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

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

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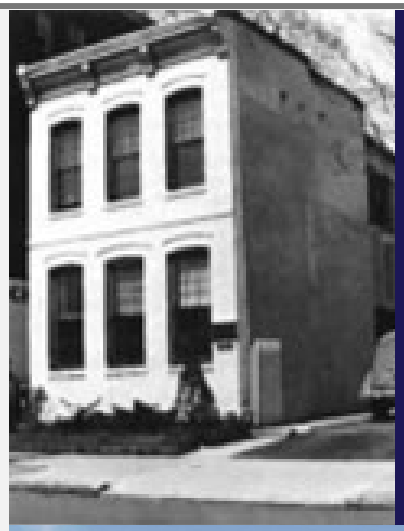
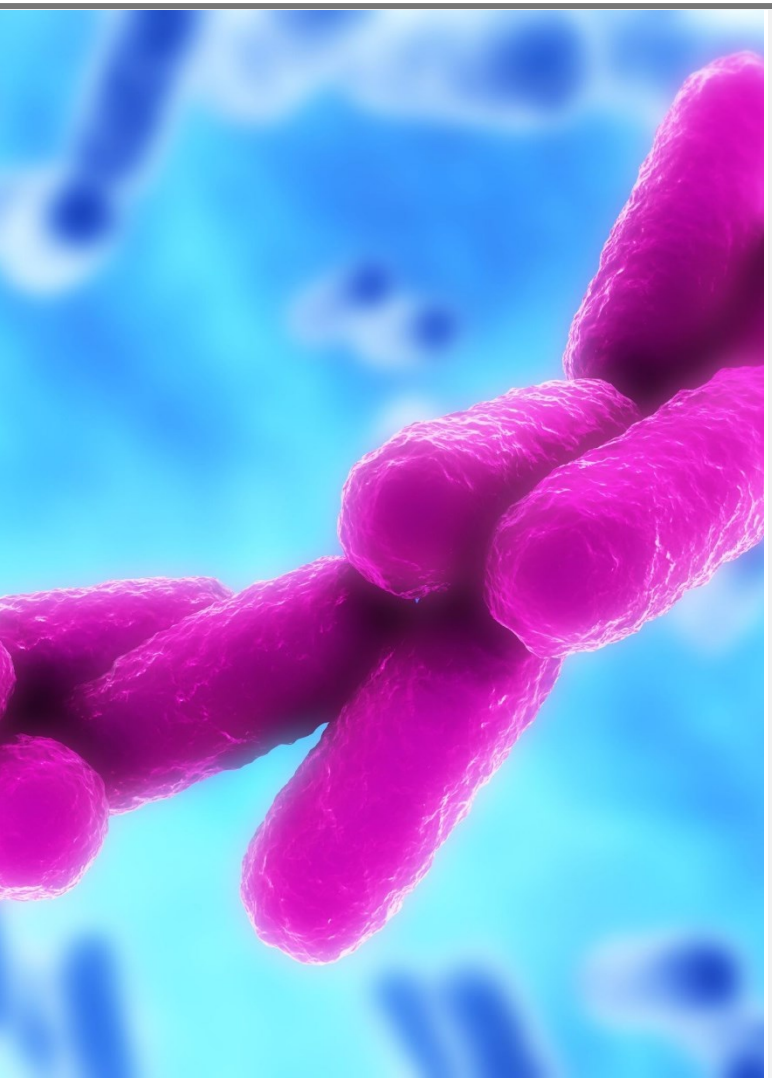
About ATCC

- 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



Established partner to global researchers and scientists

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

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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 *Staphylococcus aureus* (MRSA)

Photo credit: NIAID

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CDC. Antibiotic Resistance Threats in the United States, 2013.

Bad Bugs, No Drugs: No ESKAPE!

Staphylococcus aureus
Photo credit: F. DeLeo, NIAID

E*nterococcus faecium* — Gram +
S*taphylococcus aureus* — Gram +
K*lebsiella* sp. — Gram -
A*cinetobacter baumannii* — Gram -
P*seudomonas aeruginosa* — Gram -
E*nterobacter* sp. — Gram -

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

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Boucher HW, et al. Bad bugs, no drugs: no ESKAPE! An update from the IDSA. Clin Infect Dis 48(1): 1-12, 2009.

Multidrug Resistance is Widespread

Minimum Estimates of Morbidity and Mortality from Antibiotic-Resistant Infections

Antibiotic-Resistant Microorganism	Estimated Annual Number of Cases	Estimated Annual Number of Deaths
Carbapenem-resistant Enterobacteriaceae (CRE)	9,300	610
Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL)	26,000	1,700
Vancomycin-resistant *Enterococcus* (VRE)	20,000	1,300
Methicillin-resistant *Staphylococcus aureus* (MRSA)	80,000	11,000
Multidrug-resistant *Acinetobacter* (≥3 drug classes)	7,300	500
Multidrug-resistant *Pseudomonas aeruginosa* (≥3 drug classes)	6,700	440

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CDC. Antibiotic Resistance Threats in the United States, 2013.

