Establishment of murine thigh and lung infection models with carbapenem resistant clinical isolates of *Pseudomonas aeruginosa*

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Abstract

The study objective was to establish a set of carbapenem resistant *Pseudomonas aeruginosa* clinical isolates for the in vivo characterization of new antibacterial agents. Distinct (non-clonal) carbapenem resistant isolates from the FDA-CDC AR-Bank and a Liverpool Epidemic Strain were selected based on priority antimicrobial resistance (AMR) phenotypes, genotypes, and epidemiological relevance. The isolates were analyzed for growth in lung and thigh infection models of neutropenic mice to identify an inoculating density that results in robust growth over the 24 hr infection time course.

Eight carbapenem resistant isolates were found to result in robust growth in mouse tissue demonstrated by at least a two-log increase in bacterial density. Colistin treatment in the mouse models yielded significant and dose-responsive inhibition that was consistent between the different isolates.

We anticipate that this collection of genetically diverse carbapenem resistant isolates will provide a useful tool for pharmacological studies of novel antimicrobial agents.

Methods

Genome sequenced *P. aeruginosa* strains were sourced from the FDA-CDC AR Isolate Bank and the BCCM repository. The acquired antibiotic resistance genes were supplied by the AR Bank. The sequence types were determined by querying assembled genomes against the PubMLST database (performed by CosmosID). Mutations in Topo IV, DNA Gyrase, and OprD mutations were identified from whole genome sequence analysis (performed by MicrogenomX). Minimum inhibitory concentration values were determined following the microbroth dilution method, (CLSI M07-A10) and the interpretive criterion (CLSI M100S) was used to assess susceptibility. Murine thigh and lung infection models were conducted with neutropenic ICR mice following standard methods (RV Dudhani, 2009).

Organism sequence types

Organism	Sequence type	Description
ATCC 27853	ST155	Laboratory strain for in vitro and rodent antimicrobial testing.
LES-431	ST146	A liverpool epidemic strain of cystic fibrosis patients. This isolate displayed increased expression of the AmpC B-lactamase and efflux pumps as well as reduced expresssion of the oprD porin (2)
AR-0108	ST233	An international high risk clone that produces the VIM-2 metallo β-lactamase.
AR-0054	ST654	An international high risk clone associated with the dissemination of carbapenemase genes.
AR-0103	ST964	An international high risk clone associated with the dissemination of the Imp-1 carbapenemase gene.
AR-0064	ST277	A clone endemic in hospitals throughout Brazil and associated with São Paulo metallo-β-lactamase (SPM-1) carbapemenase.
AR-0105	ST235	An international high risk clone associated with the dissemination of multidrug-resistance genes.
AR-0094	ST155	N/A
AR-0264	Novel	A novel ST most closely related to the ST233 international high risk clone.

The strain collection is comprised of phylogenetically distinct organisms . Sequence types (ST) of organisms was determined from Multi Locus Sequence Typing using whole genome sequence and the PubMLST database. The analysis was performed by CosmosID.

Carbapenem resistance, genotypes and phenotypes

Organism	Acquired β-lactamase genes	OprD porin mutation	Serine β-lactamase inhibition		Metallo β-lactamase inhibition (EPI Chelator)		Carbapenem resistance class
			CAZ MIC µg/mL	CAZ + AVI MIC µg/mL	IPM MIC µg/mL	IPM + EPI MIC µg/mL	
ATCC 27853	--	--	1	4/4	1	1	--
LES-431	--	--	**>64**	**16/4**	16	16	Serine β-lactamase
AR-0108	VIM-2 (MBL) OXA-4	Body text	>64	>16/4	**>128**	**32**	Metallo-β-lactamase
AR-0054	VIM-4 (MBL)	W417*	>64	>16/4	**>128**	**16**	Metallo-β-lactamase
AR-0103	IMP-1 (MBL)	W417*	>64	>16/4	**128**	**16**	Metallo-β-lactamase
AR-0064	SPM-1 (MBL) OXA-56	A126 fs	>64	>16/4	**>128**	**32**	Metallo-β-lactamase
AR-0105	OXA-2	--	**64**	**16/4**	2	2	Serine β-lactamase
AR-0094	--	L46 fs	>64	>16/4	64	64	Unknown
AR-0264	--	L45 fs	1	2/4	16	16	Unknown

