Carbapenemases and More

Karen Bush, PhD
January 18, 2018
Texas Medical Center Antimicrobial Resistance & Stewardship Conference, Houston
Major $\beta$-Lactam Resistance Mechanisms

- **Gram-Positive Bacteria**
  - Penicillin-Binding Proteins (cell wall synthesis)
  - Penicillinases (staphylococci)

- **Gram-Negative Bacteria**
  - $\beta$-Lactamases
  - $\beta$-Lactamases + Porin Changes
  - $\beta$-Lactamases + Efflux
**β-Lactamases**

- Enzymes that can hydrolyze penicillins, or carbapenems, or cephalosporins, or monobactams, or any other β-lactam
- The primary resistance mechanism operative for β-lactam antibiotics in Gram-negative bacteria
Two Distinct $\beta$-Lactamase Families

- Serine
- Metallo (Zn)

Classified according to active site configurations and hydrolysis mechanism

The First Reported $\beta$-Lactamases

- Abraham and Chain, Oxford 1940
  - Described an enzyme that destroyed penicillin from a *Bacillus (Escherichia) coli*
  - Most likely the chromosomosomal AmpC enzyme
- Staphylococcal penicillinases
  - Rapidly emerged in the 1940s
  - 80% of *S. aureus* produced these by 1953
- *Bacillus cereus* (academic interest)
  - Coproduction of a serine and metallo-$\beta$-lactamase

Resistance Selection by New Penicillins and Cephalosporins (1960s and 1970s)

- Agents with activity against *S. aureus*
  - Selected for high level penicillinase production
  - Acquisition of a low affinity penicillin-binding protein (PBP2a / PBP2’) leading to MRSA

- Agents with activity against Gram-negative bacteria
  - Selected for β-lactamases capable of hydrolyzing the new agents
  - Hyperproduction of species-specific chromosomal cephalosporinases
  - Strains with porin mutations
FDA Approvals of New β-Lactams
1978 to 1989 Spawned New β-Lactamases

Data from “50 Years of ICAAC”, ASM Press, 2010;
http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm129875.htm
Predicted Resistance Mechanisms (Gram-Negatives)

- "3rd Generation Cephalosporins" served as selective pressure.
- Predictions:
  - Chromosomal AmpC cephalosporinase hyperproduction
  - Porin changes leading to decreased penetration
- Reality:
  - Selection of "Extended-Spectrum β-Lactamases" or ESBLs
  - Single amino acid variants of known enzymes that can hydrolyze the cephalosporins

Correlation Of Function With Structure

β-Lactamases / ESBLs

- Serine
  - Group 1
    - Class C: AmpC cephalosporinases
  - Group 2
    - Class A: TEM/SHV
    - Class B: OXA
  - Group 2d
    - Class D: CTX-M
- Metallo (Zn)
  - Group 3
    - Class B

Cephalosporin-Nonsusceptibility in European *Klebsiella pneumoniae* [EARSS/EARS-Net data 2005 - 2014]

*K. pneumoniae* 2005

*K. pneumoniae* 2016

New β-Lactams Introduced After 1990 (Post ESBLs)

27% (3/11) of the drugs introduced were Carbapenems
Advantages of “New” β-Lactams (Post 1990)

• Cefepime penetrated the *Enterobacteriaceae* better than other expanded-spectrum cephalosporins

• Carbapenems were stable to hydrolysis by ESBLs and AmpC cephalosporinases
  – Broad spectrum activity including non-fermentative bacteria (except for ertapenem)
  – Used empirically for serious infections in hospitals with ESBL epidemics

CARBAPENEM RESISTANCE DUE TO CARBAPENEMASES
Carbapenemases in Gram-Negative Pathogens

- Before 1990, carbapenemases were species-specific chromosomal enzymes
  - No major outbreaks due to these
- Transferable carbapenemases created a more serious problem
  - Plasmid-encoded Metallo-\(\beta\)-Lactamases (MBLs) first identified from Japanese isolates (IMP in 1990)
    - Hydrolyzed all \(\beta\)-lactams except monobactams
  - Plasmid-encoded serine \(\beta\)-lactamases in the United States
    - KPC isolate in 1996 first reported in 2001
    - Hydrolyzed all \(\beta\)-lactams

Carbapenem-Resistant *Enterobacteriaceae* (CRE) May be Due to Multiple Factors

- Carbapenem-Resistant *Enterobacteriaceae* (CRE)
  - Classified by the CDC as an “Urgent Threat”
  - WHO Critical Priority List
- Organisms are usually multidrug, or pan-resistant
- Most probably causes
  - High level production of AmpC cephalosporinases together with decreased carbapenem penetration across the outer membrane
  - Carbapenemase production
    - Leads to “Carbapenemase-Producing *Enterobacteriaceae* (CPE)
β-Lactamases / Carbapenemases