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

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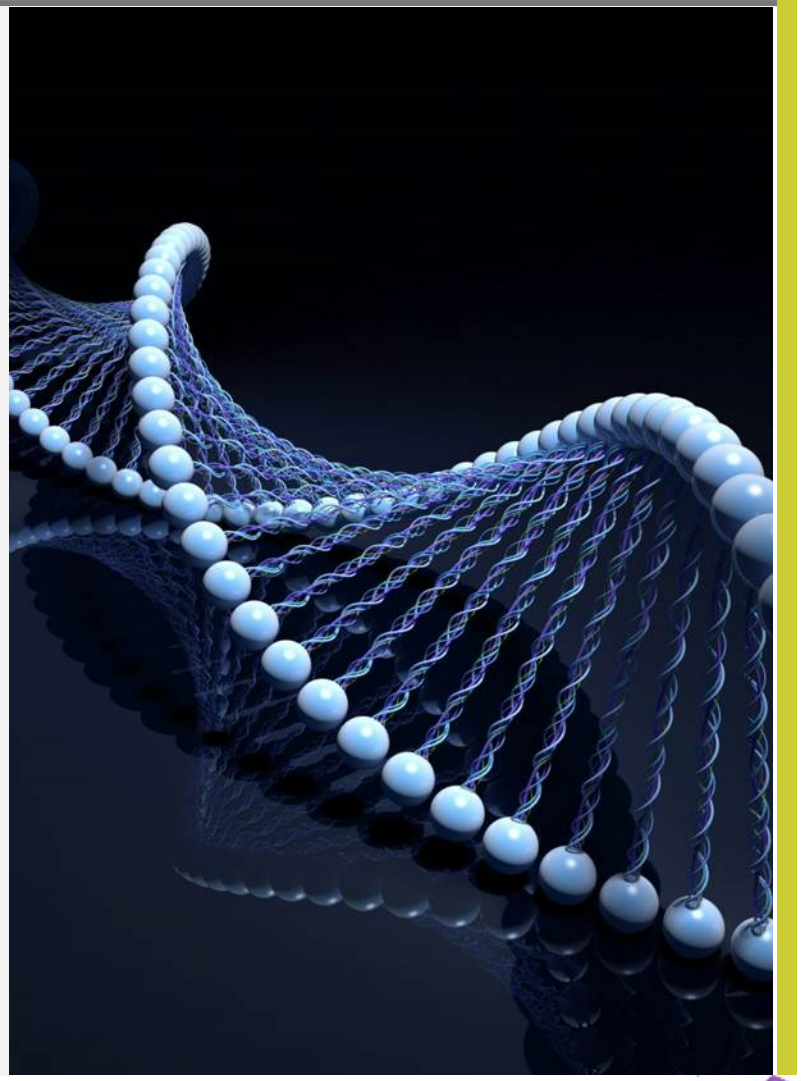
The White House. National Action Plan for Combating Antibiotic-Resistant Bacteria, March 2015.

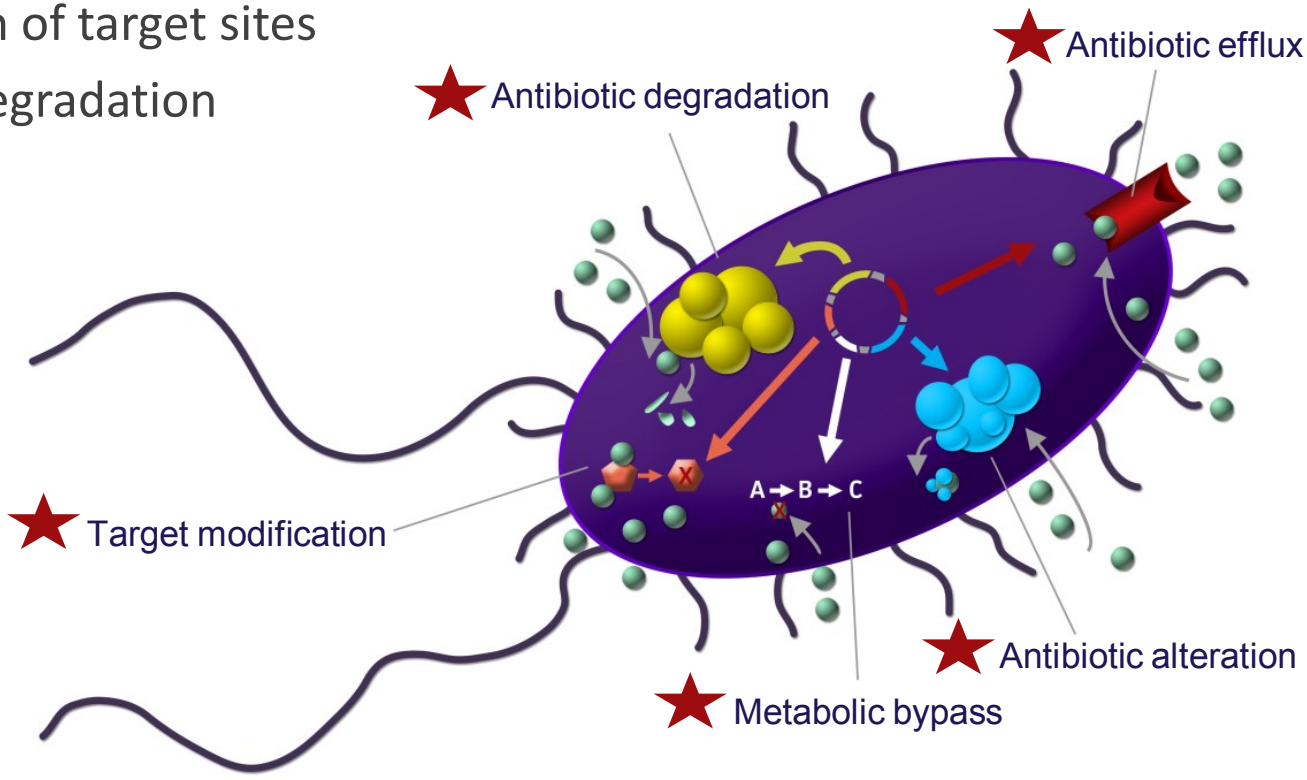
Antibiotic Resistance – Evolution & Dissemination

