# Phenotypic and Genotypic Characterization of Antimicrobial Resistance (AMR) Strains from the ATCC® Collection



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# Background

Antimicrobial resistance (AMR) is a global health emergency; to counter this growing threat, researchers need access to authenticated, well-characterized strains to use as standards during the development of diagnostic assays, therapeutics, or other applications.

Here, we describe the phenotypic and genotypic characterization of **one hundred** AMR strains available in the ATCC<sup>®</sup> collection. To develop and provide this highly curated collection of AMR strains, each strain went through additional testing to establish its phenotypic and genotypic profile.

- Susceptibility testing: Strains were tested against a variety of critical drug classes and are provided with the minimal inhibitory concentration (MIC) values and susceptibility profile.
- **Genomic analyses:** Strains were analyzed via whole-genome sequencing (WGS) on two different instruments to ensure the highest-level of accuracy, coverage, and depth. The corresponding assembled and annotated genomes are available on the ATCC® Genome Portal (genomes.atcc.org).
- **Methylation profiles:** Most strains are provided with methylation data accessible through the ATCC® Genome Portal (genomes.atcc.org).

### Methods

#### **Susceptibility Testing**

To establish the MIC profile, antibiotic susceptibility was generally obtained using VITEK 2 AST cards. We typically selected one or two cards based on the organism, aiming to cover the broadest possible range of antibiotics. MIC ranges for resistant, intermediate, and susceptible are based on criteria within the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 27<sup>th</sup> Edition. For *Neisseria gonorrhoeae*, antibiotic susceptibility was obtained using bioMérieux ETEST strips and MIC interpretations are based on CLSI M100-Ed35.

#### **Genomic Analyses**

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 (Illumina) and Oxford Nanopore Technologies (ONT; Oxford Nanopore Technologies) 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). The whole-genome sequences are available on the ATCC® Genome Portal.

#### **Methylation Profiles**

Methylation profiles were generated with ONT data using the Dorado basecaller version 0.8.0+acec121 (ONT). The methylation profile for many strains are available to customers through the ATCC® Genome Portal.

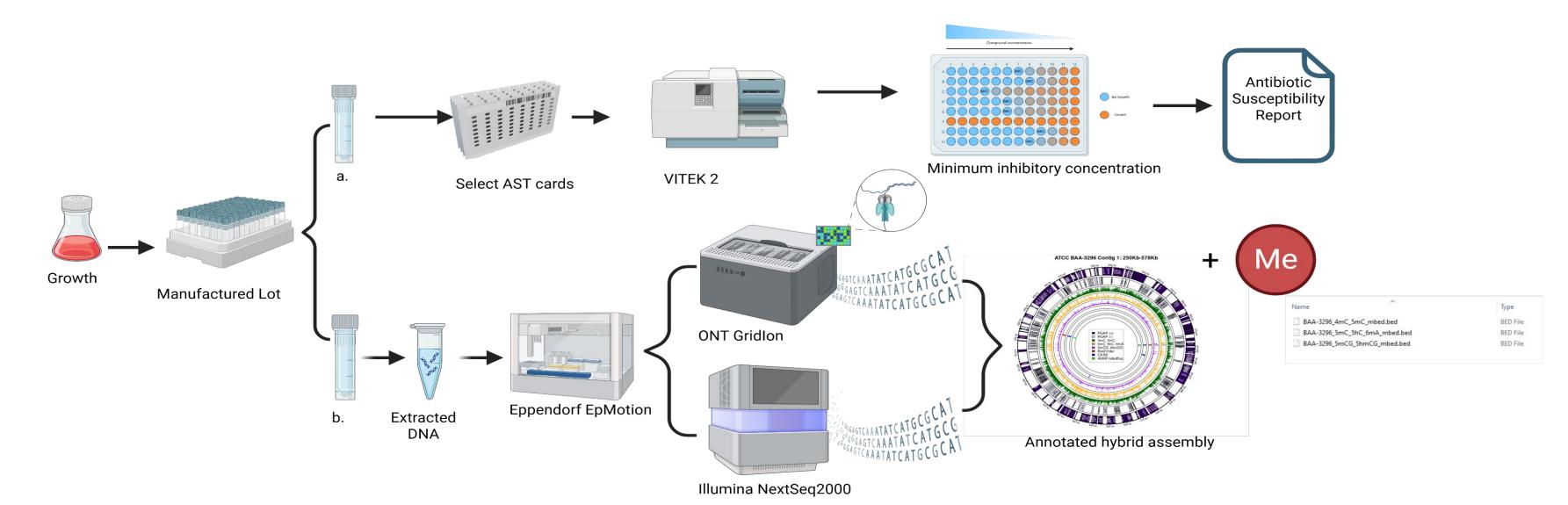
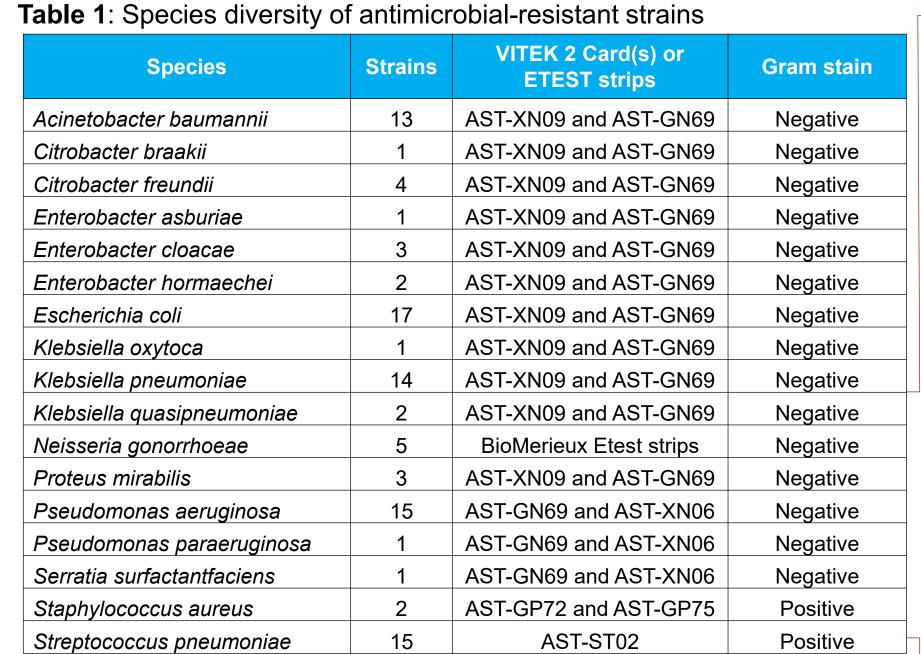