Serine
- Group 1: Class C, AmpC
- Group 2: Class A, TEM/SHV, CTX-M ESBLs
- Group 2d: Class D, OXA, KPC / SME

Metallo (Zn)
- Group 3: Class B, IMP / VIM / SPM, NDM

KPC-Producing *Enterobacteriaceae*

- Major outbreaks in New York/New Jersey began in 2004
  - From 2004 to 2006, Mt. Sinai Hospital (NYC) identified >550 isolates
  - In 2007, 33% of all *K. pneumoniae* produced KPCs
  - Usually resistant to all antibiotics except colistin and tigecycline
- Later KPC outbreaks: Israel, Greece, and Italy
- KPCs in *K. pneumoniae* often clonal
  - Associated with ST258 & located on Tn4401 transposon
- High mortality rates
  - In NY, 47% overall 14-day mortality for bacteremic patients
  - In Greece, 79% of KPC-infected hospital patients died (2008)

NDM-1
A Relatively Recent Transferable Zinc-containing Carbapenemase

- Initially identified from *K. pneumoniae* isolate from a Swedish patient recently hospitalized in India (2009)
- Soon detected throughout India, Pakistan and western Europe
- Many isolates are resistant to most antibiotics
- May be found in commensal bacteria (*E. coli*) in patients infected with other organisms
- Environmental samples collected from New Delhi in 2010 contained *bla*$_{\text{NDM-1}}$
  - 4% of drinking-water samples; 30% of seepage samples
  - Eleven “new” species including *Shigella boydii* and *Vibrio cholerae*
- Source may be either environmental or hospital-acquired

Global Prevalence of KPC and NDM Carbapenemases

Sporadic identification
Outbreaks
Endemic production

NDM Production

KPC Production

Drug susceptibility for both KPC- and NDM-producing CRE frequently only
- [Tigecycline]
- Polymyxins /colistin

Don’t Forget the OXA (Class D) Carbapenemases and Their Impact

• The OXA family of β-lactamases is the most diverse on a molecular level
  – Oxacillinases, ESBLs and carbapenemases
  – May have as little as 19% sequence identity
• Particularly important in non-fermentative bacteria
• As many as 50% of US *Acinetobacter baumannii* isolates are imipenem-resistant due to OXA enzymes
  – OXA-51-types = chromosomal carbapenemases in *A. baumannii*
  – OXA-23, OXA-40 and OXA-58 (transferable)
• *Acinetobacter baumannii* isolates in a Taiwanese study
  – All 577 carried a gene for OXA-51-like enzymes; 68% also positive for OXA-23-like genes

OXA-48

• Hydrolytic activity against broad spectrum carbapenems – *Enterobacteriaceae* only

• Not well inhibited by many diazabicyclooctane (DBO) or boronic acid β-lactamase inhibitors (avibactam and vaborbactam)

• Prevalence quite variable
  – In a European surveillance study published in 2011
    • 5 of 12,572 hospital isolates produced OXA-48 (0.0004%)
  – in a Spanish study from 2012-2014
    • 72% of the 121 carbapenemase-producing *E. coli* encoded OXA-48
  – OXA-48 identified in European pet food

CARBAPENEMASE IDENTITIES HIGHLY DEPENDENT ON COUNTRY OF ISOLATION
CPE in Canada: CPHLN Data

Courtesy of Mike Mulvey of the Public Health Agency of Canada

- KPC
- NDM
- OXA-48-like
- SME
- OXA-48/NDM
- Other

(n=2106)

Year

- 2008 (n=5)
- 2009 (n=4)
- 2010 (n=70)
- 2011 (n=142)
- 2012 (n=150)
- 2013 (n=208)
- 2014 (n=318)
- 2015 (n=430)
- 2016 (n=779)
Prevalence of Carbapenem Resistance

KPC carbapenemases reported in the United States

Carbapenem resistance in *Klebsiella* -- Europe (2016)

CDC Compilation of US Patients with Carbapenemase-Producing Infections (June 2017)

https://www.cdc.gov/hai/organisms/cre/trackingcre.html
Yearly evolution (2009–2012) of carbapenemase-producing Enterobacteriaceae in Spain and number of individual hospitals reporting cases to the national surveillance program of the Instituto de Salud Carlos III.

Incidence of carbapenemase-producing Enterobacteriaceae in Queensland, Australia, from July 2009 to March 2014.