- 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



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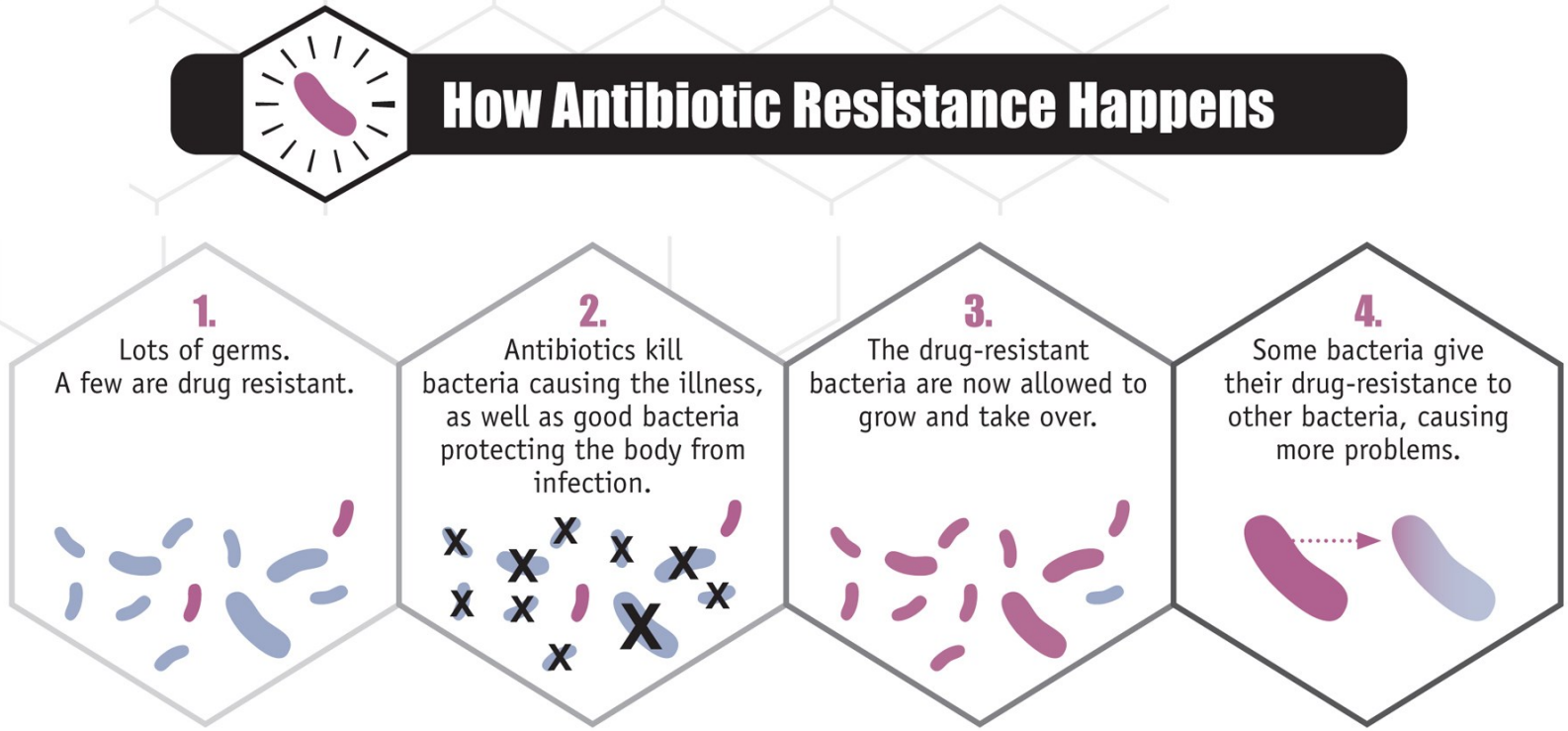
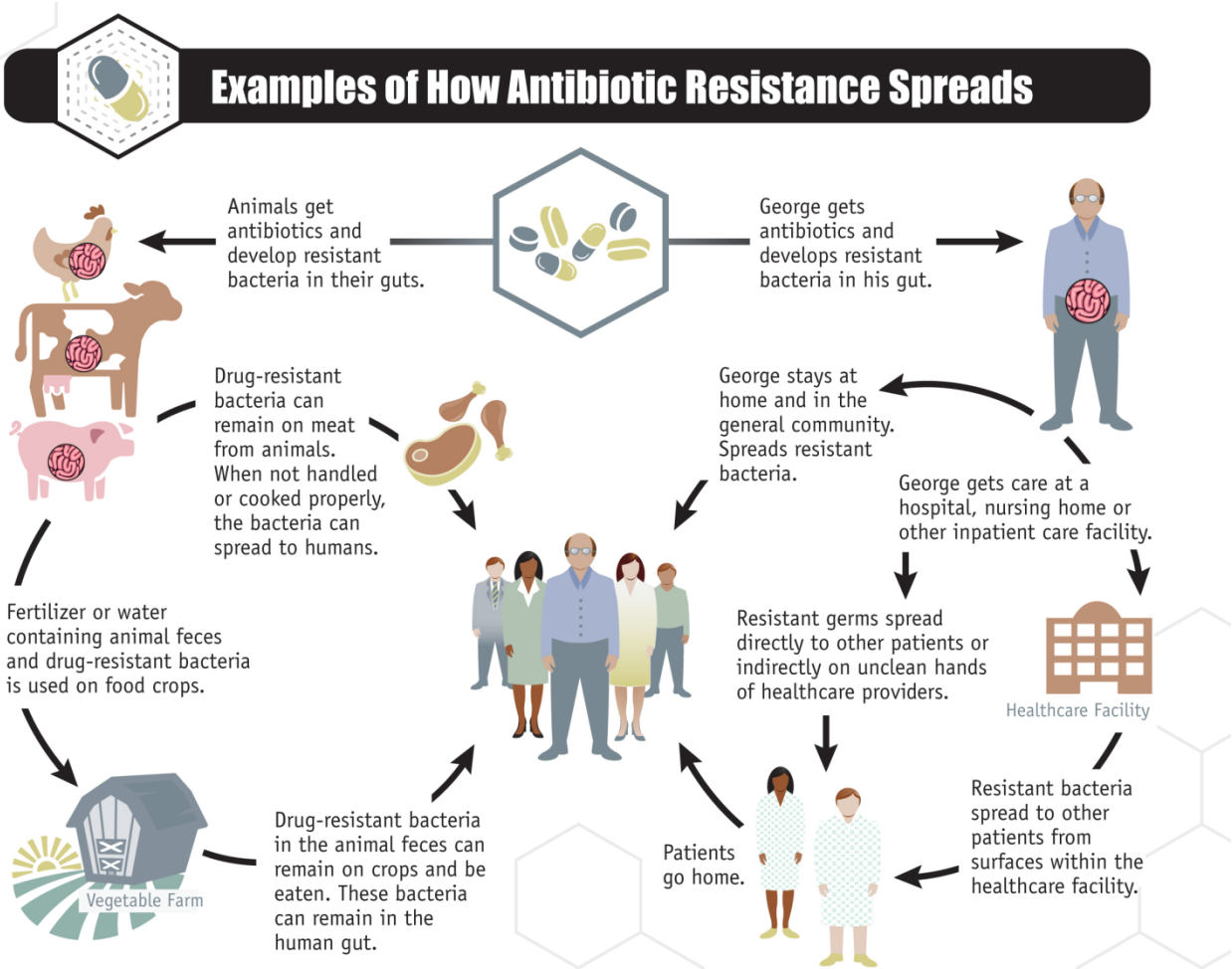
Antibiotic Resistance – Evolution & Dissemination



