Poster Number: HEALTH-SAT-331
Date: June 6, 2026

Genome, Resistome, Methylome – When the Same Gene(s) Produces a Different Phenotype

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Background

Here, we describe the process used to generate the genome, resistome, and methylome for more than 3,700 ATCC® bacterial organisms. We then selected three species from the WHO bacterial priority pathogen list and conducted an in-depth analysis of ten strains from each species. For each strain, we produced the genome, resistome, and methylome, and compared these profiles to their corresponding antibiotic susceptibility testing (AST) results.

When discordance between the resistome and AST data was detected, we conducted further analysis of the methylome to identify potential patterns that may contribute to these discrepancies. We also detail the methods used to process, curate, and analyze the resulting datasets, all of which were generated using established and documented workflows to ensure consistency and reproducibility. The genome, resistome, methylome, and phenotype data described here are available through the ATCC® Genome Portal.

Methods

For genomic analyses, we used our ISO 9001-compliant standardized sequencing, assembly, and annotation pipelines for each strain. We extracted high-quality DNA and sequenced the DNA on both the Illumina and Oxford Nanopore Technologies (ONT) instruments. The data were analyzed for quality and then both data sets were combined to produce a complete de novo hybrid assembly.

All de novo assemblies were then annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and further curated for AMR-related genes using NCBI AMRFinderPlus, the Comprehensive Antibiotic Resistance Database through the Resistance Gene Identifier software (RGI-CARD; McMaster University), and ResFinder (Technical University of Denmark).

Methylation profiles were generated with ONT data using the Dorado basecaller version 1.4.0+acec121 (ONT). The genomes, resistome, and methylomes for referenced strains are now available to customers through the ATCC® Genome Portal.

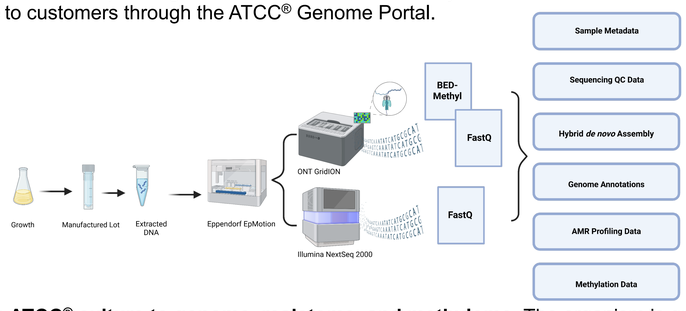

Figure 1: ATCC® culture to genome, resistome, and methylome. The organism is grown, vialed and quality controlled according to ATCC® parameters. DNA is extracted using a method best suited for organism type and NGS libraries are produced and loaded on the Illumina NextSeq 2000 and Oxford Nanopore Technologies GridION instruments. Data from both platforms were quality controlled and a subsequent hybrid genome assembly was produced and annotated. ONT base-called data were further analyzed for the presence of methylated nucleotides. Figure created using BioRender.com.

Table 1: AST data from 9 ATCC® strains of *Streptococcus pneumoniae* The appropriate VITEK 2 AST cards are selected and loaded on to the VITEK 2 instrument to produce the Minimum Inhibitory Concentration (MIC) profile (not shown).

	ATCC® Catalog Number →	BAA-3313™	BAA-3322™	BAA-3323™	BAA-3326™	BAA-3327™	BAA-3329™	BAA-3330™	BAA-3331™	BAA-3334™
Antibiotics	**Antibiotic class**	***Streptococcus pneumoniae***								
Cefotaxime (Meningitis)	Cephalosporin	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	4 (R)	2 (R)	4 (R)	4 (R)	4 (R)
Cefotaxime (Other)	Cephalosporin	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	4 (R)	2 (I)	4 (R)	4 (R)	4 (R)
Ceftriaxone (Meningitis)	Cephalosporin	4 (R)	4 (R)	4 (R)	≥8 (R)	4 (R)	2 (R)	4 (R)	4 (R)	4 (R)
Ceftriaxone (Other)	Cephalosporin	4 (R)	4 (R)	4 (R)	≥8 (R)	4 (R)	2 (I)	4 (R)	4 (R)	4 (R)
Trimethoprim-sulfamethoxazole	Diaminopyrimidines	≥320 (R)	160 (R)	160 (R)	80 (R)	160 (R)	160 (R)	160 (R)	160 (R)	≥320 (R)
Levofloxacin	Fluoroquinolones	≤0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)
Moxifloxacin	Fluoroquinolones	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)	0.12 (S)
Vancomycin	Glycopeptide	≤0.12 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)
Tigecycline	Glycylcyclines	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)	≤0.06 (S)
Clindamycin	Lincosamides	≥1 (R)	≥1 (R)	≥1 (R)	≤0.25 (S)	≥1 (R)	≥1 (R)	≥1 (R)	≤0.25 (S)	≥1 (R)
Erythromycin	Macrolides	≥8 (R)	≥8 (R)	≥8 (R)	2 (R)	≥8 (R)	≥8 (R)	≥8 (R)	4 (R)	≥8 (R)
Linezolid	Oxazolidinones	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)	≤2 (S)
Benzylpenicillin (Meningitis)	Penicillin	4 (R)	2 (R)	4 (R)	≥8 (R)	≥8 (R)	4 (I)	≥8 (R)	4 (R)	4 (R)
Benzylpenicillin (Oral)	Penicillin	4 (R)	2 (R)	4 (R)	≥8 (R)	≥8 (R)	4 (R)	≥8 (R)	4 (R)	4 (R)
Benzylpenicillin (Other)	Penicillin	4 NA	2 NA	4 NA	≥8 NA	≥8 (R)	4 (R)	≥8NA	4NA	4NA
Benzylpenicillin (Pneumonia)	Penicillin	4 (I)	2 (S)	4 (I)	≥8 (R)	≥8 NA	4NA	≥8 (R)	4 (I)	4 (I)
Tetracycline	Tetracyclines	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)
Metadata	Isolation country	South Korea	Germany	Hong Kong	United States	United States	China	Italy	Spain	Australia
	Year of isolation	2014	2014	2014	2014	2014	2013	2013	2013	2014
	Source of isolation	Respiratory sputum	Respiratory sputum	Respiratory sputum	Respiratory sputum	Respiratory sputum	Respiratory sputum	Respiratory bronchoalveolar lavage	Respiratory sputum	Abscess
	Patient Gender	Male	Male	Female	Female	Female	Female	Male	Male	Male
	Patient Age	69 years	14 years	10 years	2 years	49 years	75 years	22 years	46 years	1 year

