Antimicrobial Resistance: Arming Your Lab in the Fight Against Superbugs

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Microbiologist, ATCC

Credible Leads to Incredible™
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD.
- World’s largest, most diverse biological materials and information resource for microbes – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Overview

- Introduction
- Risk factors
- Progress and advancements
- Challenges and roadblocks
- How ATCC is contributing
- Further information
Antimicrobial-Resistant Infections

Antimicrobial-resistant infection: an infection that does not respond to appropriate antimicrobial treatment.

AMR infections are found in:

- Clinical Settings
- Communities
- Environment

Resistant infections result from exposure and susceptibility
Antimicrobial-Resistant Infections

The Threat of Antibiotic Resistance in the United States

Antibiotic resistance—when germs (bacteria, fungi) develop the ability to defeat the antibiotics designed to kill them—is one of the greatest global health challenges of modern time.

New National Estimate*

Each year, antibiotic-resistant bacteria and fungi cause at least an estimated:

- **2,868,700** infections
- **223,900** cases
- **35,900** deaths
- **12,800** deaths

Clostridioides difficile is related to antibiotic use and antibiotic resistance.

New Antibiotic Resistance Threats List

Updated urgent, serious, and concerning threats—totaling 18

- **5** urgent threats
- **2** new threats
- **NEW:** Watch List with **3** threats

*Source: cdc.gov*
Risk Factors

Introduction

Summary

Risk Factors

Progress

Challenges

Exposure

Healthcare-acquired infections (HAIs): infections that occur within 30 days of receiving health care

Community-acquired infections (CAIs): infections that cannot be traced to healthcare

Examples of How Antibiotic Resistance Spreads

Simply using antibiotics creates resistance. These drugs should only be used to treat infections.
cdc.gov

ATCC
Individuals may be at a higher risk of becoming infected with a pathogen due to:

- Age
- Illness
- Nutrition
- Immunosuppressive drugs or treatments
- Immune dysregulation

Human immunodeficiency virus causing chronic secondary immunosuppression

Cummins N, Badley A Cell Death Dis 1: e99, 2010
Detection and Surveillance

The importance of detection

**Purpose of detection:** diagnosis, treatment, surveillance
- Diagnosis: presence/absence of specific pathogens
- Treatment: drug susceptibility profile
- Surveillance: pathogenic strains and resistance variants

**Surveillance:** tracking resistant pathogens
- Tracking variants to uncover and predict trends in AMR pathogens
- Analyze what intervention methods are working
Detection and Surveillance

Current and emerging methods of testing

**Phenotypic analyses**
- Antimicrobial diffusion (discs, strips)
- Antimicrobial broth microdilution (MIC)

**Genotypic analyses**
- Nucleic acid amplification (NAA) tests: PCR, qPCR
- Hybridization
- Whole-genome sequencing

**Other technologies**
- Rapid screening: ESBL NDP detection
- Rapid identification: MALDI-TOF
Research and Development

Basic research
- Understanding the exact nature of the problem
- Understanding how resistance mechanisms work
- Understanding the efficacy of interventions and treatments
- Uncovering the potential for new detection methods, intervention techniques, or therapeutic targets

Preclinical research
- Exploring new interventions and treatments
- Screening potential antimicrobial compounds
- Reducing the need for animal models
- Predicting success of compounds and interventions in clinical trials

a All institutions
- Total 314 institutions
- Small and medium-sized enterprises (<1,000 employees) 81%
- Academic institutions 12%
- Large companies (>1,000 employees) 3%
- Non-profit institutions 3%
- Public-private partnership 1%

b Small and medium-sized enterprises
- USA: 136
- Canada: 7
- Netherlands: 5
- Denmark: 7
- Switzerland: 10
- UK: 27
- France: 12
- India: 8
- Other countries: 43

Key focal points for development pipelines

- New antimicrobial compounds
- Restoring or enhancing function
- Prevention of infection
- Supplementation of treatments