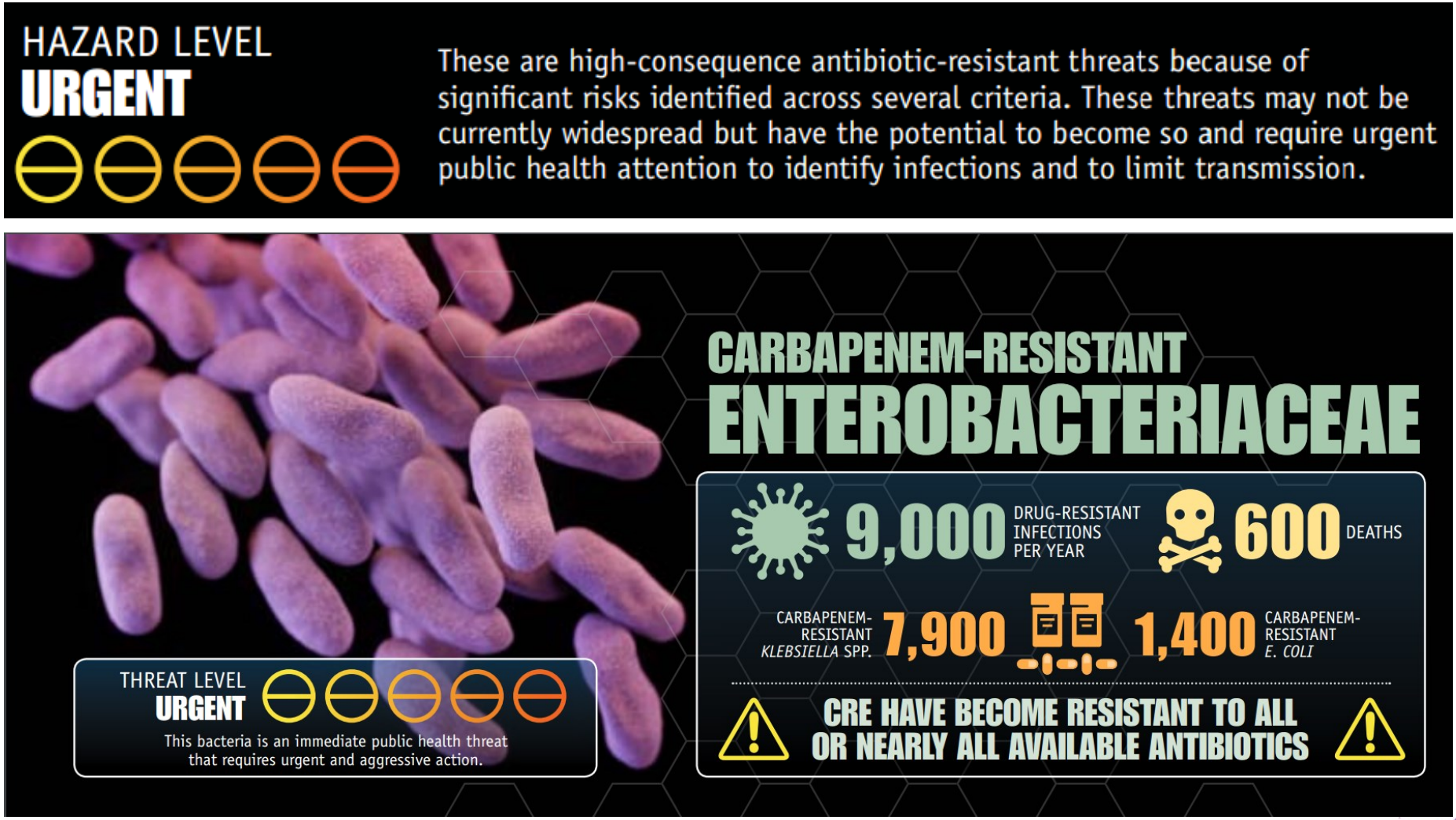
CDC. Antibiotic Resistance Threats in the United States, 2013.

Antibiotic Resistance – Evolution & Dissemination



CDC. Antibiotic Resistance Threats in the United States, 2013.

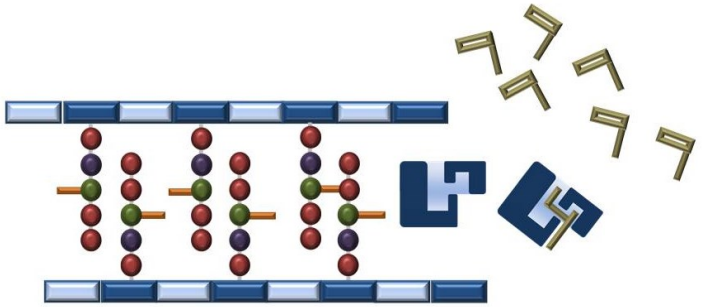
CRE – Hazard Level Urgent



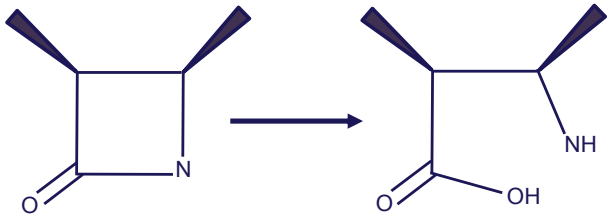
CDC. Antibiotic Resistance Threats in the United States, 2013.

