Molecular Epidemiology of Carbapenem-Resistant Enterobacteriaceae in the US and Beyond

Dr. Barry N. Kreiswirth
Director, PHRI TB Center
β-Lactam Antibiotics

- Narrow spectrum penicillins (4) – penicillin G
- Narrow spectrum penicillinase resistant penicillins (3) - methicillin
- Moderate spectrum penicillins (2) - amoxicillin
- Broad spectrum penicillins (1) - amoxicillin and clavulanic acid
- Extended spectrum penicillins (4) - piperacillin
- 1st generation cephalosporins (3) - cephalexin
- 2nd generation cephalosporins (2) - cefotetan
- 3rd generation cephalosporins (3) - ceftriaxone
- 4th generation cephalosporins (2) - cepafibrate
- Carbapenems (3) - meropenem
- Monobactams (1) - aztreonam
- Beta-lactamase inhibitors (3) - clavulanic acid

*bla*<sub>KPC</sub> encodes for a class A carbapenemase that hydrolyzes the entire class of β-lactam antibiotics.
There are more than 1,000 β-lactamases reported.
Carbapenem Resistance Determinants

- **Carbapenemases class A**
  
  _K. pneumoniae_ carbapenemase (KPC)
  
  GES, SME

- **Carbapenemases class B**
  
  Metallo-β-Lactamases: NDM-1, VIM, IMP

- **Carbapenemases class D**
  
  Oxa23, Oxa24, Oxa48, Oxa58

**CONFOUNDING PROBLEM:**

MOST OF THESE GENES ARE ON MOBILE ELEMENTS

WE ARE EXPERIENCING A PLASMID EPIDEMIC
Molecular Epidemiology
CRE – EPIDEMIOLOGY

- Northeast US the epicenter of the $bla_{KPC}$ ST258 strains which have spread to Israel, Greece, Italy and South America.

- India and Pakistan the epicenter of the $bla_{NDM-1}$ epidemic and strains are spreading to UK and Europe.

- Strains harboring $bla_{IMP}$, $bla_{VIM}$ and $bla_{OXA-48}$ are commonly reported in Europe.
KPC- EPIDEMIOLOGY

Spread of *K. pneumoniae* harboring $bla_{KPC}$

MSLT reveals that ST258 is the major predominant clone

Figure: Epidemiological features of producers of *Klebsiella pneumoniae* carbapenemases by country of origin

Other carbapenemase types include VIM, OXA-48, or NDM. KPC= *Klebsiella pneumoniae* carbapenemase.

Munoz-Price et al. Lancet Infectious Disease, 2013
NDM Producers

OXA-48 Producers

Unknown distribution of OXA-48 producers
Sporadic spread of OXA-48 producers
Outbreaks caused by OXA-48 producers
Endemicity of OXA-48 producers

Carbapenem Resistant *Klebsiella pneumoniae*

- Since 2005, NYC/NJ hospitals have been the epicenter for the emergence and spread of KPCs
- Have started to spread across the US
- Resistance is linked to $bla_{KPC}$ gene
- $bla_{KPC}$ gene on Tn4401 harbored on large plasmids
- Plasmids are transmissible in Enterobacterceae
- Strains are approaching pan-resistance
- ~50% mortality among transplant patients
- ST258 is the global KPC clone
Is ST258 a single genetic clone that has disseminated worldwide?

PNAS. 2014;111;4983-93
Comparative Genomic Analysis

Genome = 5,266,518 bp
Prophages = 8
IS-elements = 22
Conjugative elements = 2
Plasmids = 5
12 – 147 kb

Genome = 5,293,301 bp
Prophages = 7
IS-elements = 19
Conjugative elements = 2
Plasmids = 3
12 – 86 kb
Comparative Analysis on
85 K. pneumoniae Genomes
85 – Selected *K. pneumoniae*

- **KPC-3**, 52, 61%
- **KPC-2**, 28, 33%
- **KPC-1**, 5, 6%

- **ST258**, 76, 89%
- **ST379**, 4, 5%
- **ST512**, 4, 5%
- **ST418**, 1, 1%
Comparative whole genome analysis on 85 strains showed that ST258 can be distinguished in two subclones: Clades I and II.

- ~350 SNP differences among the strains ranging from 116 to 784 SNPs.

- Genetic divergence between the two clades is found in a ~215 kb region of divergence which contains the genes encoding the capsular polysaccharide biosynthesis.
The region of differences, including the capsular and lipid polysaccharide pathway genes, are the result of chromosomal replacement from other *K. pneumoniae* STs.
Chromosomal recombination between different STs drives antigenetic variation and evading the host.
Distribution of eight sequenced plasmids in 85 genomes

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Diagram showing the distribution of plasmids across 85 genomes.
3 major KPC harboring plasmids are identified in 75% of the circulating strains

- Tn4401 has been identified with diverse incompatibility groups
- Tn4401 found K. pneumoniae chromosome
Molecular Survey of the Dissemination of Two $bla_{KPC}$-Harboring IncFIA Plasmids in New Jersey and New York Hospitals

Liang Chen, a Kalyan D. Chavda, a Roberto G. Melano, b, c, d Tao Hong, e Albert D. Rojtman, f Michael R. Jacobs, g Robert A. Bonomo, h, i Barry N. Kreiswirth a
The US Epidemic

- There are at least two major clades of ST258 *K. pneumoniae* harboring $bla_{KPC}$ resistance and clade I is associated with $bla_{KPC-2}$ and clade II with $bla_{KPC-3}$

- The diversity between the two clades is in the HVR and specifically the ~20 kb capsular polysaccharide region which evolves from recombination with other ST lineages

- CPS switching is a common recombination mechanism in *K. pneumoniae*

- $bla_{KPC}$ is mobilized on Tn4401 which is found on numerous conjugative plasmids and three major sub-clones of ST258 harbor: IncI2, IncFIA and IncFIIK2
And beyond ...?
The molecular epidemiology of CRE in Jiangsu, China – A very different picture
46 CRE identified
No ST258 KPC strains, ST11 predominant background
10 different sequence types among 19 *K. pneumoniae*
Two *E. cloacae* carry NDM-1 and KPC-3
Several frameshift mutations were identified in porin genes with and with carbapenemases
Plasmid Microevolution

*E. cloacae* isolated from two patients separated by 11 days in the same ICU – harboring both KPC and NDM
And finally ......
Identified *mcr-1* gene on a plasmid from an *E. coli* isolated from a pig encoding for resistance to colistin
Retrospective analysis of colistin resistant strains in Jiangsu, China identified the $mcr-1$ gene in four Enterobacteriaceae identified in 2014-2015.

Two *K. pneumoniae* strains with conjugative plasmids harboring $mcr-1$ and $bla_{NDM}$
In Summary

- Carbapenem resistant Enterobacteriaceae is a global epidemic caused by the primary spread of multidrug resistant clones and the repeated acquisition of plasmids harboring genes for KPC, NDM, IMP, VIM and OXA resistance.

- We have shown that the US and global spread of ST258 *K. pneumoniae* harboring \( \text{bla}_{\text{KPC}} \) resistance is caused by two primary clones that are currently evolving different capsular polysaccharide types through chromosomal recombination and acquiring and spreading different incompatibility plasmids.

- Controlling this epidemic will require the combination of new antibiotics, carriage surveillance of high risk populations and novel strategies to enhance the innate host response.

- HOW DO WE CONTROL A PLASMID EPIDEMIC???