Rapid resistance development timeline
- Evolution of resistance is rapid
- Spread of resistance is rapid

Slow intervention development timeline
- Information, advancements, and treatments are not immediately available
Developing new antimicrobials is time intensive and expensive

- **Discovery**: basic research
- **Testing**: preclinical and clinical trials
- **Approval**: FDA (and other) requirements and application
- **Deployment**: knowledge, adoption

**Challenges**

**Long-term sustainability**

Developing new antimicrobials is time intensive and expensive

- **Discovery**: basic research
- **Testing**: preclinical and clinical trials
- **Approval**: FDA (and other) requirements and application
- **Deployment**: knowledge, adoption

Drug repurposing for antimicrobial discovery

Farha, Brown
Challenges

Cultural acceptance and practices

Modern day culture
- Expectations of speed, accuracy, and ease

Lack of education
- Ignorance of advancements and improvements

Adoption of new practices
- Habits are difficult to overcome for healthcare providers and the public

Cost
- Cost of drug discovery, development, testing, and approval are passed on to patients
Challenges

Quality and reproducibility

Inadequate reference materials lead to inadequate results

- Basic research data may be inaccurate
- Preclinical and clinical data may be insufficient

Cost-effective use of limited resources is only possible with high-quality materials and analysis
Meeting the Challenge

ATCC’s Incredible 2020 Initiative – Raising credibility in science

**Advance Authentication**: characterize biological materials via cutting-edge technologies

**Elevate Biological Models**: develop credible, cutting-edge models

**Evolve Education**: provide high-quality information backed by extensive experience

**Cultivate Collaboration**: accelerating innovation through knowledge sharing

**Support Global Health**: combat global health threats
Meeting the Challenge

ATCC biological materials

**Microorganisms**
- Global Priority Superbugs (GPS) collection
- Antimicrobial-resistant bacteria, fungi, and protozoa
- Bacteriophages
- Microbial panels

**Cell lines**
- Primary cells for drug toxicity screening studies
- Media and reagents to support cellular growth

**Nucleic acids**
- Quantitative genomic nucleic acids
- Nucleic acid extraction service

https://www.atcc.org/en/Products/Cells_and_Microorganisms.aspx
Meeting the Challenge

ATCC Global Priority Superbugs

Selection
- Recent, clinically relevant isolates
- Prevalent pathogenic species
- Multidrug-resistant and extensively drug-resistant strains

Analysis
- *De novo* genome sequencing and annotation
- Identification of antimicrobial resistance genes
- Evaluation of antimicrobial resistance and susceptibility

Collection
- Strains authenticated and categorized by ATCC
- Genotypically, phenotypically, and functionally characterized

Species | Strains
---|---
*Acinetobacter baumannii* | 30
*Klebsiella pneumoniae* | 28
*Pseudomonas aeruginosa* | 28
*Escherichia coli* | 21
*Streptococcus pneumoniae* | 15
*Proteus mirabilis* | 6
*Enterobacter sp.* | 6
*Citrobacter freundii* | 5
*Neisseria gonorrhoeae* | 5
*Staphylococcus aureus* | 3
*Serratia marcescens* | 2
*Citrobacter braakii* | 1
*Klebsiella oxytoca* | 1

www.atcc.org/GlobalPrioritySuperbugs
The genome of each strain is sequenced and assembled using our standardized workflow
- Antibiotic resistance and 16S rRNA genes are annotated and the species identity is confirmed
- Genome sequences and annotations are provided on the ATCC Genome Portal

https://genomes.atcc.org/
Collection Characterization

Assembly – Escherichia coli (ATCC® BAA-2779™)

Parameter | Value
---|---
Assembled size (bp) | 5,276,941
Contigs (circular) | 6 (3)
N\textsubscript{50} (bp) | 4,805,786
%GC | 50.6
Illumina coverage | 170x
ONT coverage | 141x

