactam-resistant isolates expressed greater levels of MexCD-OprJ; however, isolates susceptible to this agent displayed greater or equal expression of all remaining genes and OprD loss
- Ceftazidime-avibactam was active against isolates carrying multiple resistance mechanisms, including OprD loss, overexpression of efflux pumps, and chromosomally encoded β-lactamases
- These results demonstrate that resistance to ceftazidime-avibactam is complex and can be fully understood only in a small number of isolates; however, resistance rates among *P. aeruginosa* isolates from US hospitals are low (<2.0%)

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Figure 1 Susceptibility phenotypes and resistance mechanisms for ceftazidime-avibactam-resistant (n=46) and -susceptible (n=60) *P. aeruginosa* isolates from US hospitals

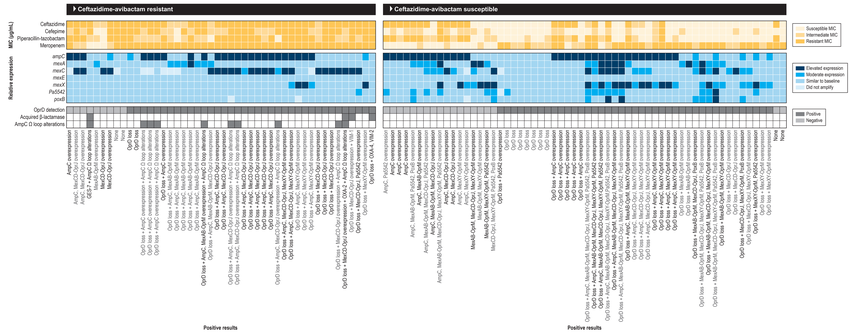
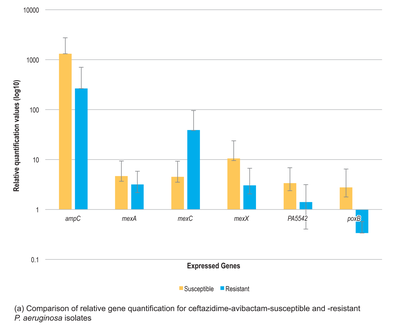
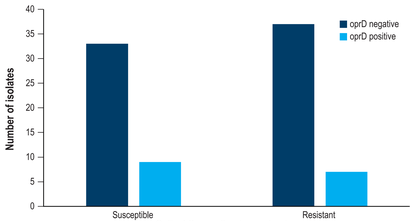


Figure 2 (a) Relative expression of intrinsic resistance genes (b) OprD loss results for ceftazidime-avibactam-resistant and -susceptible *P. aeruginosa* isolates from US hospitals



(a) Comparison of relative gene quantification for ceftazidime-avibactam-susceptible and -resistant *P. aeruginosa* isolates



(b) OprD results for ceftazidime-avibactam-susceptible vs. -resistant *P. aeruginosa* isolates

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