Where are We Today?

- Multiple resistance mechanisms in CPE pathogens and subsequent medical consequences
- More and more β-lactamases
Increase in Number of Unique, Naturally-Occurring $\beta$-Lactamases

As a Result of the Variety of Plasmid-Encoded Enzymes, Multiple $\beta$-lactamases Exist per Organism

- Carbapenemases almost always are produced together with at least one other $\beta$-lactamase
- Eight $\beta$-lactamases in one *K. pneumoniae* isolate from USA
  - ESBL, AmpC and KPC
- Three Greek *K. pneumoniae* isolates and 10 Indianapolis CRE
  - KPC and VIM with TEM-1 (and CTX-M-15 in Greece)
- ICU patient from India
  - NDM-1, KPC-2, CTX-M-15, SHV-12, TEM-1, OXA-1
- *K. pneumoniae* isolate from Morocco
  - NDM-1, VIM-1, OXA-48
- *K. oxytoca* isolate from China
  - NDM-1, KPC-2, and IMP-4

Moland et al., AAC 51:800 (2007); Giakkoupi et al., AAC 53:4048 (2009); Pournaras et al. JAC 65:1604 (2010); Wang et al. AAC 61 (Sept. 2017); Zhang, Tulpule et al. (personal communication)
Co-Production of Carbapenemases with Other Plasmid-encoded $\beta$-Lactamases (Central Indiana)

<table>
<thead>
<tr>
<th>$\beta$-Lactamase</th>
<th><em>E. cloacae</em> (n=3)</th>
<th><em>E. coli</em> (n=5)</th>
<th><em>K. pneumoniae</em> (n=96)</th>
<th><em>S. marcescens</em> (n=6)</th>
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<tbody>
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<td>KPC-2</td>
<td>0</td>
<td>1</td>
<td>15</td>
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<tr>
<td>KPC-3</td>
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<td>4</td>
<td>80</td>
<td>3</td>
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<tr>
<td>KPC-3 + VIM-1</td>
<td>3</td>
<td>0</td>
<td>(4)*</td>
<td>0</td>
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<tr>
<td>KPC-3 + NDM-1</td>
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<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
<td>SME-1</td>
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<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>KPC + SHV (plasmid)</td>
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<td>4</td>
<td>70</td>
<td>2</td>
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<tr>
<td>KPC + TEM</td>
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<tr>
<td>KPC + CTX-M-15</td>
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<tr>
<td>KPC + TEM + SHV + CTX-M-15</td>
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<td>2</td>
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<tr>
<td>KPC + TEM + SHV + OXA</td>
<td>3</td>
<td>4</td>
<td>21</td>
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</tr>
</tbody>
</table>

*VIM-encoding plasmids lost on storage

Zhang et al., AAC 61:e00389-17 (2017)
Timeline for MBLs in Indianapolis

Stable VIM production
Transient VIM production

VIM-1 (n=2)

Jan 2011

VIM-1 (n=1)

Jan 2012

VIM-1-type (n=4)

Jan 2013

VIM-1-type (n=3)

Jan 2016

NDM-1 (n=3)

NDM-1 (n=2)

Transient (4/5)

Bush lab: Kashikar et al. ICAAC 2015; Tulpule ASM Microbe 2017
All isolates were from different health care centers in central Indiana, except for 85 and 86.
WHERE DOES THIS LEAVE US?
End Result is What We See Today

- Environmental bla genes in animals and water sources that are highly mutable and transferable among species
- β-lactam pressure in community and hospitals, with carbapenems selecting for multidrug resistance in the most fit pathogens
- Patients with few therapeutic options
Introduction of New Agents to Treat CPE

• “Game-changers”
  – Non-β-lactam β-lactamase inhibitors with potent inhibitory activity against KPCs and serine carbapenemases
  – Ceftazidime-avibactam (DBO)
    • Approved in 2015
    • Effective against many KPC-producing CPE infections
    • Some resistance selected
      – KPC mutations
      – PBP insertion sequences
  – Meropenem-vaborbactam (boronic acid analog)
    • Approved Aug. 2017
    • Too soon to see clinical resistance?
Summary

- Antibiotic pressure from β-lactams in general, and carbapenems specifically, has resulted in a proliferation of various carbapenemases.
- The specific carbapenemase population is highly dependent on geographical localities.
- Multidrug resistance, together with production of multiple β-lactamases per pathogen, will continue to increase.
- Although new agents have been introduced, or are in late-stage development, to treat infections caused by CPE, additional agents will be necessary to alleviate the morbidity and mortality incurred by these infections.