

Phenotypic and genotypic profile of ceftolozane/tazobactam-non-susceptible, carbapenem-resistant *Pseudomonas aeruginosa*

Christian M. Gill¹ and David P. Nicolau ^{1,2*}; on behalf of the ERACE-PA Global Study Group†

¹Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, CT, USA; ²Division of Infectious Diseases, Hartford Hospital, Hartford, CT, USA

*Corresponding author. E-mail: david.nicolau@hhchealth.org

†Members are listed in the Acknowledgements section.

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Objectives: To evaluate the genotypic and ceftazidime/avibactam-susceptibility profiles amongst ceftolozane/tazobactam-non-susceptible (NS), MBL-negative *Pseudomonas aeruginosa* in a global surveillance programme.

Methods: Isolates were collected as part of the ERACE-PA Global Surveillance programme. Carbapenem-resistant *P. aeruginosa* deemed clinically relevant by the submitting laboratories were included. Broth microdilution MICs were conducted per CLSI standards to ceftolozane/tazobactam, ceftazidime/avibactam, ceftazidime and cefepime. Genotypic carbapenemases were detected using CarbaR and CarbaR NxG (research use only). Isolates negative for carbapenemases by PCR were assessed via WGS. Isolates were included in the analysis if they were ceftolozane/tazobactam-NS and lacked detection of known MBLs.

Results: Of the 807 isolates collected in the ERACE-PA programme, 126 (16%) were ceftolozane/tazobactam-NS and lacked MBLs. Cross-resistance to ceftazidime and cefepime was common, with only 5% and 16% testing susceptible, respectively. Ceftazidime/avibactam retained *in vitro* activity, with 65% of isolates testing susceptible. GES was the most common enzymology, detected in 57 (45%) isolates, and 89% remained susceptible to ceftazidime/avibactam. Seven isolates harboured KPC and all tested susceptible to ceftazidime/avibactam. In the remaining 62 isolates, WGS revealed various ESBLs or OXA β -lactamases. While 39% remained susceptible to ceftazidime/avibactam, marked variability was observed among the diverse resistance mechanisms.

Conclusions: Ceftazidime/avibactam remained active *in vitro* against the majority of ceftolozane/tazobactam-NS, MBL-negative *P. aeruginosa*. Ceftazidime/avibactam was highly active against isolates harbouring GES and KPC β -lactamases. These data highlight the potential clinical utility of genotypic profiling as well as the need to test multiple novel agents when carbapenem-resistant *P. aeruginosa* are encountered.

Introduction

Ceftolozane/tazobactam and ceftazidime/avibactam have provided safe and efficacious treatment options for the management of carbapenem-resistant *Pseudomonas aeruginosa*.¹ Both agents maintain potent *in vitro* activity against isolates collected in the USA and other regions globally; as such, both agents are recommended by the IDSA guidance for treatment of susceptible carbapenem-resistant *P. aeruginosa* infections when resistance to other β -lactams is detected.^{1–3}

Ceftolozane/tazobactam resistance due to MBLs has been well described in *P. aeruginosa*, and confers cross-resistance to most β -lactam agents.^{2,4} Additional β -lactamase-derived resistance to ceftolozane/tazobactam has been attributed to horizontally transferable serine β -lactamases such as Guinea extended spectrum (GES) β -lactamases.⁵ In isogenic strains, insertion of GES

β -lactamases conferred resistance to ceftolozane/tazobactam but not necessarily ceftazidime/avibactam.⁵ At present, sparse data are available to describe the phenotypic profile of these novel agents against clinical isolates of GES-producing *P. aeruginosa*.

The present study sought to define the β -lactamase genotypic background and ceftazidime/avibactam susceptibility amongst ceftolozane/tazobactam-non-susceptible (NS), MBL-negative *P. aeruginosa* from a global surveillance programme.

Methods

Isolates and phenotypic profiling

Isolates were collected as part of the ERACE-PA Global Surveillance programme.^{2,6} Briefly, clinically relevant carbapenem-resistant *P. aeruginosa* were identified by submitting sites per local standards prior to shipment

to the central laboratory. Isolates were collected from 17 sites in 12 countries including the USA, Germany, Brazil, Turkey, Israel, Spain, Kuwait, South Africa, Colombia, Greece, Saudi Arabia and Italy. Broth microdilution MICs were determined and interpreted per CLSI standards for ceftazidime, cefepime, ceftazidime/avibactam and ceftolozane/tazobactam.^{2,6} To assess for phenotypic carbapenemases, the modified carbapenem inactivation method (mCIM) was tested as previously described.^{2,6,7}

The present study assessed a subset of carbapenem-resistant *P. aeruginosa* isolates from the programme that were defined as non-susceptible to ceftolozane/tazobactam and lacked MBLs.

