Carbapenem-resistant Enterobacteriaceae (CRE) – A Growing Superbug Population

Cara Wilder, Ph.D.
Technical Writer, ATCC
May 5, 2016
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World’s premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees
Outline

1. Background on antibiotic resistance
2. Emergence and spread of carbapenem-resistant Enterobacteriaceae (CRE)
3. CRE strains available from ATCC
   - KPC
   - NDM
   - OXA-48
Multidrug Resistance (MDR) is an Emerging Threat

- Antimicrobial resistance is present in all parts of the world
- The CDC estimates that every year in the United States:
  - 2 million people become infected with antibiotic-resistant bacteria
  - 23,000 people die as a direct result of these infections
  - $20 billion in excess direct healthcare costs
  - $35 billion cost associated with lost productivity

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA)

Photo credit: NIAID

Bad Bugs, No Drugs: No ESKAPE!

*Enterococcus faecium*
*Staphylococcus aureus*
*Klebsiella* sp.
*Acinetobacter baumannii*
*Pseudomonas aeruginosa*
*Enterobacter* sp.

These pathogens cause the majority of US hospital infections and can effectively “escape” the effects of antimicrobial therapeutics.

*Staphylococcus aureus*

Photo credit: F. DeLeo, NIAID

Multidrug Resistance is Widespread

Minimum Estimates of Morbidity and Mortality from Antibiotic-Resistant Infections

<table>
<thead>
<tr>
<th>Antibiotic-Resistant Microorganism</th>
<th>Estimated Annual Number of Cases</th>
<th>Estimated Annual Number of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenem-resistant Enterobacteriaceae (CRE)</td>
<td>9,300</td>
<td>610</td>
</tr>
<tr>
<td>Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL)</td>
<td>26,000</td>
<td>1,700</td>
</tr>
<tr>
<td>Vancomycin-resistant Enterococcus (VRE)</td>
<td>20,000</td>
<td>1,300</td>
</tr>
<tr>
<td>Methicillin-resistant Staphylococcus aureus (MRSA)</td>
<td>80,000</td>
<td>11,000</td>
</tr>
<tr>
<td>Multidrug-resistant Acinetobacter (≥3 drug classes)</td>
<td>7,300</td>
<td>500</td>
</tr>
<tr>
<td>Multidrug-resistant Pseudomonas aeruginosa (≥3 drug classes)</td>
<td>6,700</td>
<td>440</td>
</tr>
</tbody>
</table>
National Action Plan for Combating Antibiotic-Resistant Bacteria

The White House – March 2015

Goals:

- Slow the emergence of resistant bacteria and prevent the spread of resistant infections
- Strengthen national One Health surveillance efforts to combat resistance
- Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria
- Accelerate basic and applied research and development for new antibiotics, other therapeutics, and vaccines
- Improve international collaboration and capacities for antibiotic-resistance prevention, surveillance, control, and antibiotic research and development
Antibiotic Resistance – Evolution & Dissemination

- Inherent resistance
- Genetic mutation
- Horizontal gene transfer
  - Transformation
  - Transduction
  - Conjugation
Antibiotic Resistance – Evolution & Dissemination

- Reduced drug accumulation
- Antibiotic alteration
- Metabolic bypass
- Modification of target sites
- Antibiotic degradation

Diagram:
- Antibiotic efflux
- Antibiotic degradation
- Target modification
- Metabolic bypass
- Antibiotic alteration
Antibiotic Resistance – Evolution & Dissemination

How Antibiotic Resistance Happens

1. Lots of germs. A few are drug resistant.
2. Antibiotics kill bacteria causing the illness, as well as good bacteria protecting the body from infection.
3. The drug-resistant bacteria are now allowed to grow and take over.
4. Some bacteria give their drug-resistance to other bacteria, causing more problems.

Antibiotic Resistance – Evolution & Dissemination

Examples of How Antibiotic Resistance Spreads

- Animals get antibiotics and develop resistant bacteria in their guts.
- Drug-resistant bacteria can remain on meat from animals. When not handled or cooked properly, the bacteria can spread to humans.
- Fertilizer or water containing animal feces and drug-resistant bacteria is used on food crops.
- Drug-resistant bacteria in the animal feces can remain on crops and be eaten. These bacteria can remain in the human gut.
- George gets antibiotics and develops resistant bacteria in his gut.
- George stays at home and in the general community. Spreads resistant bacteria.
- George gets care at a hospital, nursing home or other inpatient care facility.
- Resistant germs spread directly to other patients or indirectly on unclean hands of healthcare providers.
- Patients go home.
- Resistant bacteria spread to other patients from surfaces within the healthcare facility.

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

CRE – Hazard Level Urgent

These are high-consequence antibiotic-resistant threats because of significant risks identified across several criteria. These threats may not be currently widespread but have the potential to become so and require urgent public health attention to identify infections and to limit transmission.

CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

9,000 DRUG-RESISTANT INFECTIONS PER YEAR
600 DEATHS

7,900 CARBAPENEM-RESISTANT KLEBSIELLA SPP.
1,400 CARBAPENEM-RESISTANT E. COLI

CRE HAVE BECOME RESISTANT TO ALL OR NEARLY ALL AVAILABLE ANTIBIOTICS

Carbapenem Resistance

- Carbapenem antibiotics
  - β-lactam antibiotic
  - Inhibit peptidoglycan synthesis

- Mechanisms of carbapenem-resistance
  - β-lactamase production combined with porin mutations
  - Carbapenemase production
CDC has defined CRE as Enterobacteriaceae that are:

- Resistant to any carbapenem antimicrobial (i.e. MIC of ≥4 µg/mL for doripenem, meropenem, or imipenem OR ≥2 µg/mL for ertapenem)
- Documented to produce carbapenemase
CRE Definitions and Recommendations

- Lower CLSI break points allow easier detection
- More information is available:
  - CLSI M100-S25 2015
  - CDC 2015 CRE Toolkit
  - AHRQ Carbapenem-Resistant *Enterobacteriaceae* (CRE) Control and Prevention Toolkit

<table>
<thead>
<tr>
<th>Agent</th>
<th>Previous Breakpoints (M100-S19) MIC (µg/mL)</th>
<th>Current Breakpoints (M100-S25) MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Doripenem</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤ 2</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤ 4</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤ 4</td>
<td>8</td>
</tr>
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CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Information Supplement (M100-S25), 2015.
Concerns About CRE as an Emerging Threat

- Multidrug-resistant
- High mortality rates for invasive infections
  - Up to 50% in some studies
- Rapid spread in healthcare settings
- Potential to become widespread in the community
- Carbapenemase genes can be transmitted from one Enterobacteriaceae to another
- Increase in CRE strains
  - 1.2% in 2001 to 4.2% in 2011

*Klebsiella pneumoniae*

Photo credit: NIAID

## CRE strains

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<tr>
<th>Carbapenemase</th>
<th>Ambler Class</th>
<th>Known Bacterial Carriers</th>
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<td>KPC</td>
<td>A</td>
<td><em>K. pneumoniae</em>, <em>E. coli</em>, and <em>Enterobacter</em> spp.</td>
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<tr>
<td>NDM</td>
<td>B</td>
<td><em>E. coli</em>, <em>K. pneumoniae</em>, and <em>E. cloacae</em></td>
</tr>
<tr>
<td>VIM</td>
<td>B</td>
<td><em>P. aeruginosa</em> and <em>P. putida</em></td>
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<td>IMP</td>
<td>B</td>
<td><em>Pseudomonas</em> and <em>Acinetobacter</em> spp.</td>
</tr>
<tr>
<td>CMY</td>
<td>C</td>
<td><em>E. aerogenes</em></td>
</tr>
<tr>
<td>OXA</td>
<td>D</td>
<td><em>K. pneumoniae</em>, <em>E. coli</em>, <em>Acinetobacter</em> spp.</td>
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![Chemical structures](image)
**CRE strains**

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**Klebsiella pneumoniae Carbapenemase (KPC)**

- KPC hydrolyzes all β-lactam agents
- Encoded by the plasmid-associated gene \( bla_{KPC} \)
- May be difficult to detect using higher (older) breakpoints
- The CDC has confirmed the presence of KPC throughout most of the United States
New Delhi Metallo-β-lactamase (NDM)

- Encoded by the plasmid-associated gene $bla_{NDM}$
- First identified in 2008
- It has since been detected worldwide

Oxacillinase-48 (OXA-48)

- Penicillinases that can hydrolyze penicillins and imipenem
- Encoded by the plasmid-associated gene $bla_{OXA-48}$
- First isolated in 2001 in Istanbul, Turkey, has since spread in Europe, the Middle East, India, North Africa, and the US

Acinetobacter sp.

Photo credit: CDC

CRE – Identification

- CHROM agar
- MacConkey agar plates supplemented with meropenem
- Modified Hodge Test
- Carba NP
- Antibiotic susceptibility disc testing
- PCR
- Check-Direct CPE assay (Check-Points)
- Next-generation sequencing
CRE – Prevention

Improve sanitation procedures and barrier precautions

- Hand hygiene
- Contact precautions
- Disposable equipment
- Environmental cleaning
- Chlorhexidine bathing
- Limit indwelling devices
CRE – Prevention

Implement a surveillance program and communicate outbreaks

- CRE screening of patients that meet pre-specified criteria
- Screen contacts of CRE patients
- Laboratory notification
- Inter-facility communication/identification of CRE patients at admission
CRE – Prevention

Antimicrobial stewardship
- Promote the appropriate use of antimicrobials
- Appoint a drug expert
- Monitor and report antibiotic prescriptions and resistance patterns
- Educate clinicians about resistance and optimal prescribing
ATCC – Aiding the Scientific Community

ATCC provides top-quality, authenticated reference strains and associated molecular materials

- Enhance diagnostics
- Analyze novel therapeutics
- Improve sterility protocols
# ATCC KPC Strains