Resistome – VITEK Concordance

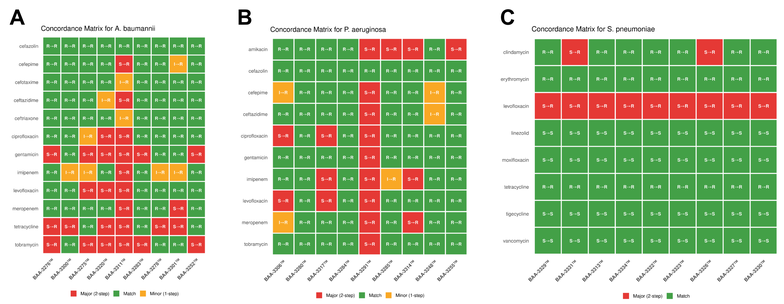

Figure 2: Concordance matrices. VITEK susceptibility data (first value in column) were compared with AMR genes (second value in column) detected across multiple databases for (A) *Acinetobacter baumannii*, (B) *Pseudomonas aeruginosa*, and (C) *Streptococcus pneumoniae*. Detection of an AMR gene associated with a specific antibiotic or antibiotic class was interpreted as resistant (R), while absence of the gene was interpreted as susceptible (S).

Table 2: Observed AMR gene for clindamycin in *S. pneumoniae*. Shown is the detected AMR gene for clindamycin by AMRFinder, ResFinder, and CARD for 4 strains. *erm*(B) was detected for all nine *S. pneumoniae* strains used in this study.

			Total AMR Genes Detected By		
ATCC® Catalog	**Vitek**	**AMR Gene**	**AMRFinder**	**ResFinder**	**CARD**
BAA-3326™	S	*erm*(B)	0	1	1
BAA-3331™	S		0	1	1
BAA-3322™	R		1	1	1
BAA-3327™	R		1	1	1

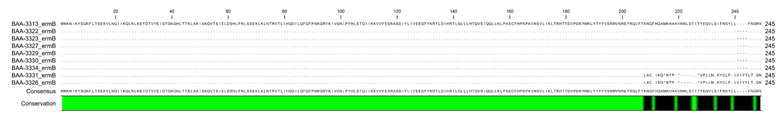

Figure 3: Erm(B) protein sequence alignment across nine *S. pneumoniae* strains. *erm*(B), a rRNA methyltransferase that modifies 23S rRNA to prevent clindamycin from binding, was detected in all strains. However, sequences from *S. pneumoniae* strain 1041992 (ATCC® BAA-3331™) and strain 1161255 (ATCC® BAA-3326™) contain multiple mutations resulting in premature stop codons. Despite the presence of the *erm*(B) gene in these strains, both harbor nonfunctional copies, consistent with their susceptibility as determined by VITEK.

Epigenome Analysis for Levofloxacin Resistance

Based on the concordance matrices in Figure 2C, all *S. pneumoniae* strains were classified as susceptible to levofloxacin by VITEK. However, resistome analysis identified three efflux pump genes (*pmrA*, *patA*, and *patB*) that are associated with levofloxacin resistance. Here, epigenomic profiles surrounding these loci were examined.

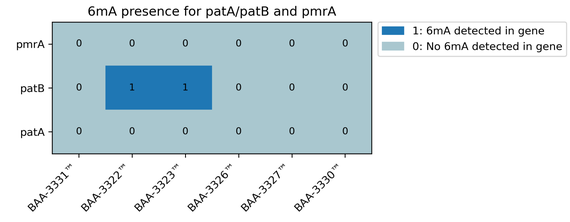

Figure 4: Gene-body 6mA methylation presence for *patA/patB* and *pmrA*. Calls indicate detection of at least one 6mA site within the gene (1 = detected, 0 = not detected). *patA* and *pmrA* show no detectable 6mA methylation across strains, while *patB* exhibits strain-specific gene-body methylation. No consistent relationship is observed between gene-body methylation and levofloxacin susceptibility.

Conclusions

- In addition to generating annotated genomes for more than 5,600 bacterial strains, we have produced resistome and epigenome data for a subset of these organisms.
- To date, this represents the most diverse collection of microbial methylation data available.
- Care should be used when using AI AMR prediction models because genotype cannot always accurately predict antimicrobial susceptibility.

Learn more about the ATCC® Genome Portal

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