Carbapenem Resistance

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



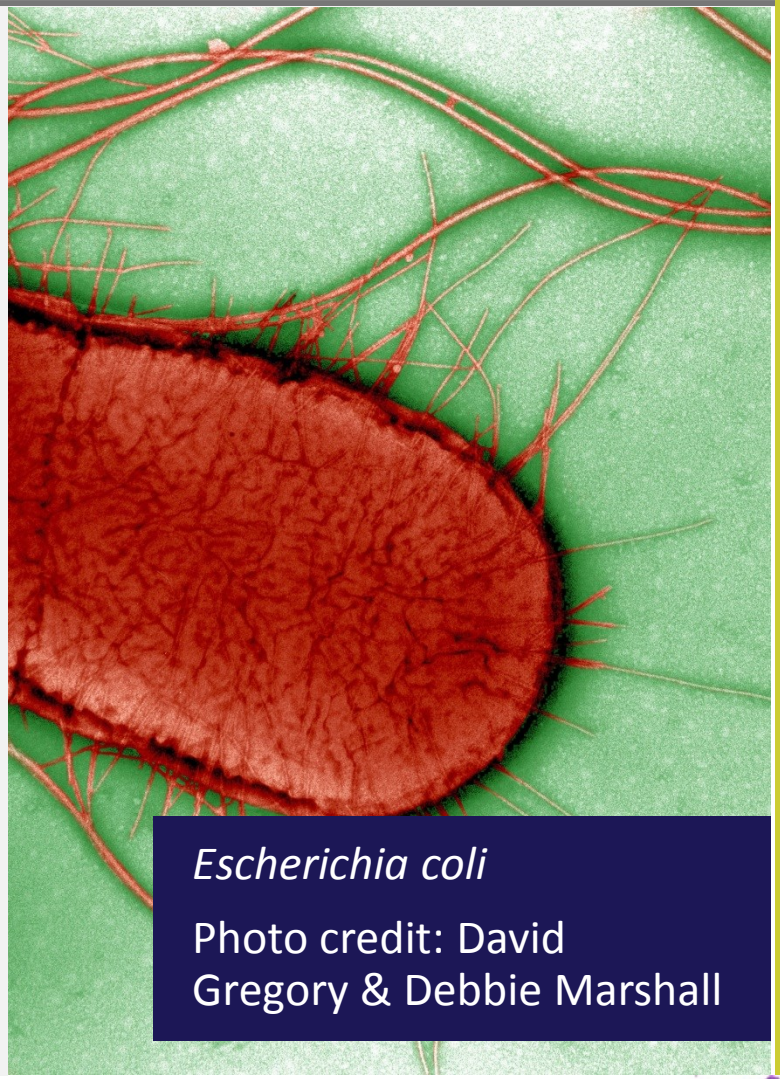
- Mechanisms of carbapenem-resistance
  - β-lactamase production combined with porin mutations
  - Carbapenemase production



CRE Definitions and Recommendations

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



Escherichia coli

Photo credit: David Gregory & Debbie Marshall

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CDC. Facility Guidance for Control of Carbapenem-resistant *Enterobacteriaceae* (CRE), November 2015 Update – CRE Toolkit.

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

Agent	Previous Breakpoints (M100-S19) MIC (µg/mL)			Current Breakpoints (M100-S25) MIC (µg/mL)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Doripenem	--	--	--	≤ 1	2	≥ 4
Ertapenem	≤ 2	4	≥ 8	≤ 0.5	1	≥ 2
Imipenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Meropenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4

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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

Klebsiella pneumoniae

Photo credit: NIAID

- 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

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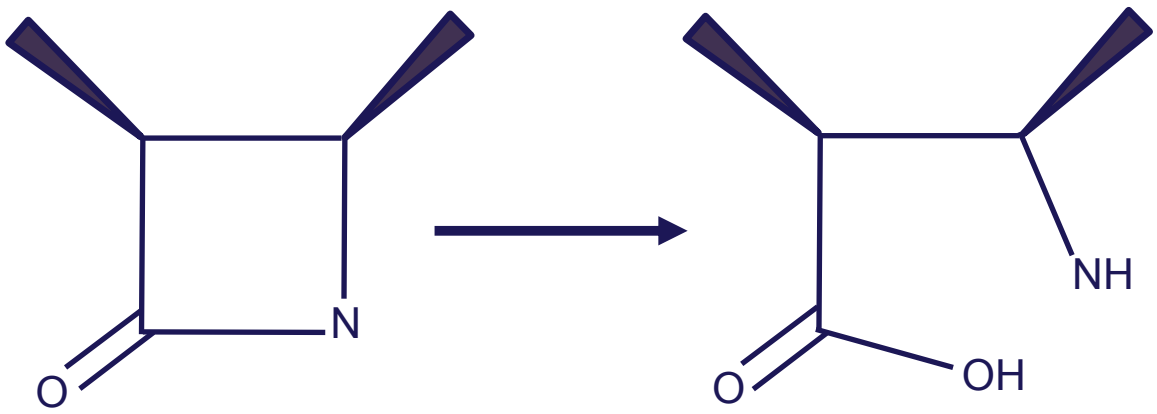
CDC. Vital Signs: Carbapenem-Resistant Enterobacteriaceae. Morbidity and Mortality Weekly Report (MMWR): 62(09): 165-170, March 8, 2013.

CRE strains

Carbapenemase	Ambler Class	Known Bacterial Carriers
KPC	A	*K. pneumoniae*, *E. coli*, and *Enterobacter* spp.
NDM	B	*E. coli*, *K. pneumoniae*, and *E. cloacae*
VIM	B	*P. aeruginosa* and *P. putida*
IMP	B	*Pseudomonas* and *Acinetobacter* spp.
CMY	C	*E. aerogenes*
OXA	D	*K. pneumoniae, E. coli, Acinetobacter* spp.



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CRE strains

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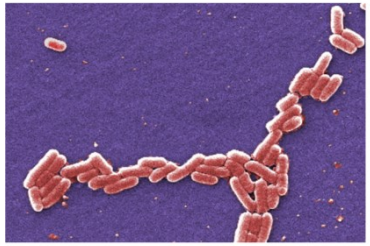
OXA	D	*K. pneumoniae, E. coli, Acinetobacter* spp.

New superbug found in two patients here

Both cases successfully contained as experts urge health officials to track deadly bacteria



Last-Ditch Resistance: More Countries, More Dire Results

POSTED THU, 01/7/2016

An E. coli bacterium. PHOTOGRAPH BY THE PUBLIC HEALTH IMAGE LIBRARY, CDC GOV.