Genotypic resistance determinants

Isolates were assessed with the CarbaR and CarbaR NxG to determine the presence of carbapenemase targets as previously described.^{2,8} Since non-carbapenemase β -lactamases have been described to cause ceftolozane/tazobactam non-susceptibility in isogenic strains, isolates that lacked detection of a carbapenemase using the prior methods underwent WGS as previously described.^{2,8}

Results

Ceftolozane/tazobactam-NS, MBL-negative isolates

A total of 126 of the 807 total isolates were ceftolozane/tazobactam-NS, MBL-negative *P. aeruginosa*. Of these, 65% retained ceftazidime/avibactam *in vitro* susceptibility, with MIC_{50/90} values of 4 and 64 mg/L, respectively. Ceftolozane/tazobactam non-susceptibility was associated with nearly complete cross-resistance to ceftazidime and cefepime, with only 5% and 16% of isolates testing susceptible, respectively.

Genotypic resistance determinants

Of the 126 ceftolozane/tazobactam-NS, MBL-negative *P. aeruginosa*, GES was the most common enzymology detected in 57 isolates, while 7 harboured KPC. ESBLs were detected in 15 isolates [VEB ($n=11$), CTX-M-2 ($n=2$) and PER ($n=2$)]. The remaining 47 isolates harboured either acquired Class D oxacillinases or only chromosomal β -lactamases [OXA-10-like oxymes ($n=9$), OXA-2-like (with or without OXA-10-like) ($n=3$), chromosomal AmpC and OXA-50-like enzymes without other detected exogenous β -lactamases ($n=35$)]. Available enzyme subtypes, ST and country of origin for the transmissible β -lactamases are listed in Table 1.

Ceftazidime/avibactam susceptibility by geno- and phenotypic profiles

Figure 1(a) depicts the MIC distribution of serine carbapenemase-harboring isolates (GES and KPC) by ceftazidime/avibactam MIC. Of the serine carbapenemase harbouring isolates, 89% and 100% tested susceptible to ceftazidime/avibactam among GES- and KPC-harboring isolates, respectively. Interestingly, 26% (15/57) of GES-positive isolates tested as mCIM negative. Ceftazidime/avibactam MICs were lower in the GES-positive, mCIM-positive versus the GES-positive, mCIM-negative isolates, with 98% and 67% of each group testing susceptible to the agent, respectively [Figure 1(b)].

In the remaining 62 isolates negative for GES and KPC with various genotypic backgrounds, ceftazidime/avibactam susceptibility was highly variable, with 39% of isolates testing susceptible

to the agent. In isolates harbouring an ESBL gene, 40% (6/15 isolates) were susceptible to ceftazidime/avibactam. Similarly, in isolates harbouring acquired OXA- β -lactamases or only intrinsic β -lactamases (AmpC and OXA-50-like), 38% (18/47) tested susceptible to ceftazidime/avibactam.

Discussion

The present study adds to the growing literature describing the genotypic and phenotypic profile of *P. aeruginosa* that are ceftolozane/tazobactam-NS. Although cross-resistance was common for cefepime and ceftazidime, many isolates remained susceptible to ceftazidime/avibactam, presenting a therapeutic option for these challenging pathogens. Notably, GES was the most commonly detected enzymology and ceftazidime/avibactam remained active amongst 89% of isolates harbouring this class of β -lactamases. In isolates negative for GES or KPC, ceftazidime/avibactam susceptibility was more variable, albeit 39% remained susceptible to the compound.

Although MBLs are notable mechanisms of ceftolozane/tazobactam non-susceptibility,^{2,4} transmissible GES β -lactamases are an increasingly recognized challenge. Ortiz de la Rosa and colleagues⁵ previously reported ceftazidime/avibactam phenotypic profiles of four clinical and four isogenic *P. aeruginosa* carrying various GES alleles, which showed that despite ceftolozane/tazobactam non-susceptibility, isolates were still susceptible to ceftazidime/avibactam. Similarly, a recent surveillance programme from Spain detected 30 GES-harboring *P. aeruginosa* and similar to the present study, all isolates were non-susceptible to ceftolozane/tazobactam.⁹ In the Spanish cohort, ceftazidime/avibactam remained highly susceptible, with 97% of isolates testing susceptible, similar to the 89% in our global cohort.⁹ These *in vitro* data suggest ceftazidime/avibactam may be a therapeutic option for these difficult-to-treat isolates harbouring GES. By integrating *in vitro* susceptibility data with reliable and rapid detection of GES β -lactamases, clinicians can develop therapeutic pathways that may aid in selection of more timely and appropriate therapy.

