Poster #: 90

Phenotypic Characterization of a Reference Set of Recalcitrant Gram-Negative Bacteria

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Background

The emergence and spread of antibacterial resistance among Gram-negative bacteria has become a global challenge for health care. Multiple programs have been implemented to reduce exposure and spread, but new therapeutics are necessary to combat these challenges. For the discovery and development of novel therapeutic agents to become a reality, multidrug-resistant (MDR) clinical isolates that represent current disease strains from around the globe are required. To support this effort, ATCC has collected and characterized 33 Gram-negative isolates using standard and new technologies.

Methods

Thirty-three nonduplicative MDR Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* strains collected from five continents were evaluated using the broth microdilution methodology as described by the Clinical and Laboratory Standards Institute (CLSI). Strains were identified via 16S rRNA and MALDI-TOF, and were tested beyond CLSI breakpoints to provide better visibility into the level of resistance. Multiple replicates of the minimum inhibitory concentration (MIC) evaluations were completed on different days and with different technicians. Sequencing and bioinformatic analyses were performed and antibiotic resistance genes were identified.

Table 1. Individual strains and isolation source information

ATCC® No.	Species	Strain	Isolation Source
BAA-2774™	*Escherichia coli*	1093925	50-year-old male, abscess, general surgery, Argentina, Latin America, 2014
BAA-2775™	*Escherichia coli*	1099675	75-year-old male, decubitus, Czech Republic, 2014
BAA-2776™	*Escherichia coli*	1099705	78-year-old female, urine, general medical clinic, Czech Republic, 2014
BAA-2777™	*Escherichia coli*	1106769	40-year-old female, urine, general surgery, Nigeria, 2014
BAA-2778™	*Escherichia coli*	1123005	46-year-old female, urine, emergency room, Nigeria, 2014
BAA-2779™	*Escherichia coli*	1136922	83-year-old male, urine, emergency room, Israel, 2014
BAA-2780™	*Escherichia coli*	1138276	70-year-old female, abscess, general medicine, Mexico, 2014
BAA-2781™	*Escherichia coli*	1144993	52-year-old female, blood, medicine ICU, Turkey, 2014
BAA-2782™	*Klebsiella pneumoniae*	931476	55-year-old female, peritoneal fluid, general surgery, Brazil, 2013
BAA-2783™	*Klebsiella pneumoniae*	972863	35-year-old male, large colon, surgery ICU, Serbia, 2013
BAA-2784™	*Klebsiella pneumoniae*	1147843	53-year-old female, urine, medicine ICU, United States, 2014
BAA-2785™	*Klebsiella pneumoniae*	BK33650	United States, 2010
BAA-2786™	*Klebsiella pneumoniae*	BK34774	Deep wound, United States, 2010
BAA-2787™	*Klebsiella pneumoniae*	BK34786	Urine, United States, 2010
BAA-2788™	*Klebsiella pneumoniae*	BK34907	Urine, United States, 2011
BAA-2789™	*Klebsiella pneumoniae*	BK35106	Blood, United States, 2012
BAA-2790™	*Klebsiella pneumoniae*	BK35639	Wound, United States, 2012
BAA-2791™	*Proteus mirabilis*	927889	79-year-old male, urine, general medical clinic, Greece, 2013
BAA-2792™	*Proteus mirabilis*	1104843	50-year-old male, urine, general medicine, Greece, 2014
BAA-2793™	*Pseudomonas aeruginosa*	1077994	26-year-old female, urine, general medicine, Chile, 2014
BAA-2794™	*Pseudomonas aeruginosa*	1079232	28-year-old female, abscess, general medicine, Thailand, 2014
BAA-2795™	*Pseudomonas aeruginosa*	1106432	70-year-old male, urine, general medicine, Kenya, 2014
BAA-2796™	*Pseudomonas aeruginosa*	1106434	32-year-old male, blood, general medicine, Kenya, 2014
BAA-2797™	*Pseudomonas aeruginosa*	1109196	51-year-old male, endotrachial aspirate, surgery ICU, Colombia, 2014
BAA-2798™	*Pseudomonas aeruginosa*	1124989	34-year-old male, sputum, general medicine, Spain, 2014
BAA-2799™	*Pseudomonas aeruginosa*	1145009	55-year-old male, blood, medicine ICU, Turkey, 2014
BAA-2800™	*Acinetobacter baumannii*	1074318	57-year-old male, abscess, general surgery, Spain, 2014
BAA-2801™	*Acinetobacter baumannii*	1103521	36-year-old female, endotrachial aspirate, medicine ICU, Venezuela, 2014
BAA-2802™	*Acinetobacter baumannii*	1109222	37-year-old male, blood, Colombia, 2014
BAA-2803™	*Acinetobacter baumannii*	1125209	73-year-old male, sputum, general medicine, Italy, 2014
BAA-2806™	*Enterobacter cloacae*	976689	33-year-old male, urine, general medicine, Russia, 2013
BAA-2807™	*Citrobacter freundii*	990954	54-year-old male, skin ulcer, general surgery, Brazil, 2013
BAA-2808™	*Serratia marcescens*	938677	72-year-old male, urine, Romania, 2013

