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Induction of Resistance Against Antipseudomonal Agents: Comparison of Novel β-Lactam/β-Lactamase Inhibitor Combinations and Other β-Lactam Agents

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CONCLUSIONS

Our results demonstrate that exposure to meropenem, cefepime, and piperacillin-tazobactam terminal mutants displayed higher increases in MIC values compared to the isolates obtained after exposure to ceftolozane-tazobactam, imipenem-relebactam, and ceftazidime-avibactam.

Mutations detected in isolates exposed to 10-day serial passaging involved various genes previously associated to BL resistance, including PBPs and AmpC, efflux, and porin regulators.

This data might indicate that these newer agents could help prevent the emergence of high-level resistance, but additional data is needed for confirmation.

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INTRODUCTION

- Mutation is the main driver of β-lactam (BL) resistance among *Pseudomonas aeruginosa* isolates.
- New β-lactam/β-lactamase inhibitor (BL/BLI) combinations, such as ceftazidime-avibactam, ceftolozane-tazobactam, and imipenem-relebactam, exhibit good activity against most *P. aeruginosa* isolates from US hospitals.
  - Limited information is available regarding the potential for these new β-lactam/β-lactamase inhibitor combinations to induce resistance in *P. aeruginosa* compared to older agents.
- We subjected 7 *P. aeruginosa* isolates to a 10-day serial passage with 6 antipseudomonal agents to evaluate resistance levels and mechanisms in terminal mutant strains.

MATERIALS AND METHODS

- Seven *P. aeruginosa* isolates susceptible to antipseudomonal β-lactam agents were subjected to passaging experiments.
- Serial passaging was performed in broth microdilution panels produced according to the CLSI M07 (2018) guidelines.
  - The broth from the highest concentration of each antimicrobial agent that displayed visible growth after overnight incubation was used to prepare a new 0.5 McFarland standard (5 x 10^5 CFU/mL) bacterial inoculum.
  - This process was repeated for 9 days.
- Antimicrobial agents used for passaging were ceftazidime-avibactam, imipenem-relebactam, ceftolozane-tazobactam, meropenem, cefepime, and piperacillin-tazobactam.
  - All BLIs were added to wells at a 4 mg/L fixed concentration.
- The MIC of terminal mutants was determined by using the broth microdilution reference method after subculturing isolates twice on drug-free agar.
- Parent strains and terminal mutants that displayed >2-fold changes from the baseline were subjected to short-read whole genome sequencing at 100X coverage (MiSeq Sequencer; Illumina, San Diego, CA, USA).
- Parent isolates were sequenced using long-read WGS (MinION; Oxford Nanopore Technologies Ltd, Oxford, UK).
  - Data from short- and long-reads were combined using Unicycler v0.4.8-beta and SPAdes.
- Single nucleotide polymorphism (SNP) analysis was performed by comparing the baseline short- and long-read assembled sequences and their follow-up pairs by employing MAUVE V2.4.0.
- Insertion/deletion (INDEL) sites were realigned using IndelRealigner from the GATK toolbox v3.8-1-0gf15c13ef and reported.