Our group and others have previously described challenges with phenotypic detection of GES β -lactamases using mCIM.^{7,10} Indeed, there are upwards of 30 different GES alleles, all with varying hydrolytic activity towards carbapenems, which differ by as little as one amino acid substitution.¹¹ The varying hydrolytic potential of GES subtypes may explain the variable detection using broad hydrolysis-based phenotypic assays.^{7,10,11} Novel genotypic methods of detection would be advantageous as they often have faster turn-around time and do not rely on the hydrolytic spectrum, thus expansion of the carbapenemase detection targets (i.e. CarbaR NxG) will aid in increasing the real-time recognition of this expanding carbapenemase in clinically indicated populations.⁸ Although the reflex testing of ceftolozane/tazobactam and ceftazidime/avibactam is increasingly common after the detection of carbapenem resistance at many institutions, depending on the methods utilized, phenotypic profiling of these or other alternative agents may not be available for 24–72 h, whereas rapid genotypic detection methods can guide therapy within a matter of hours.¹² In addition to providing insights regarding optimal therapy, the implementation of rapid genotypic testing will also better inform the utilization of infection prevention strategies.

Table 1. Characteristics of exogenous β -lactamases detected

β -Lactamase category (number of isolates)	Subtypes detected (number of isolates)	ST (number of isolates)	Country
GES ^a (57)	GES-5 (25) ^{b,c}	ST-235 (33)	Turkey, Israel, Spain, Kuwait, South Africa, Saudi Arabia, Italy
	GES-5/20 (1) ^c	ST-17 (1)	
	GES-1 (9) ^{d,e}	ST-654 (1)	
	GES-2 (1) ^c	Inconclusive, nearest ST-1816 (1)	
	GES-12 (2) ^e	ST-664 (2)	
KPC (7)	ND	ND	Colombia
CTX-M (2)	CTX-M-2 (2)	ST-235 (2)	Turkey
PER (2)	PER-1 (2) ^d	ST 253 (2)	Turkey
VEB (11)	VEB-1 (11) ^d	ST-357 (11)	Turkey, Kuwait
OXA-10-like (9)	OXA-10 (1)	ST-235 (7)	Brazil, Turkey, Israel, Greece, USA
	OXA-129 (1) ^f	ST-316 (1)	
	OXA-14 (3) ^f	ST-664 (1)	
	OXA-256 (1)		
	OXA-74 (2)		
	OXA-147/35 (1) ^f		
OXA-2 \pm OXA-10-like (3)	OXA-2 (2)	ST-235 (3)	Turkey, Israel
	OXA-2, OXA-74 (1)		

ND, not determined.

^aWGS available for $n=38$. Remaining 19 isolates were detected by PCR and the four isolates from the same site that were sequenced were all GES-5 and ST-235.

^bSome isolates also harboured CARB-2.

^cGES subtype considered a carbapenemase.

^dSome isolates also harboured OXA-10-like.

^eGES subtype considered an ESBL.

^fOXA subtype considered extended spectrum.

Although GES β -lactamases are hydrolytically variable, common phenotypes of carbapenem resistance and ceftolozane/tazobactam non-susceptibility have been identified in our work and that of others.^{2,9,10} Interestingly, mCIM negativity (i.e. failure to degrade meropenem sufficiently for a positive mCIM result) was associated with reduced ceftazidime/avibactam susceptibility in our cohort. Previous investigations have detected elevated ceftazidime/avibactam MICs in surveillance rectal swab cultures of a patient receiving ceftazidime/avibactam for a bloodstream infection because of single amino acid substitution.¹³ The *in vivo* and clinical consequences of mCIM-positive and mCIM-negative isolates harbouring GES warrant investigation.

An increasing number of studies have been conducted to link the molecular detection of resistance genes and phenotypic antimicrobial susceptibility.^{14,15} These efforts specifically for *P. aeruginosa* are challenging as it is the additive effects of exogenous and intrinsic β -lactamases as well as non-enzymatic mechanisms that contribute to the overall phenotype.^{14,15} Thus we cannot practically discern which specific mechanism is driving the ceftolozane/tazobactam non-susceptibility in *P. aeruginosa*. Future efforts must assess expression and functionality to better estimate the phenotypic consequences of the genotype.¹⁵ Similarly, differences between enzyme variants may carry different hydrolytic spectrums, which is prominent in both GES and OXA- β -lactamases observed in *P. aeruginosa* including in the present study.^{11,16} Indeed, both carbapenemase and ESBL-type GES variants were detected in this cohort (Table 1), but

notably all isolates were determined to be carbapenem resistant. Previous experiments have described that the difference between different GES variants is as little as a single amino acid substitution thus changing the hydrolytic spectrum.^{11,13,17} Such mutations have been described in the literature including during antibiotic therapy.^{13,17} Specific to GES, future studies evaluating therapeutic agents, or combinations, that are active against both ESBL- and carbapenemase-type GES variants may be needed to optimize outcomes since mutations to other variants have a low genetic barrier.