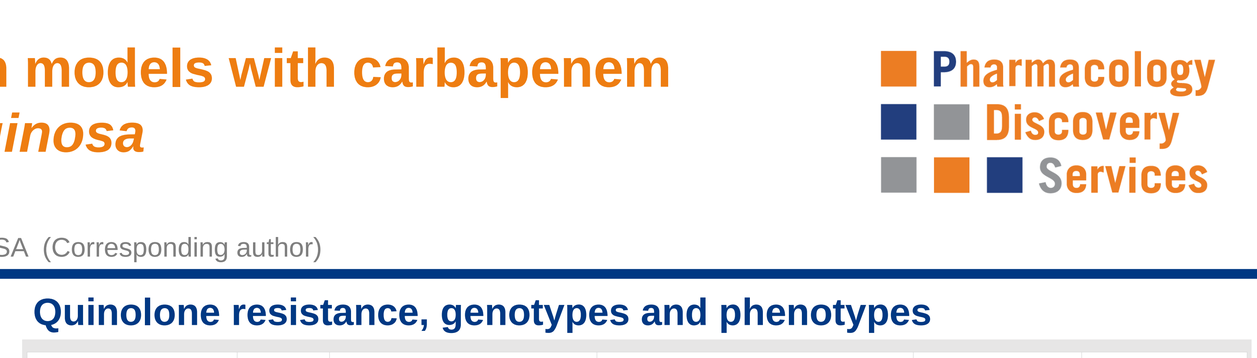
- Acquired β-lactamase genes were reported by the AR-Bank and were identified using Resfinder analysis of whole genome sequence.
- OprD mutations that result in loss of function and reduced uptake of β-lactam antibiotics were identified from whole genome sequence analysis. Nonsense mutations (*) and frameshift (fs) mutations likely contribute to the carbapenem resistance.
- Serine β-lactamase production was phenotypically assessed with susceptibility analysis of ceftazidime (CAZ) combined with avibactam (AVI), a serine β-lactamase inhibitor. Inhibition of resistance was detected among strains LES-431 and AR-0105. Strain LES-431 demonstrates elevated expression of the AmpC serine β-lactamase which may contribute to resistance.
- β-lactam resistance due to metallo-β-lactamase (MBL) expression was phenotypically tested using the EPI (EDTA-phenanthroline imipenem) microdilution test. The chelator mixture reduced the imipenem MIC of strains AR-0108, AR-0054, AR-0103, and AR-0105 consistent with metallo-β-lactamases identified from genomic analysis.

Quinolone resistance, genotypes and phenotypes

Strain / Clinical isolate	ST	Topo IV mutations ParC and ParE	DNA Gyrase mutations GyrA and GyrB	CIP MIC µg/mL	CIP S/I/R
ATCC 27853	155	--	--	0.5	S
LES-431	146	ParE D142N	GyrA T83I GyrB I299M	2	I
AR-0108	233	ParC S87L	GyrA T83I D652Y	>8	R
AR-0054	654	ParC S87L	GyrA T83I Δ(909ES)	>8	R
AR-0103	964	ParC S87L	GyrA T83I Δ(909ES)	>8	R
AR-0064	277	ParC S87L H262Q	GyrA T83I	>8	R
AR-0105	235	ParC S87W ParE D533E	GyrA T83I GyrB E46	>8	R
AR-0094	155	ParE V460G	GyrA T83I	>8	R
AR-0264	21~	ParC S87L	GyrA D87Y	>8	R

