A Panel of MDR *P. aeruginosa* Clinical Isolates for Pharmacology Studies with Murine Lung and Thigh Infection Models

Pharmacology Discovery Services

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Abstract #93

Abstract

This study objective is to generate a set of characterized multidrug resistant *Pseudomonas aeruginosa* clinical isolates for *in vivo* efficacy evaluations of new antibacterial drug candidates. Ready access to validated murine models of lung and thigh infections with these organisms will enable researchers to demonstrate the therapeutic value of their drug candidates against clinically relevant organisms with priority antibiotic resistance mechanisms. This presentation will show the selection of organisms based on the antimicrobial resistance genotypes and phenotypes and the validation of the infection models with the analysis of colistin efficacy.

Methods

Clinical isolates were from the FDA-CDC Antimicrobial Resistance (AR) Isolate Bank and the BCCM Belgian Coordinated Collections of Microorganisms. The sequence types were determined from whole genome sequence (NCBI) using the PubMLST database. The acquired antibiotic resistance genes were supplied by the AR Bank and were also identified using the ResFinder database. Substitutions in the *parC*, *parE*, *gyrA*, and *gyrB* genes, potentially associated with quinolone resistance, and loss of function mutations in the *oprD* porin gene, potentially associated with β-lactam resistance, were identified through multi-sequence alignment against target sequences isolated from *P. aeruginosa* PAO1.

The antibiotic susceptibility of selected organisms was determined using the micro dilution method of the CLSI (M07-A10 and M100S). β-lactam resistance due to metallo-β-lactamase or serine β-lactamase production was tested with β-lactam / β-lactamase inhibitor combinations using metal chelators (EDTA plus 1,10-phenanthroline) or avibactam.

Lung and thigh infection models were conducted with the selected strains using groups of 5 neutropenic ICR mice. Colistin was administered subcutaneously at 2 and 8 hours after infection. Groups of animals were sacrificed at 2 hours after infection to measure the initial counts or at 26 hours after infection for final counts. The bacterial burden (CFU/g) in tissue was calculated. Significance of antimicrobial effects relative to a vehicle treatment and the 2 hour initial counts groups was assessed with ANOVA.

Organism Sequence Types

Organism	Sequence Type	Description
ATCC 27853	ST155	A 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 expression of the OprD porin (Salunkhe, 2005).
AR-0108	ST233	An international high risk clone associated with the dissemination of metallo β-lactamase genes.
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) carbapenemase.
AR-0105	ST235~	This isolate is similar to an international high risk clone associated with the dissemination of multidrug-resistance genes.
AR-0094	ST155	ST155 is a common strain frequently found as respiratory isolates and in cystic fibrosis patients.
AR-0264	ST21~	A novel ST.

The strain collection consists 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 Cosmos ID. The sequence type description is based on literature reports.

Carbapenem Resistance, Genotypes and Phenotypes

Organism	Acquired β-Lactamase Genes	OprD Porin Mutation	AmpC Normalized Expression LOG_{10}(RQ)	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	--	--	0	1	4/4	1	1	--
LES-431	--	--	3.08	>64	16/4	16	16	Serine β-lactamase
AR-0108	VIM-2 (MBL) OXA-4		2.92	>64	>16/4	>128	32	Metallo-β-lactamase
AR-0054	VIM-4 (MBL)	W417*	2.75	>64	>16/4	>128	16	Metallo-β-lactamase
AR-0103	IMP-1 (MBL)	W417*	2.95	>64	>16/4	128	16	Metallo-β-lactamase
AR-0064	SPM-1 (MBL) OXA-56	A126 fs	2.15	>64	>16/4	>128	32	Metallo-β-lactamase
AR-0105	OXA-2	--	2.91	64	16/4	2	2	Serine β-lactamase
AR-0094	--	L46 fs	4.16	>64	>16/4	64	64	Unknown
AR-0264	--	L45 fs	2.76	1	2/4	16	16	Unknown

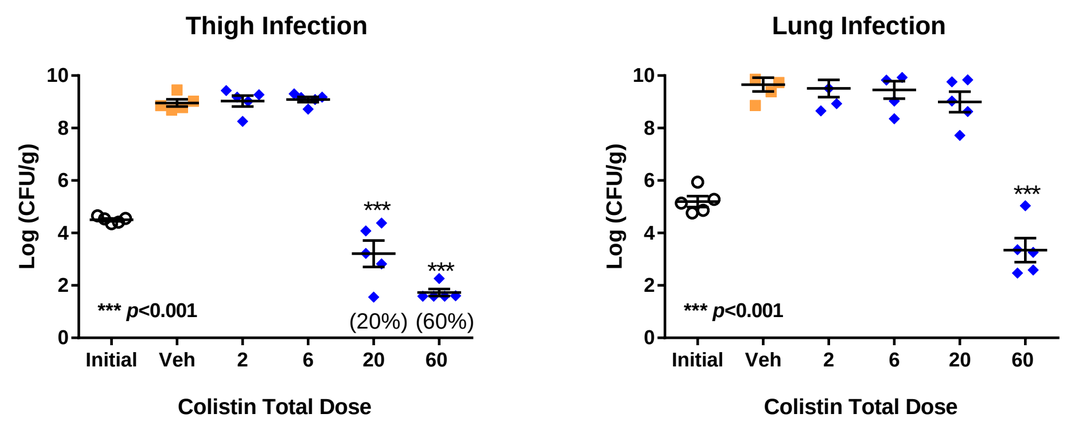
- Acquired β-lactamase genes were reported by the AR-Bank and were identified using Resfinder analysis of whole genome sequence.
- OprD mutations that result in loss of function and reduced uptake of β-lactam antibiotics were identified from whole genome sequence analysis. Nonsense mutations (*) and frameshift (fs) mutations likely contribute to the carbapenem resistance.
- AmpC expression is likely to contribute to resistance in all of the clinical isolates. Relative Quantification (RQ) values of RNA is relative to strain ATCC 27853 and was measured with RT-qPCR. The rpsL gene for the 30S ribosomal subunit protein was used for normalization. The values are based on 3 repetitions. LOG_{10}(RQ) values greater than 2 are highly significant, $p < 0.001$, One-way ANOVA followed by Dunnett's comparison test. This analysis was performed by Mission Biotech, Taipei Taiwan.
- 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