Parameter | Value
---|---
tRNAs | 88
rRNAs | 21
Protein-encoding | 5,036
AMR-associated | 74

AMR-Associated Genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Major Target Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC(3)-Ii, AAC(6')-Ib-cr, APH(3&quot;)-Ib, APH(6)-Id</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>catB3</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>CMY-2, CMY-47</td>
<td>Cephalosporins</td>
</tr>
<tr>
<td>KPC-2</td>
<td>Carbapenems, Cephalosporins, Monobactams</td>
</tr>
<tr>
<td>OXA-1</td>
<td>Cephalosporins</td>
</tr>
<tr>
<td>qnrB20</td>
<td>Quinolones</td>
</tr>
<tr>
<td>TEM-1</td>
<td>Cephalosporins, Monobactams</td>
</tr>
<tr>
<td>tetG</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>acrA, acrB, acrD, acre, acrF, baer, emrB, macA, macB, mdtB, mdtC, mdtF, msbA, tolC</td>
<td>Multiple (Components of efflux pump systems)</td>
</tr>
<tr>
<td>marA</td>
<td>Multiple (Transcriptional regulator that increases expression of existing resistance associated genes)</td>
</tr>
</tbody>
</table>
Collection Characterization

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration:

- Determines drug resistance and susceptibility profiles
- Reduces use of ineffective, high-dose, and broad-spectrum antibiotics
- Useful for drug discovery and compound screening
- Trends and patterns in resistance

<table>
<thead>
<tr>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Amikacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 µg/mL</td>
<td>8 µg/mL</td>
<td>16 µg/mL</td>
<td>32 µg/mL</td>
</tr>
</tbody>
</table>
Collection Characterization

MIC – Escherichia coli (ATCC®, BAA-2779™)

<table>
<thead>
<tr>
<th>Antimicrobial Class</th>
<th>Antimicrobial Compound</th>
<th>MIC Value</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycoside</td>
<td>Amikacin</td>
<td>≤16 µg/mL</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Penicillin + BLI</td>
<td>Amoxicillin-clavulanate</td>
<td>≥32/16 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Monobactam</td>
<td>Aztreonam</td>
<td>≥16 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Cefepime</td>
<td>≥16 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftazidime</td>
<td>≥16 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftriaxone</td>
<td>≥4 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Quinolone</td>
<td>Ciprofloxacin</td>
<td>≥4 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Doripenem</td>
<td>2 µg/mL</td>
<td>Intermediate</td>
</tr>
<tr>
<td>N/A</td>
<td>Fosfomycin</td>
<td>≤64 µg/mL</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Imipenem</td>
<td>2 µg/mL</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Quinolone</td>
<td>Levofoxacin</td>
<td>≥8 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Meropenem</td>
<td>≥4 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin + BLI</td>
<td>Piperacillin-tazobactam</td>
<td>≥128/4 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Sulfanomides</td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≥4/76 µg/mL</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline</td>
<td>≥16 µg/mL</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
Antimicrobial resistance is a global health threat

- Antimicrobial resistance threatens our ability to treat infection and perform essential medical procedures
- Advancements have been made, but progress must continue
- We must proactively protect ourselves and our world from resistant infections
- Everyone is a part of the solution
ATCC AMR Resources

Antimicrobial-Resistant Reference Materials
https://www.atcc.org/Products/Cells_and_Microorganisms/Multidrug-Resistant_Strains

Global Priority Superbugs
www.atcc.org/globalprioritysuperbugs

Quantitative and Synthetic Nucleic Acids
www.atcc.org/molecularstandards

ATCC Genome Portal
https://genomes.atcc.org/

ATCC Webinars
www.atcc.org/webinars
Antimicrobial Resistance: A Broad-spectrum Public Health Crisis

On demand

Amplify Your Viral Vaccine Production with CRISPR/Cas9-Engineered Host Cells

Liz Gillies, Ph.D.
March 12, 12:00 ET

ATCC Webinars
www.atcc.org/webinars

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