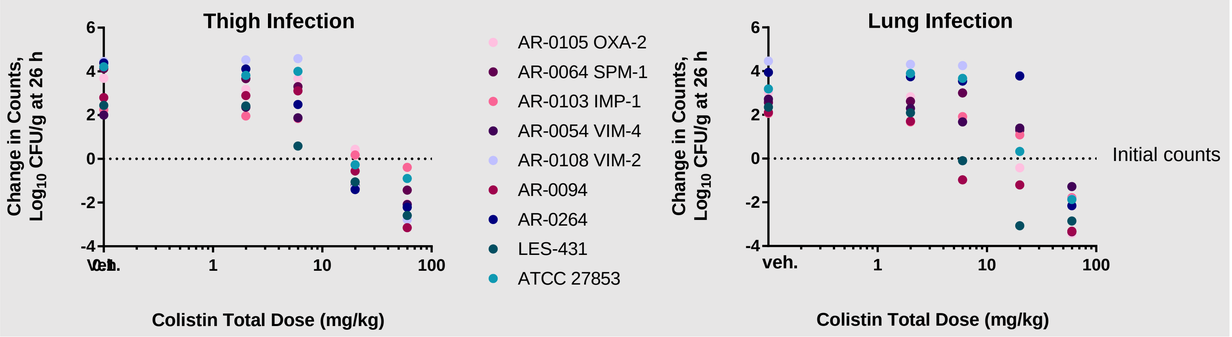
All of the carbapenem resistant strains were also resistant to ciprofloxacin (CIP). Mutations in the quinolone resistance determinant region of DNA gyrase and topoisomerase IV are strong contributors to this resistance. Elevated efflux may also contribute but was not assessed.

Mouse infection models, colistin efficacy, AR-0108 ST233 VIM-2



Colistin sulfate demonstrated significant dose-responsive reductions in bacterial counts in the thigh and lung infection models using the VIM-2 producing clinical isolate, AR-BANK 0108. Animals were rendered neutropenic then infected with bacterial suspension by intramuscular or intranasal inoculation. Colistin was administered twice by subcutaneous injection, 2 and 8 hours after infection, with dose levels of 1, 3, 10 and 30 mg/kg per administration. Bacterial counts (CFU/g) in tissue were measured from tissues harvested at 26 hr post infection, 24 hr after the first dose administration. "Veh" denotes counts of the vehicle treatment group. "Initial" denotes the initial counts of an untreated animal group at 2 hr after infection, the time of the first dose administration. Significance was defined with ANOVA using GraphPad Prism.

Colistin efficacy, nine strains



Colistin sulfate demonstrated dose-responsive efficacy in the thigh and lung infection models of the nine strains. The studies were performed as described above. The studies were performed as described above. The change in counts is difference between the mean total counts (CFU/g) of the treatment group relative to the relative to the mean initial counts at the time of dose administration. The static dose of colistin that results in no increase in counts was 20 or 60 mg/kg. Colistin treatment at 60 mg/kg resulted in a one-log killing of most strains. These organisms tested in these studies are susceptible to colistin in vitro (MIC 1 to 2 µg/mL).

Summary

Lung and thigh infection models were developed using eight carbapenem resistant *P. aeruginosa* strains. The set includes four metallo-β-lactamase producing strains and two serine β-lactamase producing strains. The set includes five international high risk clones associated with antibiotic resistance (ST233, ST654, ST954, ST277, and ST235). It also includes a Liverpool epidemic cystic fibrosis strain. All strains were virulent in mouse lung and thigh tissues. Colistin demonstrated dose responsive inhibition of bacterial growth of all strains at a total dose of 20 to 60 mg/kg. The 60 mg/kg colistin dose resulted in a bactericidal effect (>1 log kill) against most strains in both the lung and thigh models.

Acknowledgements

- FDA-CDC AR Isolate Bank supplied bacterial strains, genome sequence and the AMR annotation. Phenotype data may also be found on the AR Bank website: https://wwwn.cdc.gov/ARIsolateBank/
- CosmosID (Rockville MD) generated full genome assemblies and defined the organism sequence types with PubMLST.
- MicrogenomX (Union NJ) identified mutations in the OprD porin gene and QRDR regions of topoisomerase genes.
- R.V. Dudhani, et al. 2009. Pharmacokinetic/Pharmacodynamic Determinant of Colistin Activity against Pseudomonas aeruginosa in Murine Thigh and Lung Infection Models. Antimicrob. Agents Chemother. March 2010 vol. 54 no. 3 1117-1124