Results

Table 2. MIC result ranges by drug and bacterial group

Antibiotic	MIC Range in µg/mL*		
	Enterobacteriaceae n=22	*Pseudomonas aeruginosa* n=7	*Acinetobacter baumannii* n=4
Amikacin	1–>512	8–512	1–>512
Amoxicillin-clavulanate	4–128	NB	NB
Aztreonam	1–>512	4–>512	NB
Cefepime	1–>512	8–>512	32–256
Ceftazidime	2–>512	8–>512	64–>512
Ceftazidime-avibactam	0.12–64	2–>512	NB
Ceftriaxone	64–>512	64–>512	256–>512
Ciprofloxacin	8–>512	16–256	64–>512
Colistin	0.25–>512	0.25–1	0.25–4
Doripenem	0.008–128	8–>512	16–64
Fosfomycin	1–128	NB	NB
Imipenem	0.12–256	16–>512	64–128
Levofloxacin	4–256	32–512	8–256
Meropenem	0.03–128	16–>512	16–128
Piperacillin-tazobactam	0.05–>512	32–>512	64–512
Tetracycline	2–512	NB	16–512
Tigecycline	0.06–8	8–16	0.5–4
Trimethoprim-sulfamethoxazole	≤0.25–>512	NB	NB

*MIC data were generated using standardized methods for broth microdilution antimicrobial susceptibility testing published by the Clinical and Laboratory Standards Institute® (CLSI), 26th Edition guidelines.
n, number of strains; NB, no breakpoints

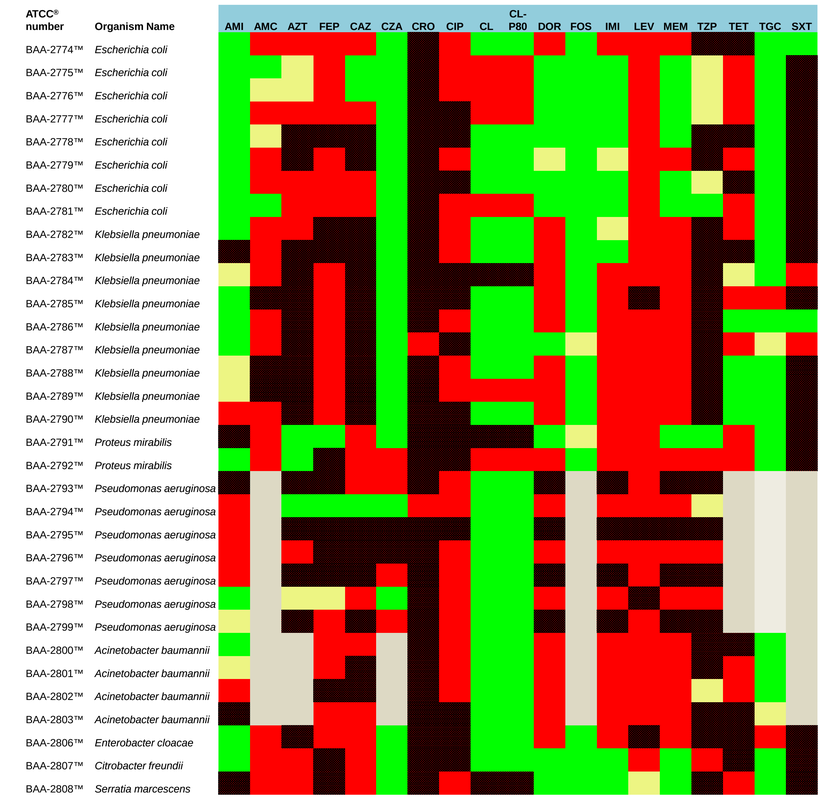

Figure 1. Heat map exhibiting the varying levels of resistance among strains. AMI=amikacin; AMC=amoxicillin-clavulanate; AZT=aztreonam; FEP=cefepime; CAZ=ceftazidime; CZA=ceftazidime-avibactam; CRO=ceftriaxone; CIP=ciprofloxacin; CL=colistin; CL-P80 = colistin-P80; DOR=doripenem; FOS=fosfomycin; IMI=imipenem; LEV=levofloxacin; MEM=meropenem; TZP=piperacillin-tazobactam; TET=tetracycline; TGC=tigecycline; SXT=trimethoprim-sulfamethoxazole; Resistance reported per CLSI guidelines based on approved breakpoints.
= Susceptible; = Intermediate Resistance; = Resistant; = Highly Resistant (>128µg/ml); = not tested;
Susceptibility for CZA, CL, CL-P80, FOS, and TGC determined by breakpoints set by British Society for Antimicrobial Chemotherapy, European Committee for Antimicrobial Susceptibility Testing, and manufacturer's product labels.

