

# Identification of Antimicrobial Resistance and Biosynthetic Gene Clusters in the ATCC® Global Priority Superbug Collection

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## Background

Microorganisms are an important source of bioactive metabolites essential for drug development; however, as antimicrobial-resistant (AMR) strains continue to spread, they have become a major concern for public health. In 2017, the World Health Organization (WHO) released a report<sup>1</sup> to guide the global priority list of AMR bacteria (Figure 1). Most recently in 2019, a report<sup>2</sup> by the Centers for Disease Control and Prevention (CDC) described three specific categories of AMR bacterial threats (Figure 1).

To thwart the global and local AMR threat via molecular detection, assay development, drug discovery, and therapeutics research, accurate phenotypic and genotypic characterization of authenticated laboratory reference strains is crucial. To that end, ATCC developed the Global Priority Superbugs collection, which comprises 148 fully characterized multidrug-resistant strains representing pathogens characterized as critical or urgent threats. Here, we discuss the genetic characterization of these strains through standardized high-quality dual platform sequencing, hybrid genome assembly, annotation, and specialized genomic mining pipelines for AMR genes and biosynthetic gene clusters (BGCs) annotation.

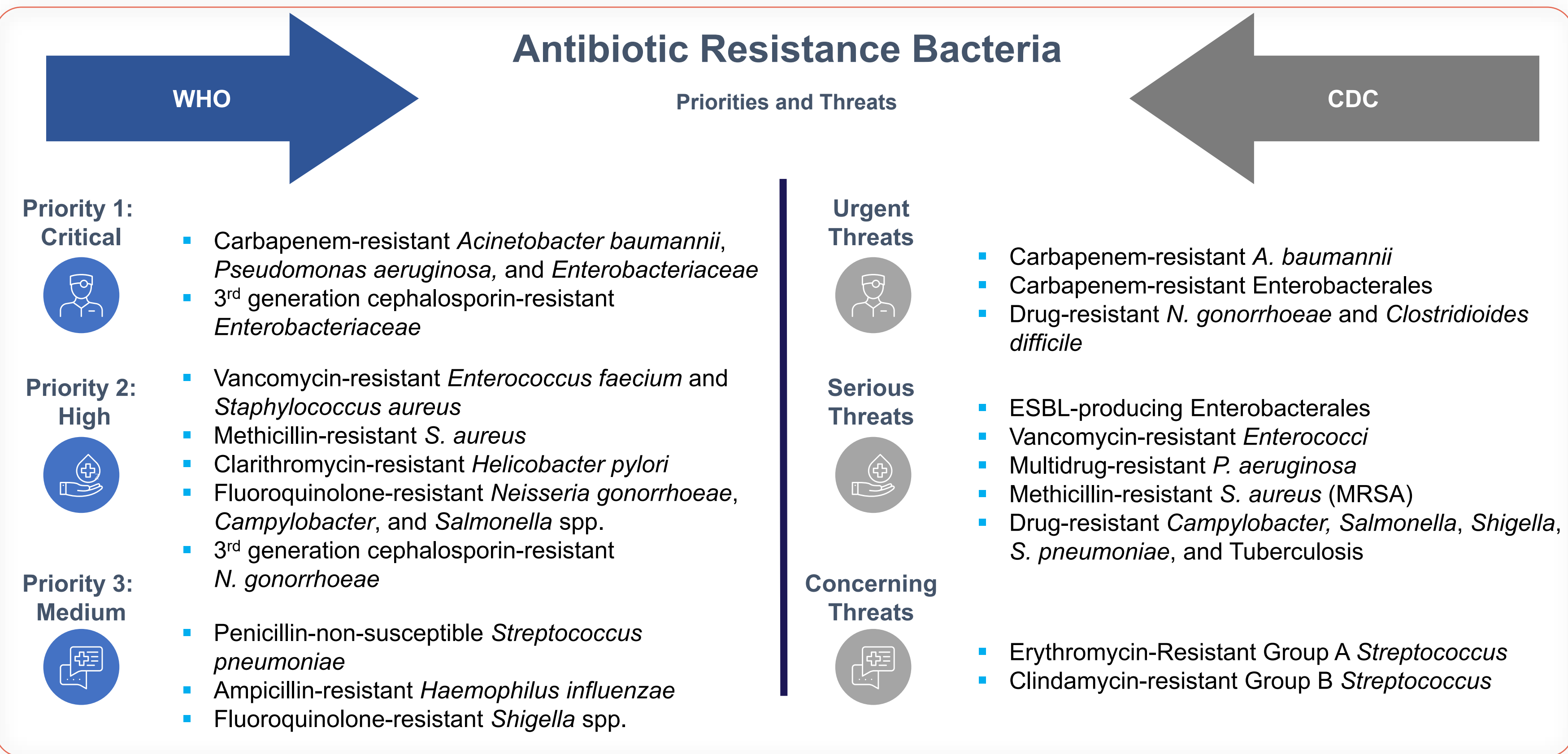


Figure 1: Classification of AMR bacteria for WHO and CDC priority lists.