Figure 1: Bacterial strains are grown, vialed, and QC'd according to ATCC® parameters. (A) The appropriate VITEK 2 AST cards (bioMérieux) are selected and loaded on to the VITEK 2 instrument to produce the MIC profile. Note: for *Neisseria gonorrhoeae*, antibiotic susceptibility was obtained using ETEST strips (bioMérieux). (B) DNA is extracted using a method best suited for organism type and next-generation sequencing (NGS) libraries are produced and loaded on the NextSeq 2000 (Illumina) and Gridlon (Oxford Nanopore Technologies) instruments. Data from both platforms were QC'd and a subsequent hybrid genome assembly was produced and annotated. ONT base called data was further analyzed for the presence of methylated nucleotides. Image created using BioRender.com

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## Results



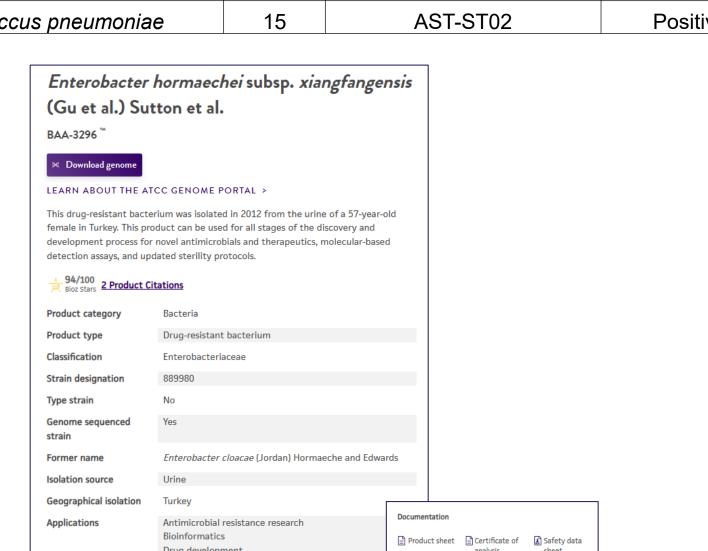
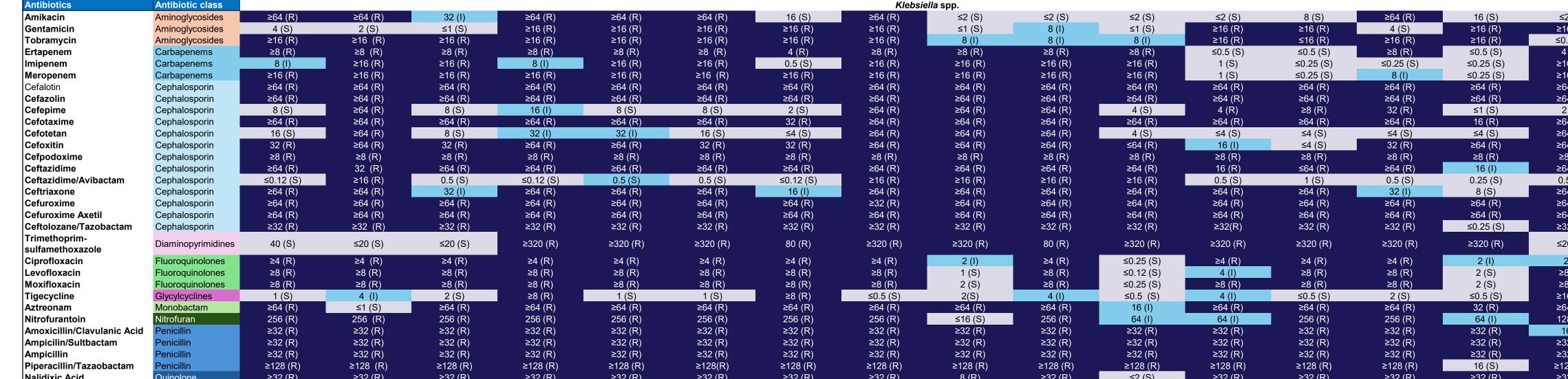
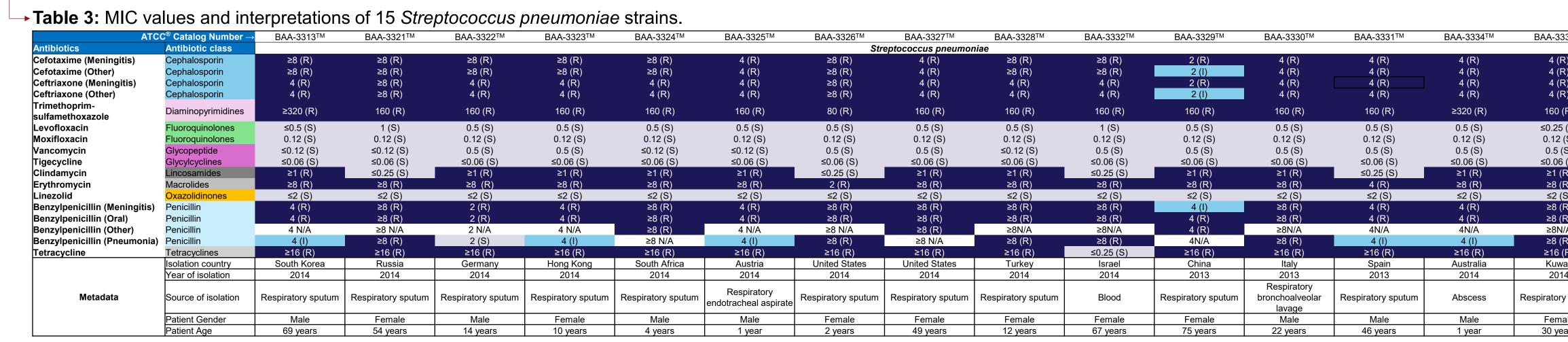


Figure 2: Example of product page with metadata and available documentation.



(R) resistant, (I) Intermediate, and (S) susceptible. A report outlining the MIC values and interpretation of susceptibility accompanies each strain. (Supplemental handout availance)

→Table 2: MIC values and interpretations of 16 Klebsiella spp



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## Conclusion

Here, we highlight one hundred clinically relevant and extensively characterized antimicrobial-resistant isolates that are available to the research community. Each strain comes with:

- Source information Geography, collection date, patient age and gender, and collection site
- Susceptibility data MIC values and susceptibility profiles for targeted drugs. The susceptibility profile of each strain is available on atcc.org
- Genetic data The complete and assembled genome sequence, annotated with antibiotic resistance genes and methylation data. Data is available on genomes.atcc.org

Overall, this diverse collection of highly characterized AMR strains provides a valuable resource for diagnostics and therapeutic development.

Explore our AMR clinical isolates at www.atcc.org/AMR



Find the whole-genome sequences on the ATCC Genome Portal at genomes.atcc.org



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