Data Delivery

A.

Clinical Isolates Collection
Antimicrobial Resistance Report

Drug	MIC (µg/mL)	CLSI® Reference Range (µg/mL)
Fosfomycin	2	Susceptible:≤64 Intermediate:128 Resistant≥:256
Imipenem	4	Susceptible:≤1 Intermediate:2 Resistant:≥4
Levofloxacin	32	Susceptible: ≤2 Intermediate:4 Resistant:≥8
Meropenem	8	Susceptible:≤1 Intermediate:2 Resistant:≥4
Piperacillin Tazobactam	>512	Susceptible:≤16 Intermediate:32-64 Resistant:≥128
Tetracycline	512	Susceptible:≤4 Intermediate:8 Resistant:≥16

B. **16S rRNA Sequence Data**

GGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAARMAGCTTGCTKYTTYGCTGA
CGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACC
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GGTGGGGTAACGGCTCACCWAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGT
TACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA
CTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGAT
ACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATAC
CGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAA
GTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGARAATGTGCCTT
CGGGAACCGTGAGACAGGTGCTGCATGGCT

C. **>Contig_3_Length_192674|Start_51752|End_53698|-strand|_ORF-1_macB**

ATGACGCCTTTGCTCGAATTAAAGGATATTCGTCGCAGCTATCCTGCCGGTGATGAGCAGGTTGAGGTGCTGAAGGGCA
TCAGCCTCGATATTTATGCGGGTGAGATGGTCGCGATTGTTGGCGCTTCGGGTTCCGGTAAATCGACCCTGATGAATATT
CTCGGCTGTCTGGATAAGGCCACCAGCGGCACCTATCGCGTCGCCGGTCAGGATGTTGCCACGCTGGACGCCGATGC
GCTGGCGCAACTGCGCCGCGAGCATTTCGGCTTTATTTTCCAGCGTTACCATTTGCTTTCGCATTTAACCGCCGAGCAG
AACGTTGAAGTACCCGCCGTCTATGCTGGTCTTGAGCGGAAACAGCGACTGCTTCGTGCCCAGGAGTTGCTGCAACG
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GGGCATTGATGAACGGTGGTCAGGTAATTCTTGCCGATGAACCAACCGGCGCACTGGACAGCCATTCTGGCGAAGAG
GTGATGGCGATCCTGCATCAGCTGCGCGATCGTGGGCATACGGTGATTATCGTCACCCACGATCCGCAGGTCGCTGCT
CAGGCCGAGCGGGTGATCGAAATTCGCGACGGCGAAATTGTGCGCAATCCTCCCGCCATTGAAAAAGTGAATGTTACT
GGCGGGACGGAACCTGTTGTCAACACGGTGTCTGGCTGGCGGCAGTTTGTCAGCGGTTTTAACGAGGCGCTGACGAT
GGCATGGCGGGCGCTGGCAGCGAATAAAATGCGTACTTTACTGACCATGCTGGGGATTATTATCGGTATTGCGTCGGTG
GTTTCCATTGTCGTGGTGGGTGACGCCGCCAAACAAATGGTGCTGGCGGATATTCGTTCTATTGGTACGAATACTATTGA
TGTCTATCCCGGGAAAGATTTTGGCGATGACGATCCGCAATATCAGCAGGCGCTGAAGTACGACGACTTAATCGCCATC
CAAAAACAACCGTGGGTCGCCTCAGCCACACCTGCCGTCTCGCAAAACCTGCGCCTGCGTTATAACAATGTTGATGTTG
CTGCCA

Figure 2. Sample characterization data for *Escherichia coli*. A) MIC results in µg/mL (excerpt). B) Confirmation of identity by 16S rRNA sequencing. C) Antimicrobial resistance gene identification (excerpt).

Summary

- A set of 33 MDR Enterobacteriaceae, *P. aeruginosa,* and *A. baumannii* strains have been characterized phenotypically and genotypically
- Authenticated strains with MIC values and genome sequences are ideal tools for:
  - Antibiotic drug discovery
  - Novel detection assays
  - Validation of diagnostic methods for species or antibiotic resistance mechanism(s)
  - Study of genetic variations between different strain types

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