The frantic international hunt triggered by the discovery of genetically mobile resistance to colistin, a last-resort antibiotic, is producing many more findings this evening. The resistance factor is showing up in more countries, but, much

'Phantom Menace' Superbug Has Alarming Ability

Antibiotic resistance travels between bacteria

By Rob Quinn, Newser Staff
Posted Dec 4, 2015 5:50 AM CST

13 comments

This illustration released by the CDC depicts a group of carbapenem-resistant Enterobacteriaceae bacteria. (AP Photo/Centers for Disease Control)

(NEWSER) – The latest strains of antibiotic-resistant bacteria to give researchers sleepless nights aren't the most common—or even the most antibiotic-resistant—but they have an ability that could make them a serious danger to public health. They contain enzymes known as "OXA-48-like carbapenemases" that can break down antibiotics and transfer that ability to normal bacteria in the body through mobile pieces of DNA, reports the *Washington Post*. and Live Science. The enzymes have been nicknamed the "phantom menace" by researchers because they can be tough to detect. In a report issued this week, the CDC says it identified 43 cases in the US involving the superbug between June 2010 and August of this year, mainly involving people who had

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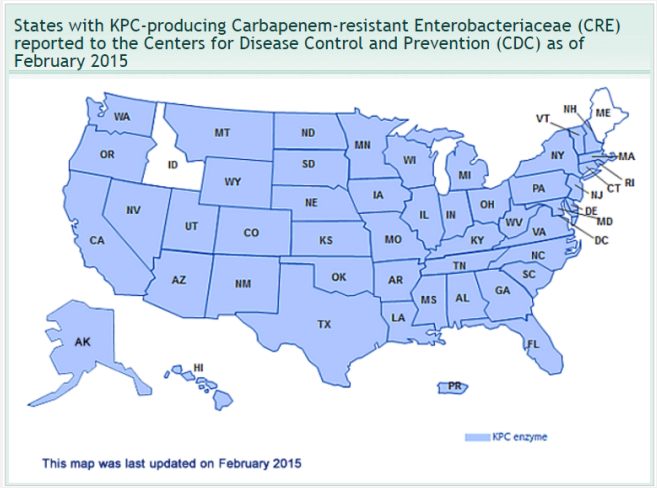
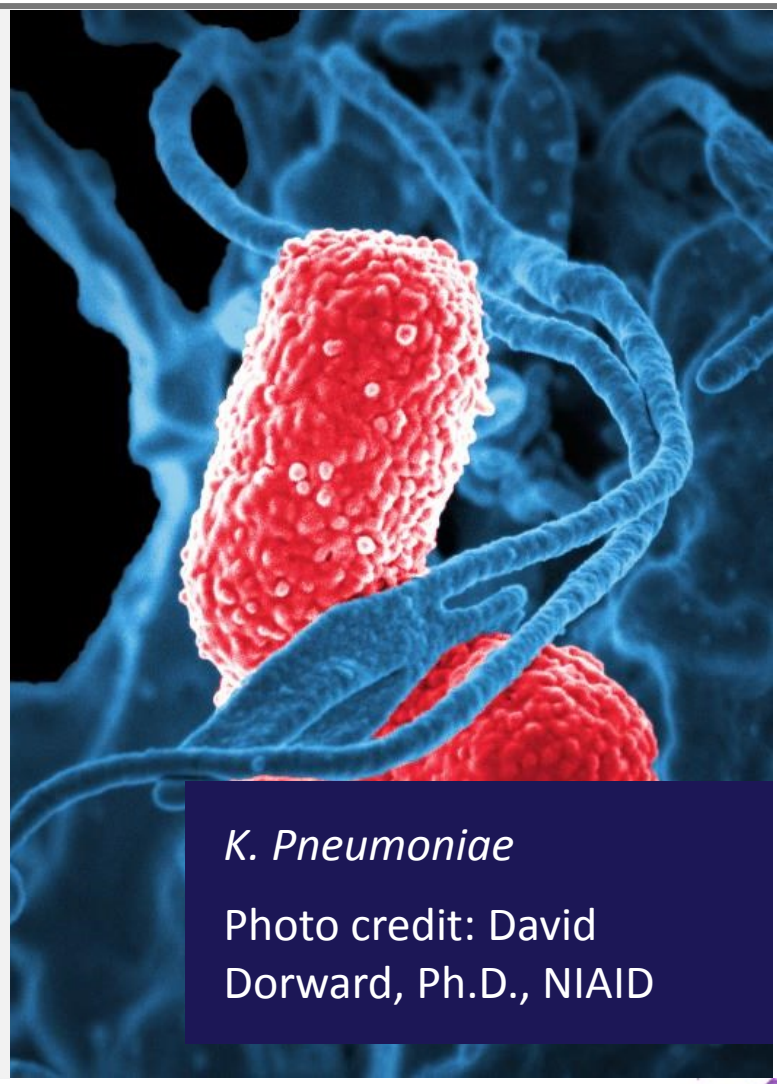
Klebsiella pneumoniae Carbapenemase (KPC)

- 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





K. Pneumoniae

Photo credit: David Dorward, Ph.D., NIAID

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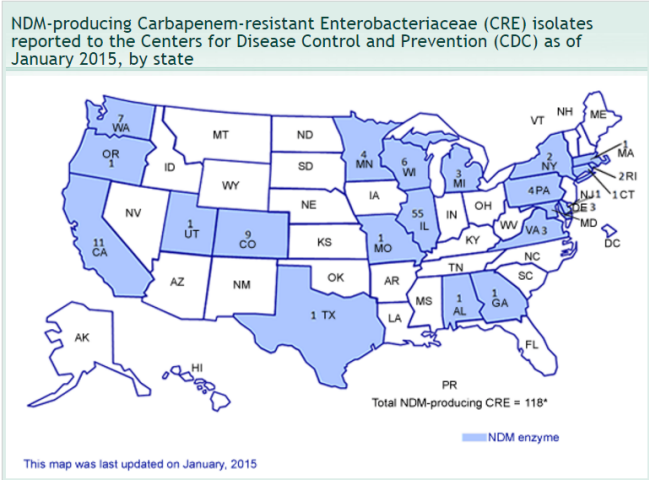
CDC. Healthcare-associated Infections (HAIs). Tracking CRE, 2015.