## Development of the ATCC® Global Priority Superbugs Collection

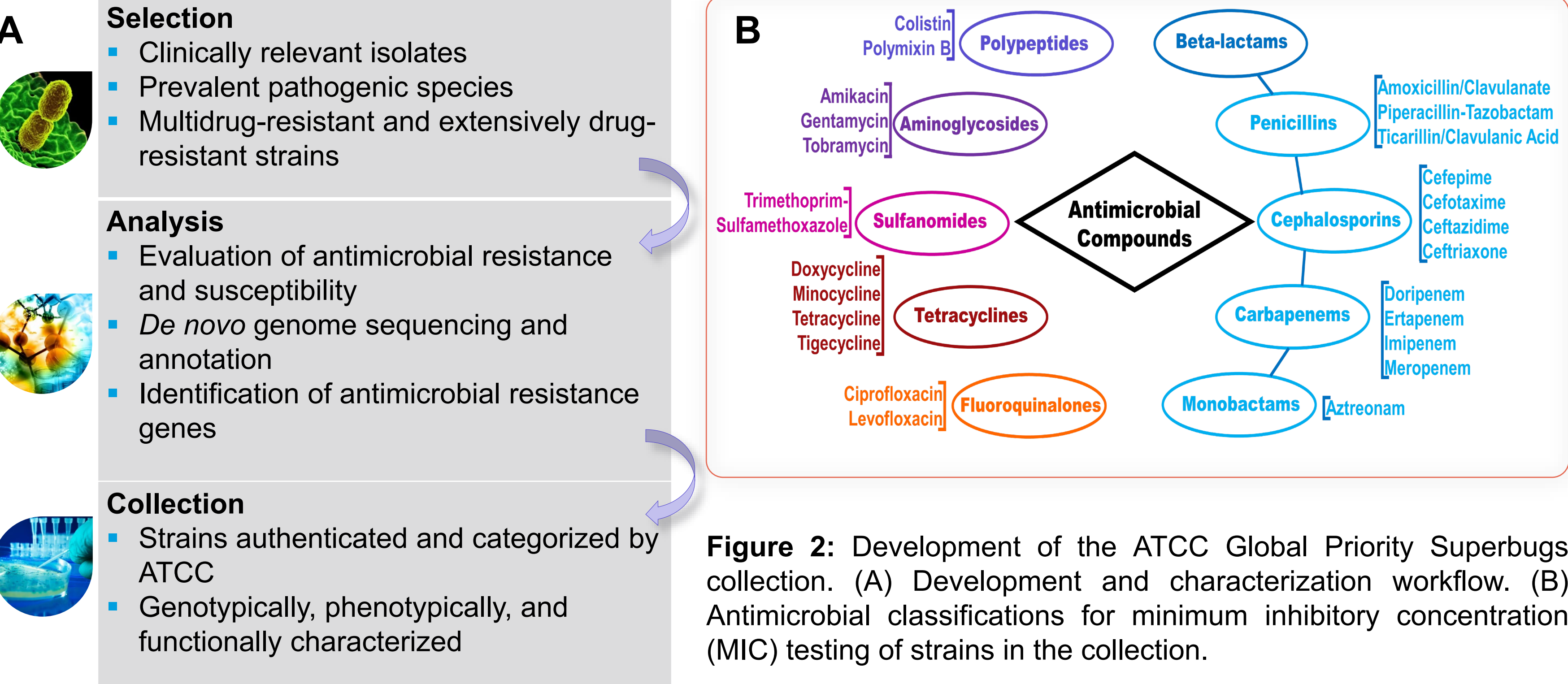


Figure 2: Development of the ATCC Global Priority Superbugs collection. (A) Development and characterization workflow. (B) Antimicrobial classifications for minimum inhibitory concentration (MIC) testing of strains in the collection.