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Species</th>
<th>Strain Designation</th>
<th>Presence of Select Virulence Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAA-1705™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>ART 2008133 [D-05, 1338]</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
</tr>
<tr>
<td>BAA-1898™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-1899™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-1900™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-1902™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-1903™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
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<td>BAA-1904™</td>
<td><em>Klebsiella pneumoniae</em></td>
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<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-1905™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-2078™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-2082™</td>
<td><em>Enterobacter hormaechei</em></td>
<td>-</td>
<td>$bla_{KPC+}$</td>
</tr>
<tr>
<td>BAA-2340™</td>
<td><em>Escherichia coli</em></td>
<td>1101362</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
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<tr>
<td>BAA-2341™</td>
<td><em>Enterobacter cloacae</em></td>
<td>1101152</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
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<tr>
<td>BAA-2342™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>1101160</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
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<tr>
<td>BAA-2343™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>1101172</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
</tr>
<tr>
<td>BAA-2344™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>1101200</td>
<td>$bla_{KPC+}/bla_{NDM-}$</td>
</tr>
</tbody>
</table>

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**KPC Strains Panel (ATCC® MP-24™)**
# ATCC NDM Strains

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Species</th>
<th>Strain Designation</th>
<th>Presence of Select Virulence Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAA-2146™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>1000527, 7561</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2452™</td>
<td><em>Escherichia coli</em></td>
<td>NDM-1</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2468™</td>
<td><em>Enterobacter cloacae</em></td>
<td>1000654</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2469™</td>
<td><em>Escherichia coli</em></td>
<td>1001728</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2470™</td>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae</td>
<td>1002565</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
</tr>
<tr>
<td>BAA-2471™</td>
<td><em>Escherichia coli</em></td>
<td>1100101</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2472™</td>
<td><em>Klebsiella pneumoniae</em> subsp. pneumoniae</td>
<td>1100975</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
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<tr>
<td>BAA-2473™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>1100770</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
</tr>
<tr>
<td>BAA-2566™</td>
<td><em>Escherichia coli</em></td>
<td>--</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
</tr>
<tr>
<td>BAA-2578™</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>--</td>
<td>$bla_{NDM}+/bla_{KPC}^-$</td>
</tr>
</tbody>
</table>

NDM-1 Strains Panel (ATCC® MP-18™)
## ATCC OXA-48 Strains

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Species</th>
<th>Strain Designation</th>
<th>Relevant Phenotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAA-2523™</td>
<td><em>Escherichia coli</em></td>
<td>bMx# 1109131</td>
<td>Produces OXA-48</td>
</tr>
<tr>
<td>BAA-2524™</td>
<td><em>Klebsiella pneumoniae subsp. pneumoniae</em></td>
<td>bMx# 1103199</td>
<td>Produces OXA-48</td>
</tr>
<tr>
<td>BAA-2525™</td>
<td><em>Providencia rettgeri</em></td>
<td>bMX# 1103204</td>
<td>Produces OXA-48</td>
</tr>
</tbody>
</table>

*Depositor statement

### Antibiotic Susceptibility

<table>
<thead>
<tr>
<th>Antibiotic*</th>
<th>BAA-2523™</th>
<th>BAA-2524™</th>
<th>BAA-2525™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>R</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Imipenem</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

R = Resistant, S = Susceptible, I = Intermediate susceptibility

*Antibiotic susceptibility determined using E-Test Strips; results may vary depending on the assay and susceptibility cut-offs used
Microbial Strain Authentication

ATCC utilizes both classical and modern techniques
- Phenotypic analysis
- Genotypic analysis
- Functional analysis

No single method of identification is sufficient
Phenotypic Testing

Culture Purity and Biochemical Properties

- Colony Morphology
- Cell Attributes
- Biochemical Analysis
Genotypic & Protein Testing

- Sequencing
- Toxinotyping
- MALDI-TOF

Targeted Gene Sequencing
Verification of Drug Resistance

**Modified Hodge Test**
- Recommended by CLSI and the CDC for the detection of carbapenemase production

**Endpoint PCR**
- Endpoint PCR used to detect the presence or absence of genes required for antibiotic production

**Antibiotic Profiling**
- VITEK® 2 used to analyze resistance to various antibiotic classes
  - Penicillins
  - Cephalosporins
  - Carbapenems
  - Quinolones
  - Aminoglycosides
Enhance Your Research with ATCC Strains

- Rapid detection methods
- Innovative therapeutic techniques
- Novel antibiotics
- Updated sterility protocols

ATCC – Leading the fight against superbugs!
Conclusion

- Multidrug-resistant strains are an emerging problem throughout the world
- ATCC acquires, authenticates, and distributes clinically relevant strains that are essential to the scientific community
  - Phenotypic, genotypic, and functional testing
- KPC, NDM, and OXA strains are now available at ATCC
  - Individual strains
  - Microbial panels
Thank you for joining today!

Register for more ATCC “Excellence in Research” webinars, or watch recorded webinars, at www.atcc.org/webinars.

Please email additional questions to: tech@atcc.org