New Delhi Metallo-β-lactamase (NDM)

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



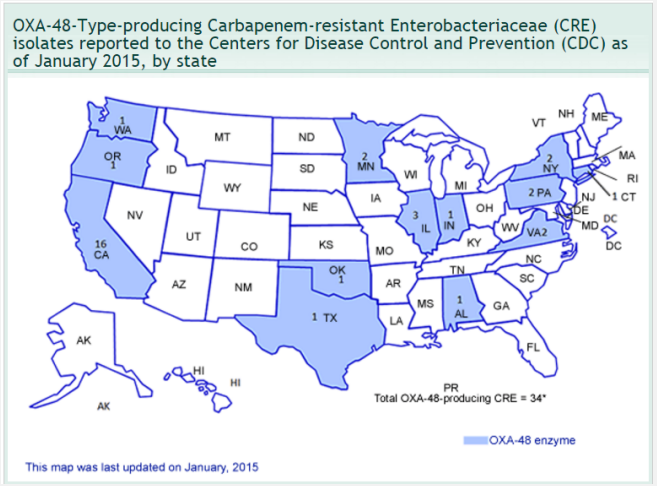
Enterobacteriaceae

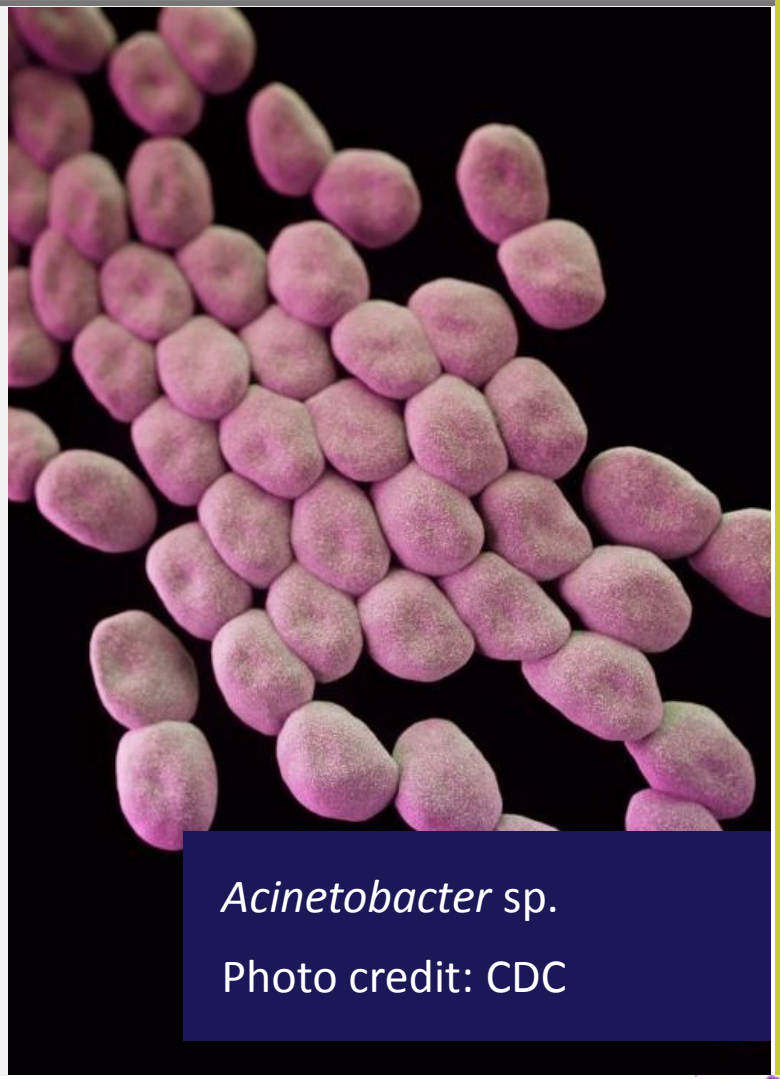
Photo credit: CDC

CDC. Healthcare-associated Infections (HAIs). Tracking CRE, 2015.

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

CDC. Healthcare-associated Infections (HAIs). Tracking CRE, 2015.

CRE – Identification

Modified Hodge Test

Photo credit: James Gathany

- 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

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CRE – Prevention

Photo credit:
Amanda Mills

Improve sanitation procedures and barrier precautions

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

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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

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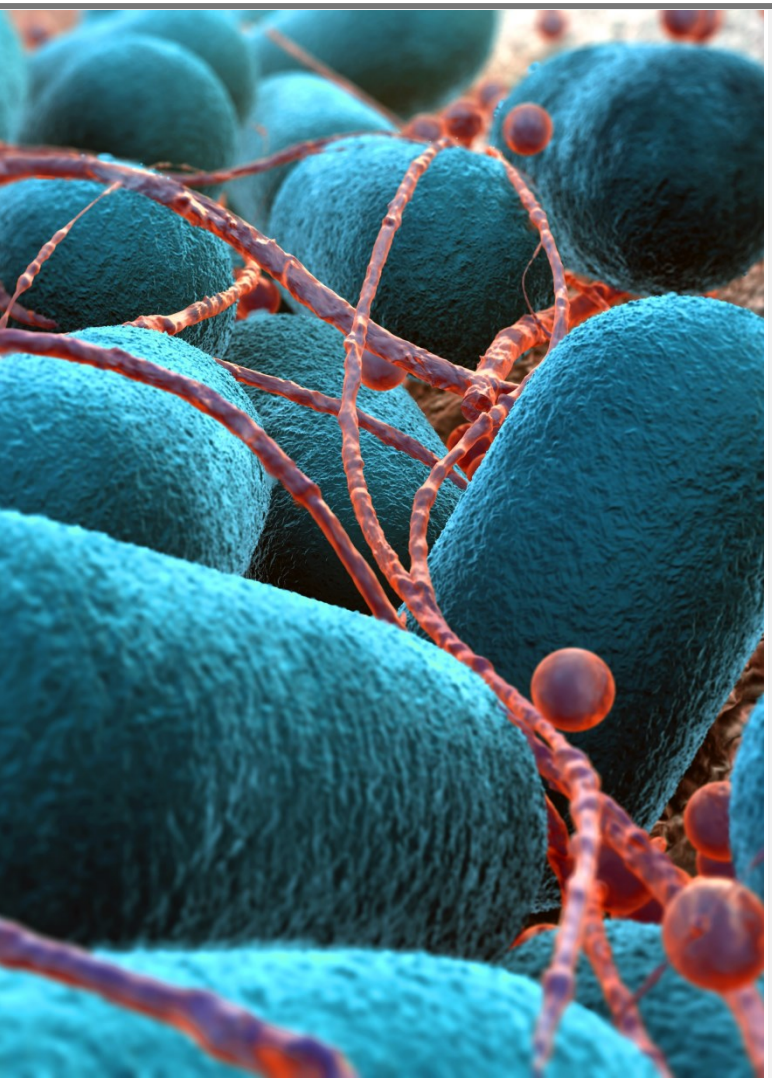
ATCC – Aiding the Scientific Community



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

- Enhance diagnostics
- Analyze novel therapeutics
- Improve sterility protocols

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ATCC KPC Strains

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

KPC Strains Panel
(ATCC® MP-24™)

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ATCC NDM Strains

ATCC® No.	Species	Strain Designation	Presence of Select Virulence Genes
BAA-2146™	*Klebsiella pneumoniae*	1000527, 7561	bla_{NDM}+/bla_{KPC}-
BAA-2452™	*Escherichia coli*	NDM-1	bla_{NDM}+/bla_{KPC}-
BAA-2468™	*Enterobacter cloacae*	1000654	bla_{NDM}+/bla_{KPC}-
BAA-2469™	*Escherichia coli*	1001728	bla_{NDM}+/bla_{KPC}-
BAA-2470™	*Klebsiella pneumoniae* subsp. *pneumoniae*	1002565	bla_{NDM}+/bla_{KPC}-
BAA-2471™	*Escherichia coli*	1100101	bla_{NDM}+/bla_{KPC}-
BAA-2472™	*Klebsiella pneumoniae* subsp. *pneumoniae*	1100975	bla_{NDM}+/bla_{KPC}-
BAA-2473™	*Klebsiella pnemoniae*	1100770	bla_{NDM}+/bla_{KPC}-
BAA-2566™	*Escherichia coli*	--	bla_{NDM}+/bla_{KPC}-
BAA-2578™	*Klebsiella pneumoniae*	--	bla_{NDM}+/bla_{KPC}-

NDM-1 Strains Panel
(ATCC® MP-18™)

ATCC

ATCC OXA-48 Strains

ATCC® No.	Species	Strain Designation	Relevant Phenotype*
BAA-2523™	*Escherichia coli*	bMx# 1109131	Produces OXA-48
BAA-2524™	*Klebsiella pneumoniae* subsp. *pneumoniae*	bMx# 1103199	Produces OXA-48
BAA-2525™	*Providencia rettgeri*	bMX# 1103204	Produces OXA-48

*Depositor statement

Antibiotic*	BAA-2523™	BAA-2524™	BAA-2525™
Meropenem	R	R	S
Ertapenem	R	R	I
Imipenem	R	R	R

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

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Microbial Strain Authentication

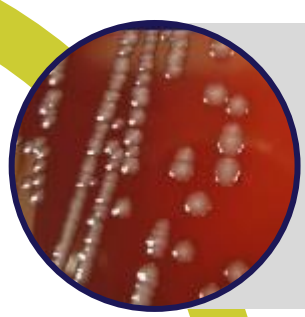
ATCC utilizes both classical and modern techniques

- Phenotypic analysis
- Genotypic analysis
- Functional analysis

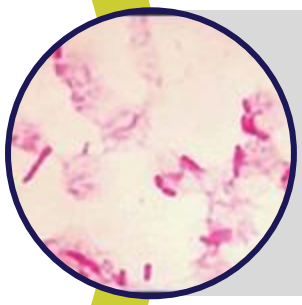
No single method of identification is sufficient

Phenotypic Testing

Culture Purity and Biochemical Properties



Colony Morphology



Cell Attributes

Biochemical Analysis

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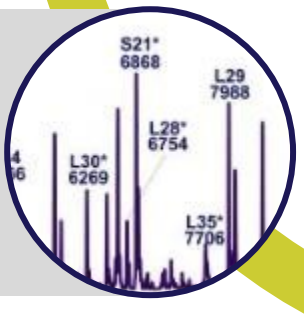
Genotypic & Protein Testing

Sequencing

Toxinotyping

MALDI-TOF



Targeted Gene Sequencing

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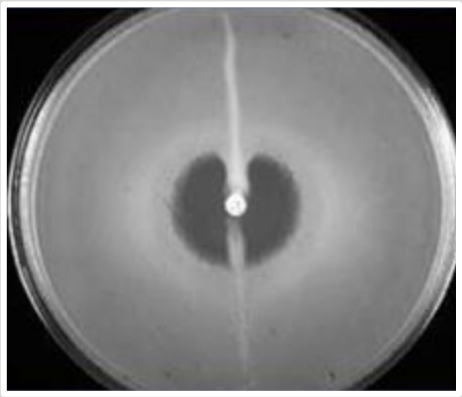
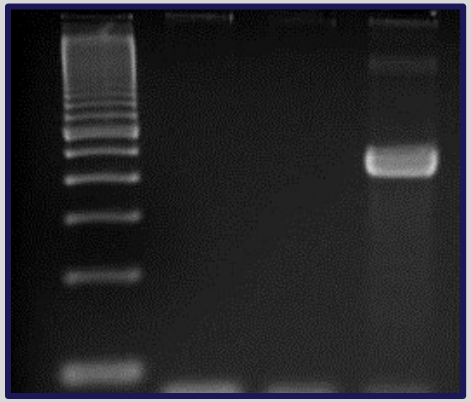
Verification of Drug Resistance

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

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VITEK® 2 is a registered trademark of bioMérieux

Enhance Your Research with ATCC Strains

ATCC – Leading the fight against superbugs!

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

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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

Identity
Purity
Authenticity
Homogeneity
Stability
Functionality

→

Comparability
Quality
Reproducibility
Standardization
Development
Verification

Enterobacteriaceae

Photo credit: CDC

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