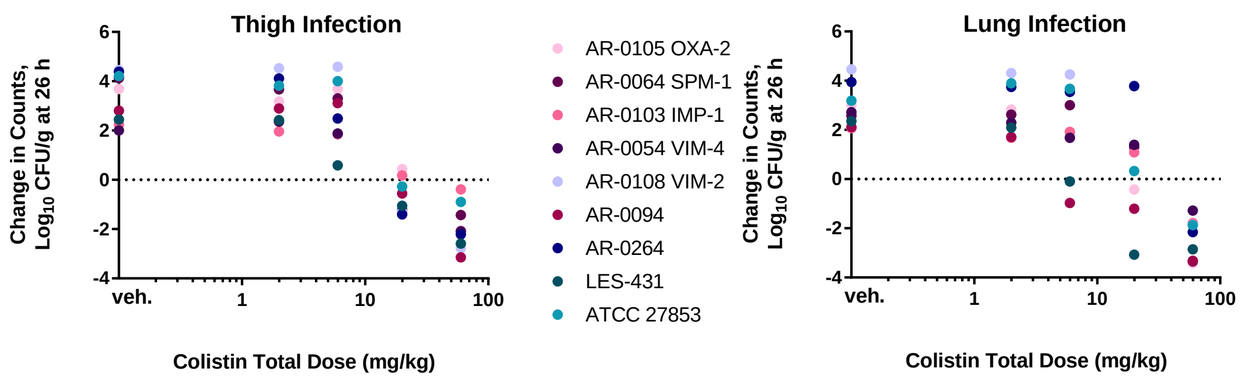
All of the carbapenem resistant strains were also resistant to ciprofloxacin (CIP). Mutations were identified in the quinolone resistance determinant region of DNA gyrase and topoisomerase IV are likely to mediate this resistance. Elevated efflux may also contribute to resistance but was not assessed.

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 hours post infection, 24 hours 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 hours after infection, the time of the first dose administration. Significance was defined with ANOVA using GraphPad Prism. This colistin efficacy analysis was performed with all nine strains in this panel.

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 change in counts (y-axis) is difference between the mean total counts (CFU/g) of the treatment group 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 (dotted horizontal line). Colistin treatment at 60 mg/kg resulted in a one-log killing of most strains. These organism tested in these studies are susceptible to colistin *in vitro* (MIC 1 to 2 µg/mL). Results are similar to those reported previously (Dudhani 2010).

Results

A set of organisms was selected that includes international and regional high risk clones associated with the dissemination of multidrug resistance – ST233, ST235, ST654, ST964, ST277 of Brazil, and ST146 the Liverpool epidemic strain (LES). The set includes four strains with acquired metallo-β-lactamase genes: *bla*-VIM-2, *bla*-VIM-4, *bla*-IMP-1, and *bla*-SPM-1.

Four strains which carried genes encoding metallo-β-lactamase enzymes also tested positive for metallo-β-lactamase production with the EDTA/phenanthroline test. Two strains that lacked detectable metallo-β-lactamase genes, LES-431 and AR-0105, were susceptible to the ceftazidime avibactam combination indicating resistance due to a serine β-lactamase production. All of the carbapenem resistant isolates were resistant to ciprofloxacin and have point mutations in the topoisomerase genes associated with quinolone resistance.

Eleven carbapenem resistant isolates were analyzed for growth in lung and thigh tissue of neutropenic ICR mice. Eight of the isolates (72%) grew well in both lung and thigh infections. Colistin treatment in the models yielded significant ($p < 0.05$) and dose-responsive inhibition of all isolates. A total colistin dose of 60 mg/kg resulted in a killing effect (a two-log reduction in counts relative to the initial counts) with 75% and 50% of the isolates in the thigh and lung models respectively.

Conclusion

This collection of phenotypically and genotypically characterized MDR *P. aeruginosa* isolates is a useful tool for the pharmacological assessments of novel antimicrobial agents with validated murine lung and thigh infection models.

References

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