RESULTS

- Final mutants had MIC increases of 2- to 8-fold for ceftazidime-avibactam, 1- to 8-fold for imipenem-relebactam, and 2- to 32-fold for ceftolozane-tazobactam (Figure 1).
  - The MIC increases for meropenem (1- to 128-fold), cefepime (2- to 32-fold), and piperacillin-tazobactam (2- to 1024-fold) were slightly higher.
- Cefepime terminal mutants (*n*=7) exhibited
  - Alterations upstream of *nalC* (*n*=2) and
  - Missense alterations in *ampD*, *mexB*, and the TetR family transcriptional regulator *amrR* either alone or in combination with mutations in other genes (Table 1).
- Three of 7 ceftazidime-avibactam mutants exhibited missense mutations in *nalD*, the MexAB-OprM regulator, as well as 1 alteration in the intragenic region upstream of *nalC* involved in the same regulatory pathway.
- Of the 6 sequenced ceftolozane-tazobactam mutants, 1 had a missense mutation in *ampG* and another had a missense mutation in the penicillin-binding protein (PBP) gene *ftsI*.
  - Mutations in genes not usually associated with BL resistance were also detected, including 2 isolates with alterations in the HAMP domain, which contained histidine kinase.
- Meropenem mutants (*n*=6) displayed alterations either in *oprD* (*n*=2);
  - Within or upstream of *nalC* (*n*=3) or its intragenic region, *ftsI* (*n*=1) *phoP* (*n*=1).
- Upon exposure to piperacillin-tazobactam, mutations in *merR* (1 upstream mutation), *nalC*, and *ampD* were observed in the terminal mutants.
- Among 2 imipenem-relebactam terminal mutants,
  - 1 displayed a nonsense mutation in *pilF*, which encodes a pilus-forming protein, and
  - 1 displayed a spermidine synthase that interacts with *arnBCDTEF-ugd* and PmrAB, increasing both the expression of MexXY/OprM and the stress response in the cell.
- Notably, ceftolozane-tazobactam and piperacillin-tazobactam mutants exhibited alterations in *mpl* and *galU* that assist the recycling of the cell wall peptidoglycan.

Figure 1. Fold change in MIC results from the parent isolate to the terminal mutant