Other ESBL-type β -lactamases have been implicated in elevated ceftolozane/tazobactam MICs amongst *P. aeruginosa*. Using isogenically inserted β -lactamases into WT *P. aeruginosa*, Ortiz de la Rosa and colleagues⁵ found VEB, PER and others resulted in elevated ceftolozane/tazobactam MICs. Similar results were found in our clinical isolates where VEB and PER were detected in ceftolozane/tazobactam-non-susceptible isolates. Rapid detection of such enzymes in the clinic can guide therapy to alternative agents or combinations. It must be noted that these enzymes and others (i.e. CTX-M) are also in the context of the intrinsic porin/efflux and enzymatic mechanisms of resistance thus it is likely a combination of the expression of each mechanism.¹⁵ Similarly, mutations to the *P. aeruginosa* AmpC have been associated with ceftolozane/tazobactam resistance.¹⁸ In the present study, all isolates were ceftolozane/tazobactam non-susceptible, thus there is a potential that even isolates with exogenous β -lactamases may also have AmpC mutations,

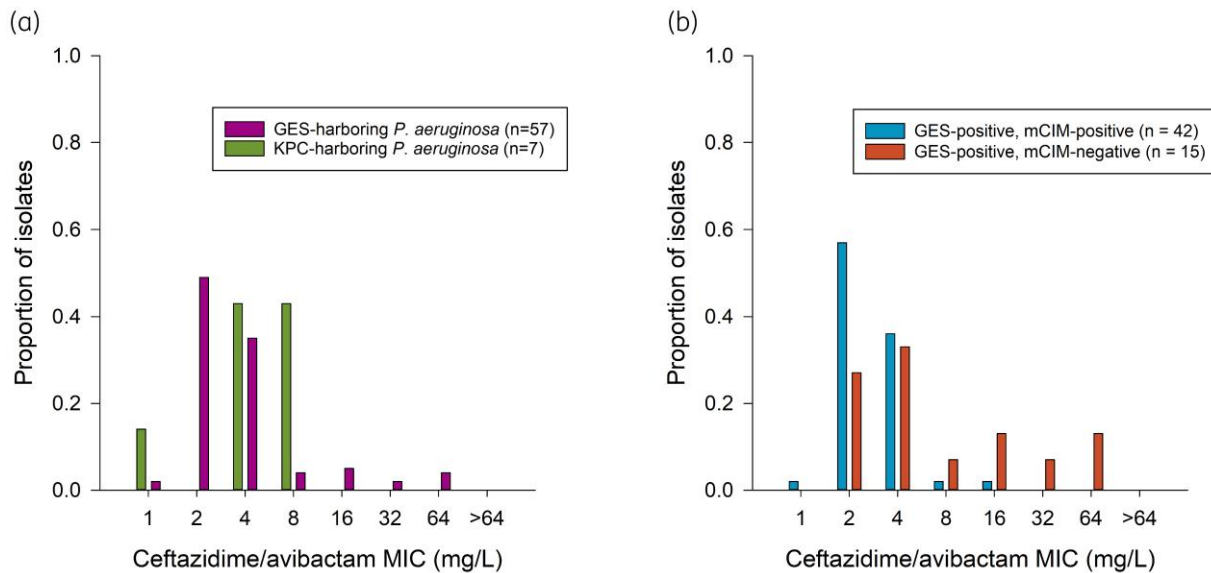


Figure 1. Proportion of isolates by ceftazidime/avibactam MIC (mg/L).

although these were not assessed in this study. Similarly, 35 of the ceftolozane/tazobactam-non-susceptible isolates lacked exogenous β -lactamases, thus it is likely that AmpC mutations among other non-enzymatic mechanisms are contributing to the phenotype.

In conclusion, although diverse β -lactamases were genotypically identified in our population of ceftolozane/tazobactam-NS, MBL-negative *P. aeruginosa*, GES was the most common. Detection of GES or KPC was largely associated with ceftazidime/avibactam susceptibility. In the absence of these enzymes, there was more variability in ceftazidime/avibactam susceptibility, albeit 39% remained susceptible to the agent. These data highlight the need to conduct susceptibility testing for multiple novel agents in the clinic when carbapenem-resistant *P. aeruginosa* are detected, as ceftolozane/tazobactam non-susceptibility does not universally preclude efficacy of ceftazidime/avibactam.

As GES-harboring *P. aeruginosa* are increasingly recognized as a global threat, additional *in vitro* and pre-clinical *in vivo* studies evaluating alternative agents and combination therapy are warranted to optimize the therapeutic decisions for this emerging MDR pathogen.

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