## ATCC's Enhanced Authentication Initiative: Genome Portal

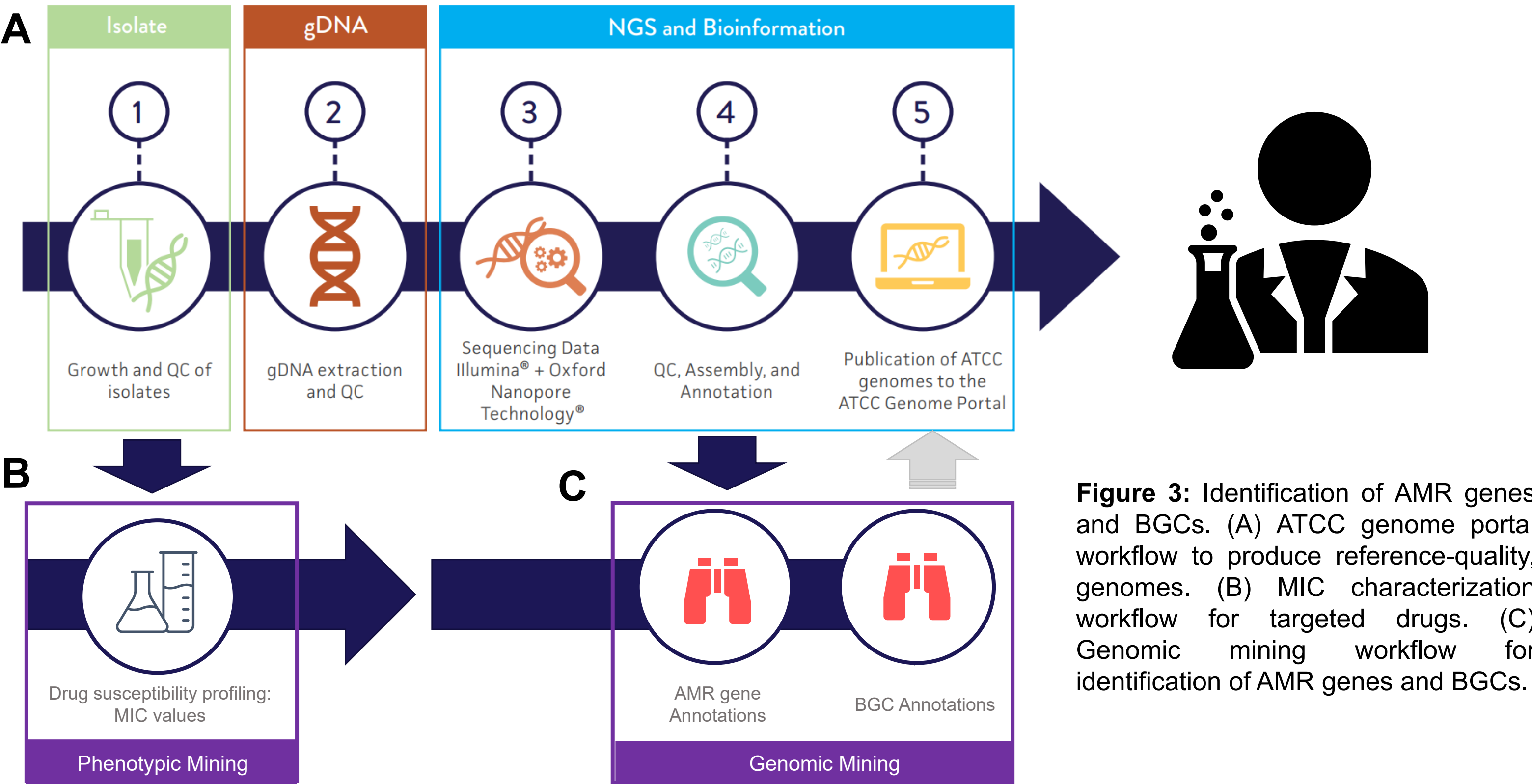


Figure 3: Identification of AMR genes and BGCs. (A) ATCC genome portal workflow to produce reference-quality genomes. (B) MIC characterization workflow for targeted drugs. (C) Genomic mining workflow for identification of AMR genes and BGCs.

## Source Metadata

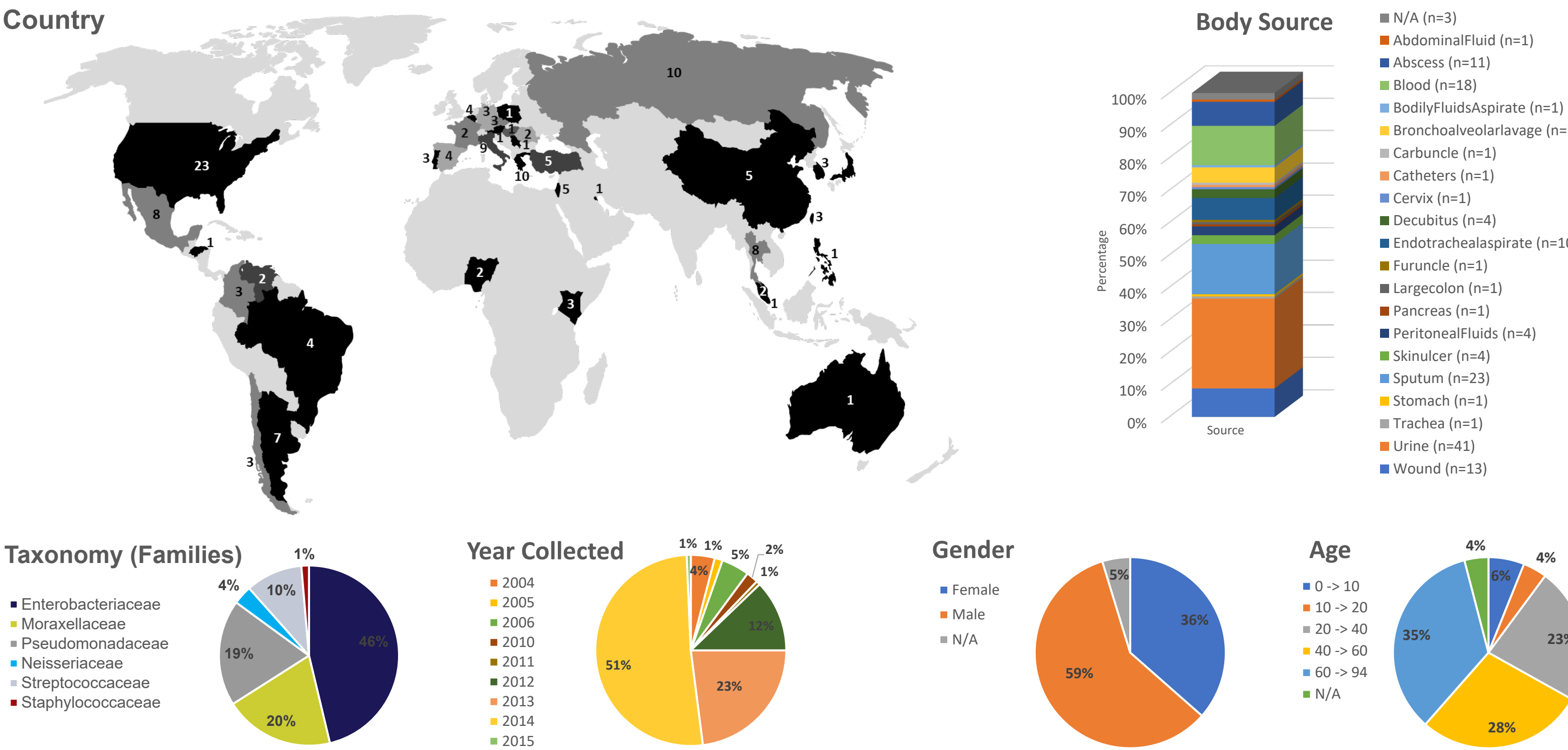


Figure 4: Metadata description for bacterial clinical isolates in the ATCC® Global Priority Superbugs collection.

## Mining AMR Genes

Table 1: Number of predicted AMR genes and beta-lactamases and penicillin-binding protein families (PBPs).

Gram status	Specie	# Strains	beta-lactamase	PBPs	Other AMR genes	Total AMR genes
Gram negative	<i>Escherichia coli</i>	18				
	<i>Klebsiella pneumoniae</i>	29				
	<i>Klebsiella oxytoca</i>	1				
	<i>Proteus mirabilis</i>	6				
	<i>Citrobacter freundii</i>	5	399	23	3766	4188
	<i>Citrobacter braakii</i>	1				
	<i>Enterobacter cloacae</i>	5				
	<i>Enterobacter asburiae</i>	1				
	<i>Serratia marcescens</i>	2				
	<i>Acinetobacter baumannii</i>	29	107	0	884	991
	<i>Pseudomonas aeruginosa</i>	28	120	0	1659	1779
	<i>Neisseria gonorrhoeae</i>	5	0	10	66	76
Gram positive	<i>Streptococcus pneumoniae</i>	15	0	31	126	157
	<i>Staphylococcus aureus</i>	2	2	0	42	44
TOTAL:		147	628	64	6543	7235

Table 2: Diversity of beta-lactamases classes (varieties).

Class A	Class B	Class C	Class D
GES (1;5;6;7;11;12;13;19;20;26)	VIM (1;2;4;5;6;23;28;31;32;42;43;44;45)	PDC (1;2;3;5;8;11;16;34;35;41;59;19a)	OXA (1;2;4;9;10;15;23;24;48;50;51;58;64;65;66;68;69;82;90;94;100;107;113;128;129;163;172;181;200;232;244;370;392;439;486;488;796;846;850)
CTX-M (2;3;9;14;15;27;65;74)	IMP (6;7;8;13;14;16;17;26;48;49;56)	CMY (2;4;6;10;13;16;34;48;81;149;152)	
SHV (1;5;11;12;27;28;64)	NDM (1;16)	ADC (6;10;11;25;30;39;58;73;78;77;159)	
OKP (A-17;B-8)	DIM-2	ACT (2;25;74)	
PER (1;3;13)		DHA (1;7)	
KPC (2;3;18)		MOX-2	
TEM (1;2;156)		ampH	
VEB (9;14;1b)		ampC	
CARB-16		LAP-2	
blaZ-PC1		STR-2	
PSE-1		OXY-1	
SCO-1			

## Beta-lactamase Diversity Between Bacterial Families

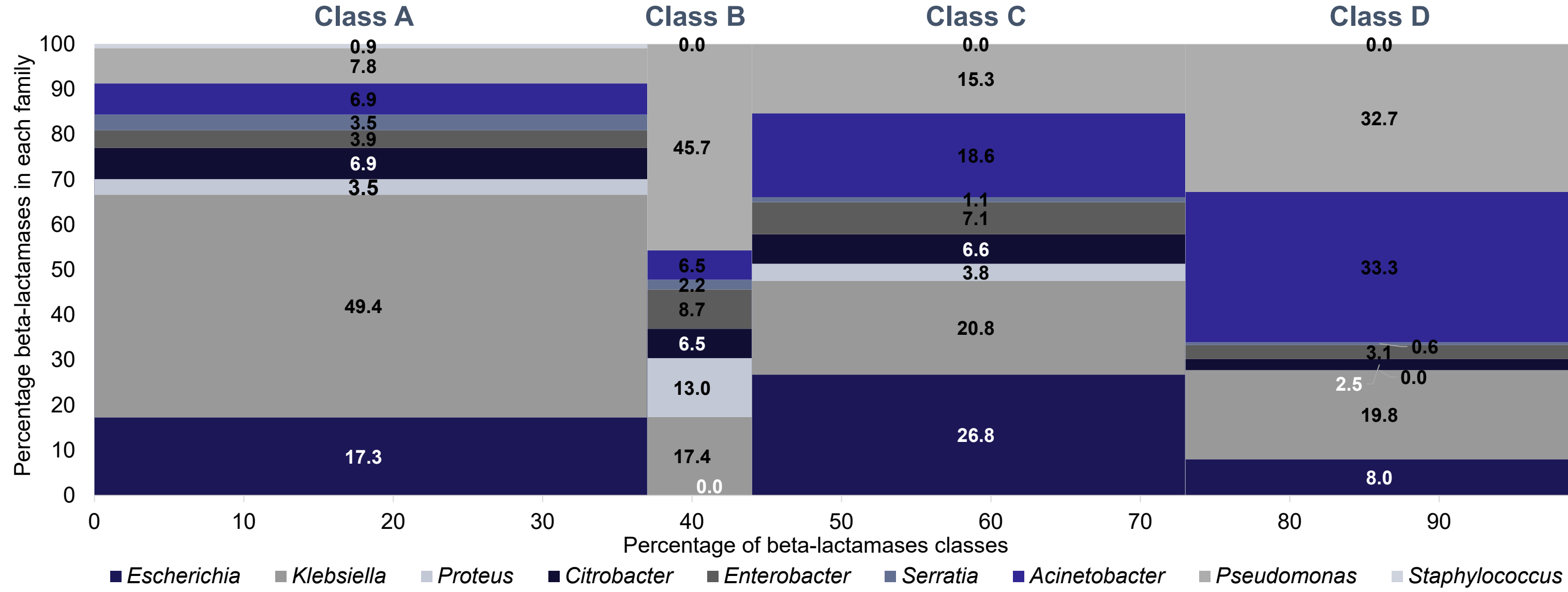


Figure 5: Diversity of beta-lactamases found in the nine bacterial families comprising the collection.

## Mining Biosynthetic Gene Clusters

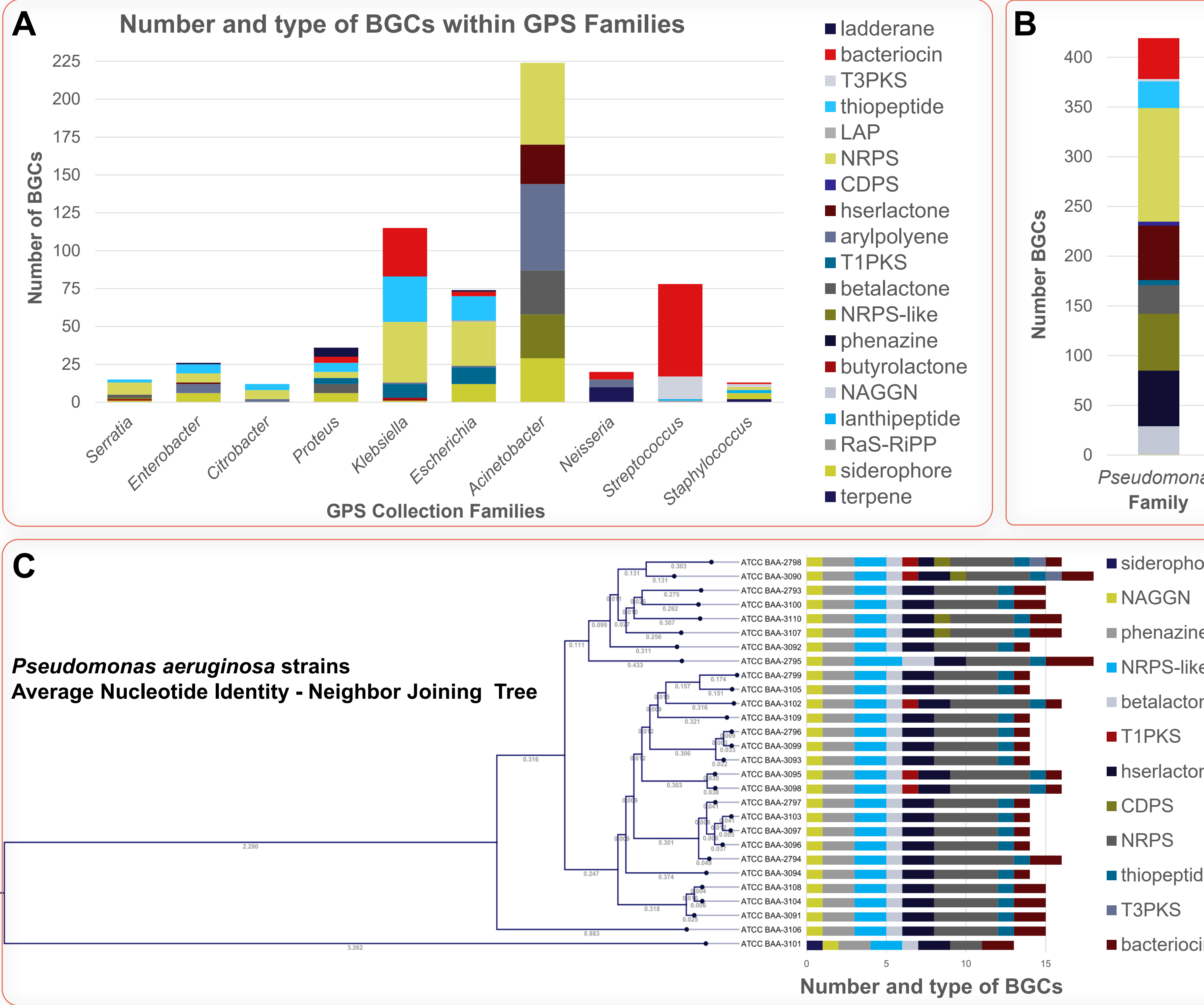


Figure 6: Identification and comparison of BGCs. (A, B) Comparison of the number and type of BGCs found in the genome of GPS strains grouped by taxonomic families. (C) Genomic similarity and diversity of BGCs within the *Pseudomonas* family.

## Conclusions

The ATCC Global Priority Superbugs collection reference-quality genomes and associated antibiotic susceptibility data will enable researchers to identify and compare strains containing relevant AMR markers and BGCs of interest while maintaining a high level of confidence in the authenticity of the data and its connection to the physical isolates from which they derive.

**References**  
<sup>1</sup>WHO. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Available Online: [https://www.who.int/medicines/publications/WHO-PPL-Short\\_Summary\\_25Feb-ET\\_NM\\_WHO.pdf](https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf).  
<sup>2</sup>CDC. Biggest Threats and Data. Available online: <https://www.cdc.gov/drugresistance/biggest-threats.html>