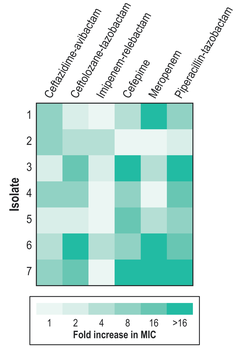

Table 1. SNP analysis of isolates exposed to antimicrobial agents

Parent	selection	Fold change	Initial MIC	Final MIC	Reference	Alteration	Annotation	Annotation effect	Gene	Function and upstream/downstream genes for intragenic mutation
Set #1	cefepime	32	1	32	G	T	Arg620Leu	missense	*mexB*	multidrug efflux RND transporter permease subunit
					T	G	Thr163Pro	missense		HAMP domain-containing histidine kinase
					G	T	Ala122Glu	missense	*mlaE*	lipid asymmetry maintenance ABC transporter permease subunit
					A	G	—	intragenic		efflux system transcriptional regulator nalC/ hypothetical protein
	ceftazidime-avibactam	8	1	8	T	G	His56Pro	missense	*nalD*	efflux system transcriptional repressor
	ceftolozane-tazobactam	0.5	8	16	T	G	Thr163Pro	missense		HAMP domain-containing histidine kinase
					C	G	Ala547Pro	missense	*ftsI*	peptidoglycan D,D-transpeptidase
	meropenem	64	0.25	16	G	C	Arg48Gly	missense	*nalC*	efflux system transcriptional repressor
	piperacillin-tazobactam	256	2	512	C	T	—	intragenic		MarR family transcriptional repressor MexR/ multidrug efflux RND transporter periplasmic adaptor subunit MexA
					A	G	Tyr207His	missense	*galU*	UTP--glucose-1-phosphate uridylyltransferase
					A	G	Leu40Pro	missense	*ampD*	1,6-anhydro-N-acetylmuramyl-L-alanine amidase
Set #2	cefepime	4	2	8	T	C	Asp155Gly	missense	*amrR*	TetR family transcriptional regulator
	ceftazidime-avibactam	8	2	16	A	G	Thr254Ala	missense		glycosyltransferase family 4 protein
	imipenem-relebactam	1	0.5	0.5	C	T	Gln153*	nonsense	*pilF*	type 4a pilus biogenesis protein
	meropenem	64	0.12	16	A	G	—	intragenic		efflux system transcriptional regulator nalC/ hypothetical protein
					T	A	Thr79Ser	missense	*phoP*	two-component system response regulator
	piperacillin-tazobactam	8	4	32	C	T	Arg36Cys	missense		MerR family transcriptional regulator
					C	T	Gln306*	nonsense	*pgi*	glucose-6-phosphate isomerase
Set #3	cefepime	1	4	4	G	A	Gln357*	nonsense		inactive transglutaminase family protein
	ceftazidime-avibactam	8	0.12	1	T	C	Ser239Pro	missense		carboxy terminal-processing peptidase
	ceftolozane-tazobactam	4	0.5	2	A	G	Leu365Pro	missense	*secY*	preprotein translocase subunit
	meropenem	1	0.03	0.03	T	G	Asp54Ala	missense	*uvrY*	UvrY/SirA/GacA family response regulator transcription factor
Set #4	cefepime	32	1	32	A	C	Val10Gly	missense	*ampD*	1,6-anhydro-N-acetylmuramyl-L-alanine amidase
					T	C	Asn214Ser	missense		glycosyltransferase
	ceftazidime-avibactam	2	1	2	G	A	Trp286*	nonsense		spermidine synthase
	ceftolozane-tazobactam	16	0.5	8	T	G	Val124Gly	missense	*mpl*	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase
	imipenem-relebactam	2	0.25	0.5	A	G	Asp333Gly	missense		spermidine synthase
	meropenem	4	1	4	T	C	Ile224Val	missense		UDP-glucose 6-dehydrogenase
					C	A	Ala530Glu	missense	*gacS*	two-component system sensor histidine kinase
	piperacillin-tazobactam	64	2	128	G	T	Tyr188*	nonsense	*mupP*	N-acetylmuramic acid 6-phosphate phosphatase
Set #5	cefepime	8	1	8	T	C	Val179Ala	missense		N-acetyl sugar amidotransferase
	ceftazidime-avibactam	8	1	8	G	C	Tyr48*	nonsense	*nalD*	efflux system transcriptional repressor
	ceftolozane-tazobactam	8	0.5	4	T	G	Thr163Pro	missense		HAMP domain-containing histidine kinase
					G	T	Arg101Ser	missense	*galU*	UTP-glucose-1-phosphate uridylyltransferase
					A	C	His24Pro	missense		hypothetical protein
	meropenem	4	0.5	2	G	A	Ser40Asn	missense	*oprD*	outer membrane porin
					T	C	Ser278Pro	missense	*oprD*	outer membrane porin
	piperacillin-tazobactam	16	2	32	T	G	Thr24Pro	missense	*nalC*	efflux system transcriptional repressor
Set #6	cefepime	16	1	16	A	G	—	intragenic		efflux system transcriptional regulator nalC/ hypothetical protein
	ceftazidime-avibactam	2	1	2	C	T	Val65Ile	missense	*nalD*	efflux system transcriptional repressor
					T	C	Ser93Gly	missense		ABC transporter ATP-binding protein
	ceftolozane-tazobactam	2	0.5	1	A	C	Thr423Pro	missense	*ampG*	MFS transporter
	piperacillin-tazobactam	8	2	16	A	T	Trp414Arg	missense	*pepA*	leucyl aminopeptidase
					C	T	Phe119Phe	synonymous	*ssuC*	aliphatic sulfonate ABC transporter permease
Set #7	cefepime	8	2	16	C	T	Phe119Phe	synonymous	*nusG*	transcription termination/antitermination protein
	ceftazidime-avibactam	4	2	8	A	G	—	intragenic		efflux system transcriptional regulator nalC/ hypothetical protein
					G	A	Gly544Ser	missense	*clpA*	ATP-dependent Clp protease ATP-binding subunit
	ceftolozane-tazobactam	32	0.5	16	C	T	Phe119Phe	synonymous	*nusG*	transcription termination/antitermination protein
					G	A	Ala159Thr	missense	*mpl*	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase
	meropenem	128	0.25	32	C	T	Gly132Glu	missense	*nalC*	efflux system transcriptional repressor
					A	C	Val471Gly	missense	*ftsI*	peptidoglycan D,D-transpeptidase
	piperacillin-tazobactam	16	4	64	A	C	Thr325Pro	missense	*mpl*	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase