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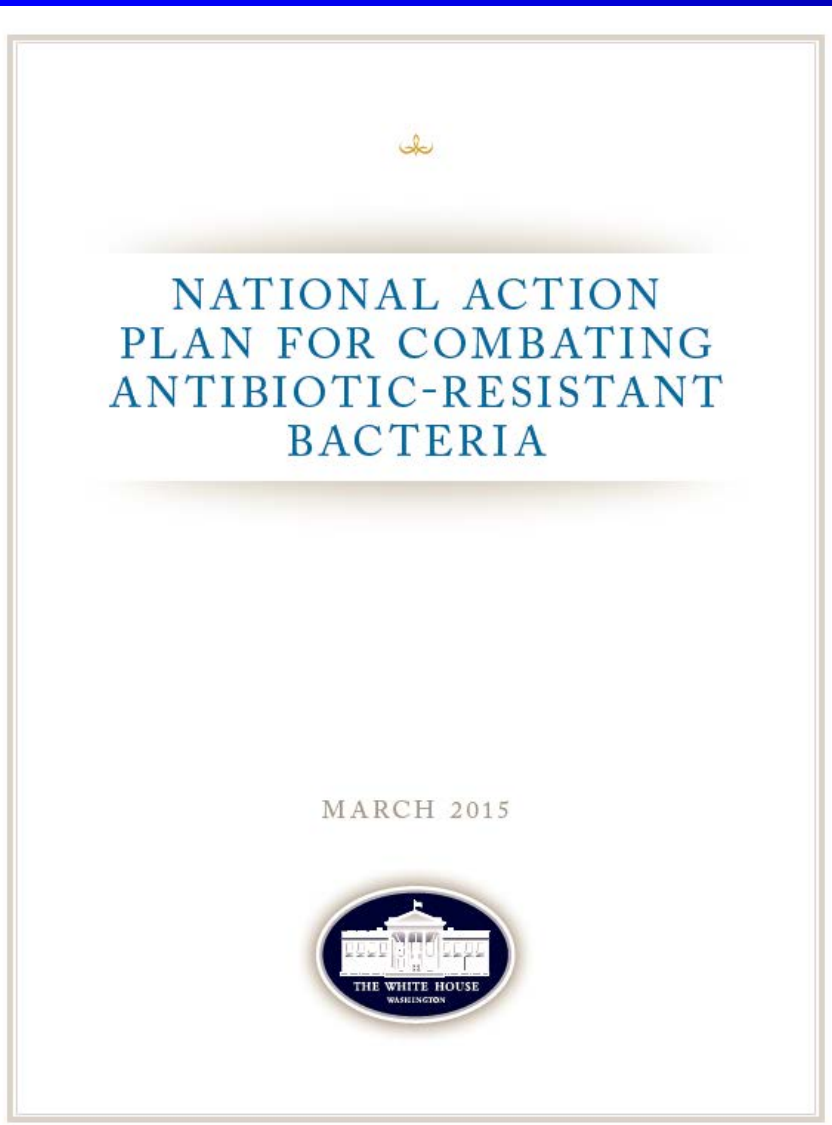
EMERGENCE OF ANTIBIOTIC RESISTANCE

Dr. Barry N. Kreiswirth
Director, PHRI TB Center

ANTIBIOTIC RESISTANCE



NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

MARCH 2015

THE WHITE HOUSE
WASHINGTON

Appendix

TABLE 3: CDC's Antibiotic-Resistant Threats in the United States, 2013

URGENT Threat Level Pathogens (3)
Clostridium difficile
250,000 infections per year requiring hospitalization or affecting hospitalized patients.
14,000 deaths per year.
At least $1 Billion in excess medical costs per year.
C. difficile deaths increased 400% between 2000-2007 because of the emergence of a strain resistant to a common antibiotic class (fluoroquinolones).
Almost half of infections occur in people younger than 65, but more than 90% of deaths occur in people 65 and older.
Half of *C. difficile* infections first show symptoms in hospitalized or recently hospitalized patients, and half show symptoms in nursing home patients or in people recently cared for in doctors' offices and clinics who received antibiotics.
The majority (71%) of pediatric *Clostridium difficile* infections, which are bacterial infections that cause severe diarrhea and are potentially life-threatening, occur among children in the general community, 73% were found to have recently taken antibiotics prescribed in doctor's offices for other outpatient settings.[6]
Carbapenem-Resistant *Enterobacteriaceae**
Out of ~140,000 healthcare-associated *Enterobacteriaceae* infections per year, more than 9,000 are caused by CRE (7,900 *CR-Klebsiella spp*; 1,400 CR-E. coli.).
Out of ~140,000 healthcare-associated *Enterobacteriaceae* infections per year, more than 9,000 are caused by CRE (7,900 *CR-Klebsiella spp*; 1,400 CR-E. coli.).
44 States have had at least one type of CRE confirmed by CDC testing.
CRE are resistant to nearly all antibiotics including carbapenems—the antibiotic of last resort.
***Neisseria gonorrhoeae** (Notifiable to CDC)*
Neisseria gonorrhoeae causes gonorrhea, is the second most common reportable infection in the United States, and is developing resistance to the cephalosporin antibiotics, the last line treatment option for this infection.
Of the 820,000 cases per year, 30% (246,000) now demonstrate resistance to at least one antibiotic.
If cephalosporin-resistant *N. gonorrhoeae* becomes widespread, the public health impact during a 10-year period is estimated to be 75,000 additional cases of pelvic inflammatory disease, 15,000 cases of epididymitis, and 222 additional HIV infections, with an estimated direct medical cost of at least $235 million.
SERIOUS Threat Level Pathogens (12)
Multidrug-Resistant *Acinetobacter*
12,000 healthcare-associated *Acinetobacter* infections occur in the U.S. of which 7,000 are multidrug-resistant
~ 500 deaths per year.
At least three different classes of antibiotics no longer cure resistant *Acinetobacter* infections.
Drug-Resistant *Campylobacter*
Campylobacter causes ~1.3 Million infections, 13, 000 hospitalizations and 120 deaths each year; 310,000 (25%) drug-resistant *Campylobacter* infections are found each year.
Campylobacter drug resistance increased from 13% in 1997 to 25% in 2011.
Campylobacter spreads from animals to people through contaminated food, particularly raw or undercooked chicken and unpasteurized milk.
Antibiotic use in food animals can results in resistant *Campylobacter* than can spread to humans.
Fluconazole-Resistant *Candida*
Out of 46,000 *Candida* yeast infections per year, 3,400 (30%) of patients with bloodstream infections with drug-resistant (DR)-Candida die during their hospitalization.
CDC estimates that each case of *Candida* infection results in 3-13 days of additional hospitalization and a total of $6,000-$29,000 in direct healthcare costs per patient.

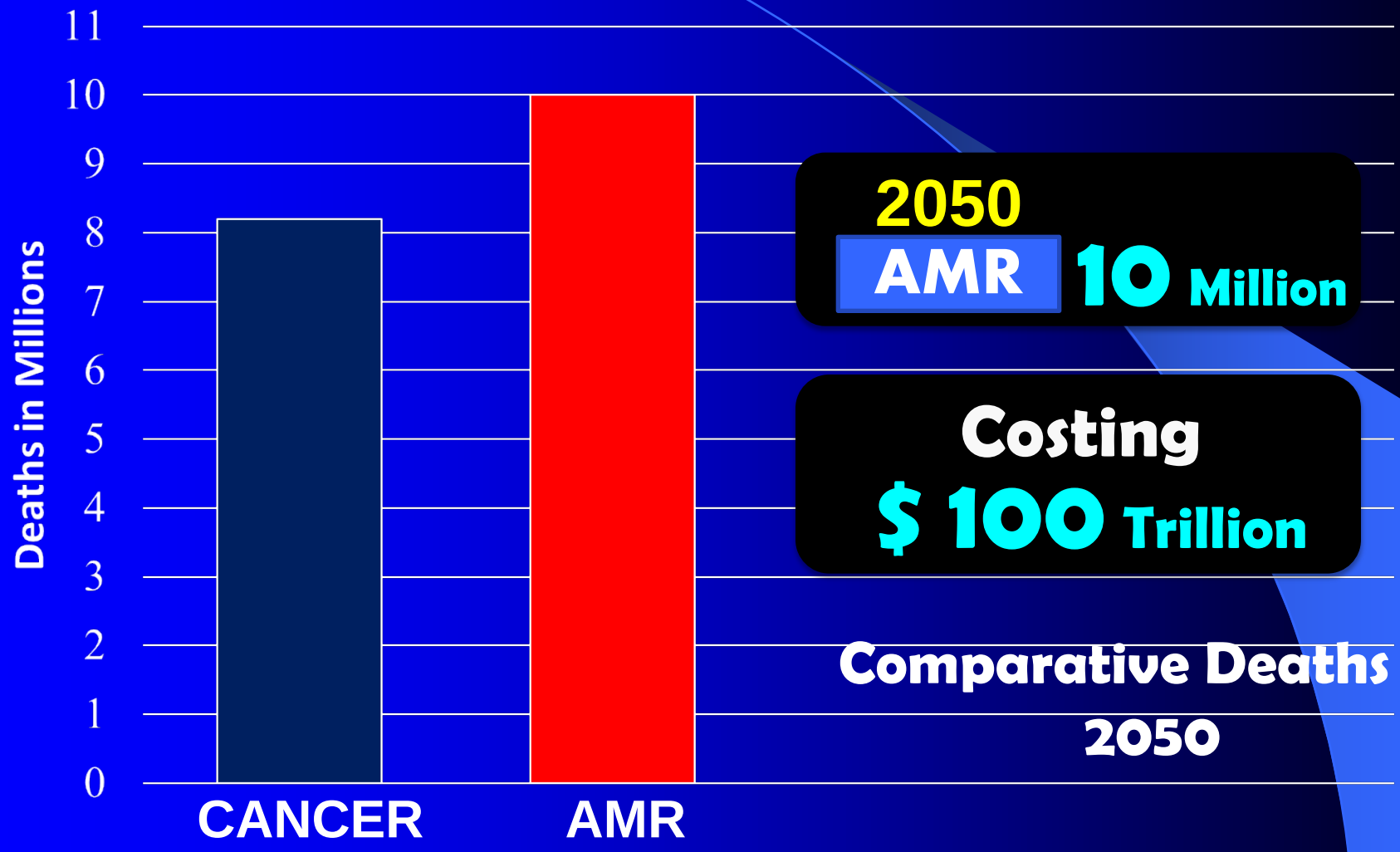
[6] Wendt, J.M. et al. *Clostridium difficile* Infection Among Children Across Diverse US Geographic Locations. *Pediatrics*. January 3, 2014

Antibiotic Resistance in the United States

* bacteria and fungus included in this report

https://www.cdc.gov/media/releases/2013/images/p0916-untreatable.jpg

Deaths from Drug-resistant infection Dramatically Rising



http://www.un.org/pga/71/event-latest/high-level-meeting-on-antimicrobial-resistance/

World Health Organization

WHO publishes list of bacteria for which new antibiotics are urgently needed

27 February 2017 | GENEVA

Priority 1: Critical

- *Acinetobacter baumannii,* carbapenem-resistant
- *Pseudomonas aeruginosa,* carbapenem-resistant
- **Enterobacteriaceae, carbapenem-resistant, ESBL-producing**

Priority 2: High

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

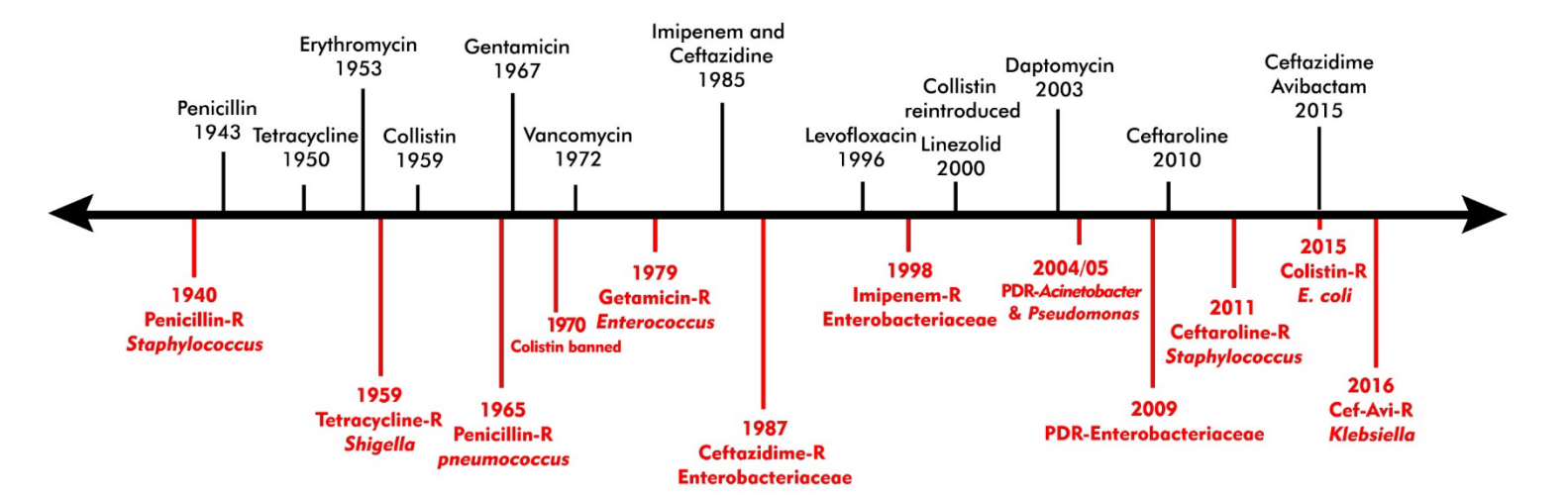
Priority 3: Medium

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/

Race against time: the introduction of new antibiotic and the emergence of resistance

ANTIBIOTIC INTRODUCED

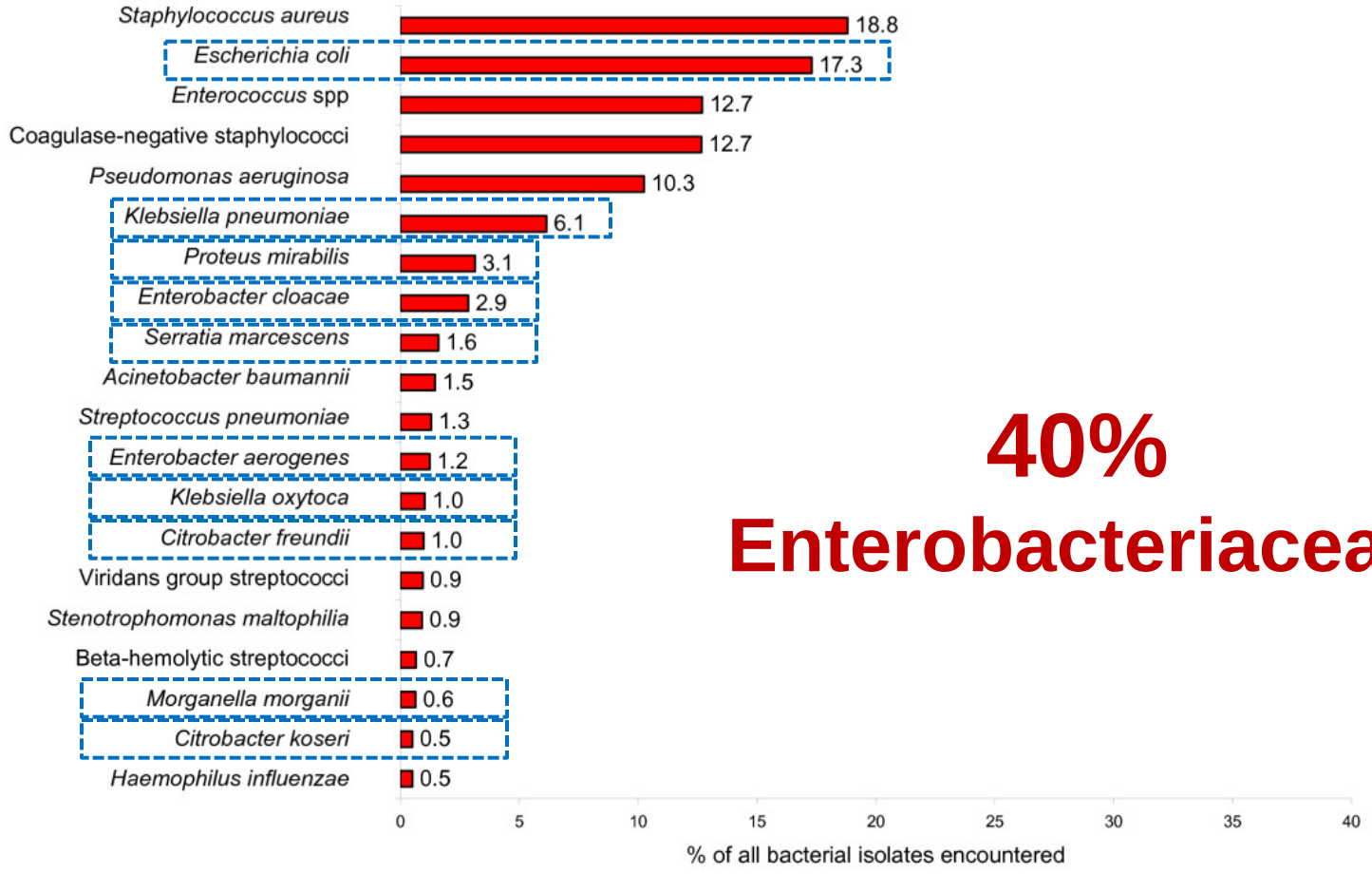


ANTIBIOTIC RESISTANCE IDENTIFIED

Enterobacteriaceae

- Gram-negative bacilli
- Gut flora, also found in soil/water
- Common causative agents of urinary tract infections, pneumonia, and blood stream infections
- Spread by hand carriage, contaminated food & water
- Examples: *Klebsiella, Enterobacter, Escherichia, Proteus, Salmonella, Shigella*

Relative Frequency of Isolated Clinical Specimens from Inpatients in the US



40%
Enterobacteriaceae

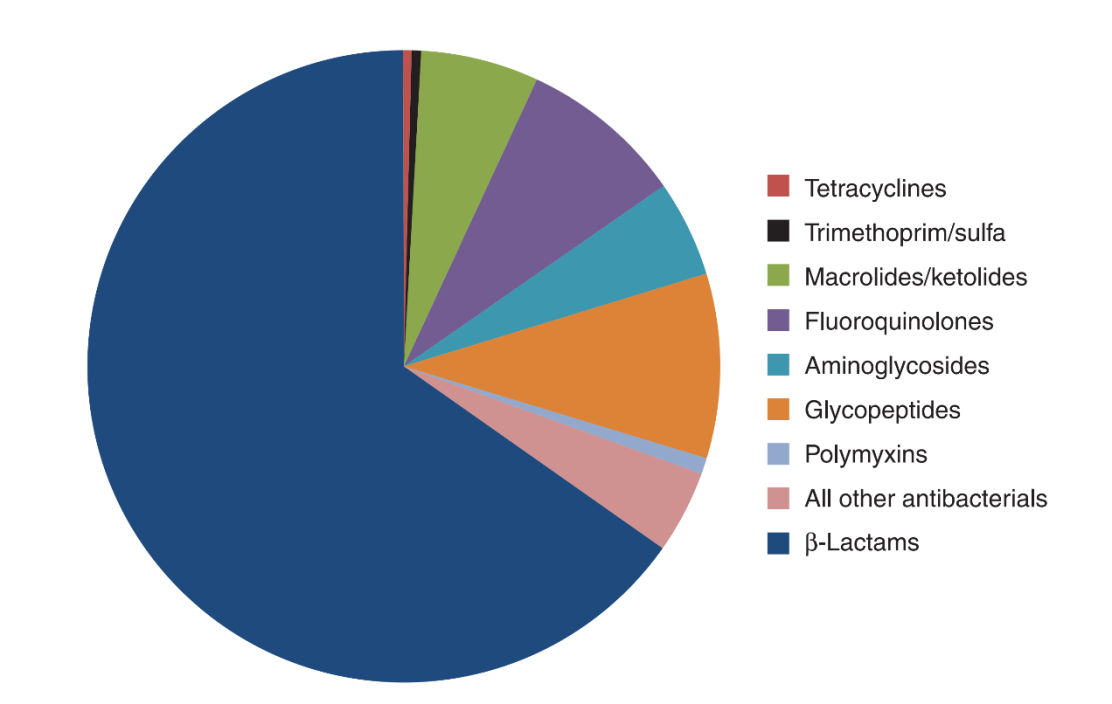
06,
5:2.

Global Public Health Threat

Since the discovery of Penicillin, β-lactam agents continue to be the most important class of antibiotics.

The global emergence of carbapenem resistance has dramatically changed our antibiotic arsenal for the worse!

Proportion of prescriptions in the United States for injectable antibiotics by class for years 2004–2014



β-lactams - 65.24%

β-Lactams and β-Lactamase Inhibitors Cold Spring Harb Perspect Med. 2016 Jun 21.

β-Lactams vs β-Lactamases

There are more than 1,600 β-lactamases reported

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)-cephalothin
- 2nd generation cephalosporins (2)-cefotetan
- 3rd generation cephalopsorins (3)-ceftriaxone
- 4th generation cephalosporins (2)-cefepime
- Carbapenems (3)-meropenem
- Monobactams (1)-aztreonam
- Beta-lactamase inhibitors (3)-clavulanic acid

bla_{KPC} encodes for a class A carbapenemase that hydrolyzes the entire class of β-lactam antibiotics

Carbapenem Resistance Determinants

- Carbapenemases class A

  K. pneumoniae carbapenemase (KPC)

  GES, SME

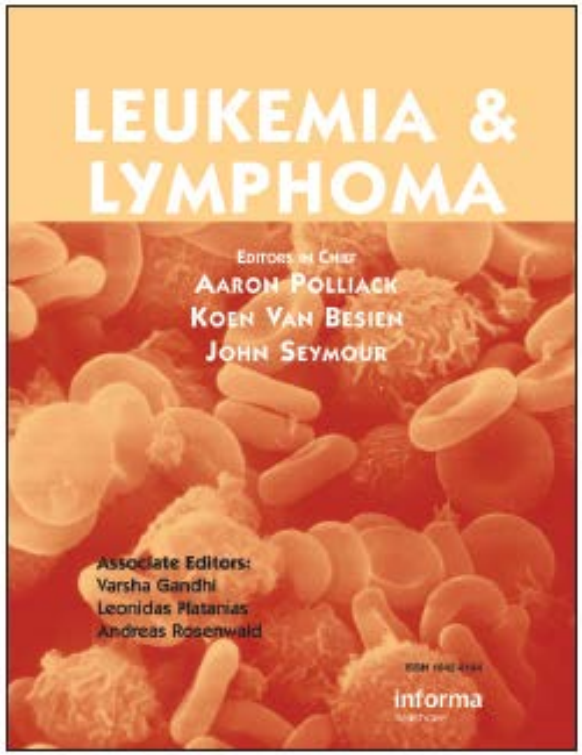
- Carbapenemases class B

  Metallo-β-Lactamases: NDM-1, VIM, IMP

- Carbapenemases class D

  Oxa23, Oxa24, Oxa 48, Oxa58

CONFOUNDING PROBLEM:
MOST OF THESE GENES ARE ON MOBILE ELEMENTS



Emergence of Carbapenem-resistant Enterobacteriaceae as Causes of Bloodstream Infections in Patients with Hematologic Malignancies

Michael J. Satlin, David P. Calfee, Liang Chen, Kathy A. Fauntleroy, Stephen J. Wilson, Stephen G. Jenkins, Eric J. Feldman, Gail J. Roboz, Tsiporah B. Shore, David C. Helfgott, Rosemary Soave, Barry N. Kreiswirth, Thomas J. Walsh

- July 2007-2010; Weill Cornell Medical College
- Patients with a hematologic malignancies and a CRE bloodstream infection
- 18 patients; 14 *K. pneumoniae*, 3 *E. cloacae*, 1 polymicrobial
- 10 patients died
- CRE had bla_{KPC} plasmids
- A median of 55 hours elapsed between culture collection and receipt of an active agent
- Active agents limited to colistin, polymyxin, tigecycline and an aminoglycoside

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

Carbapenemases : A Global Perspective

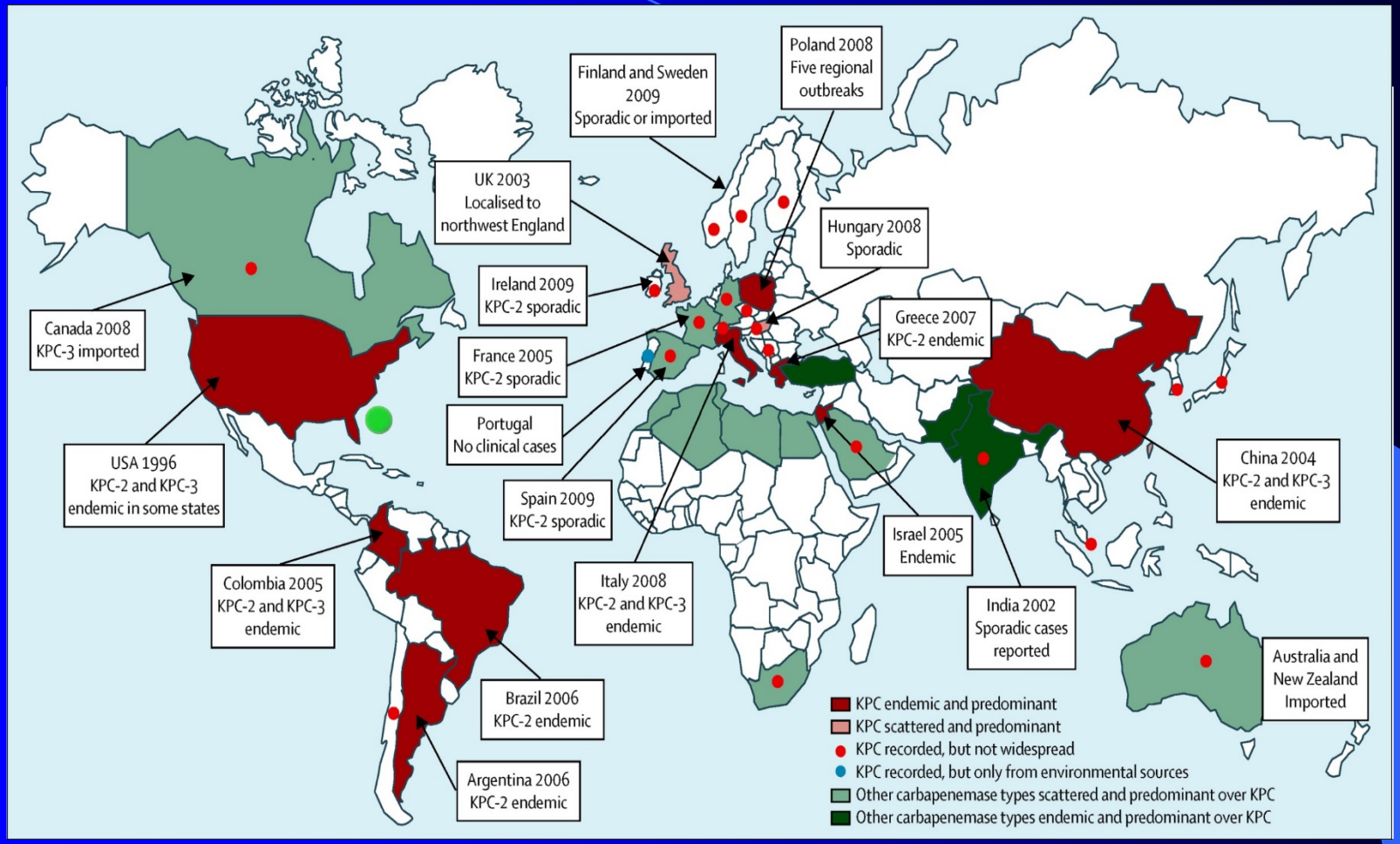
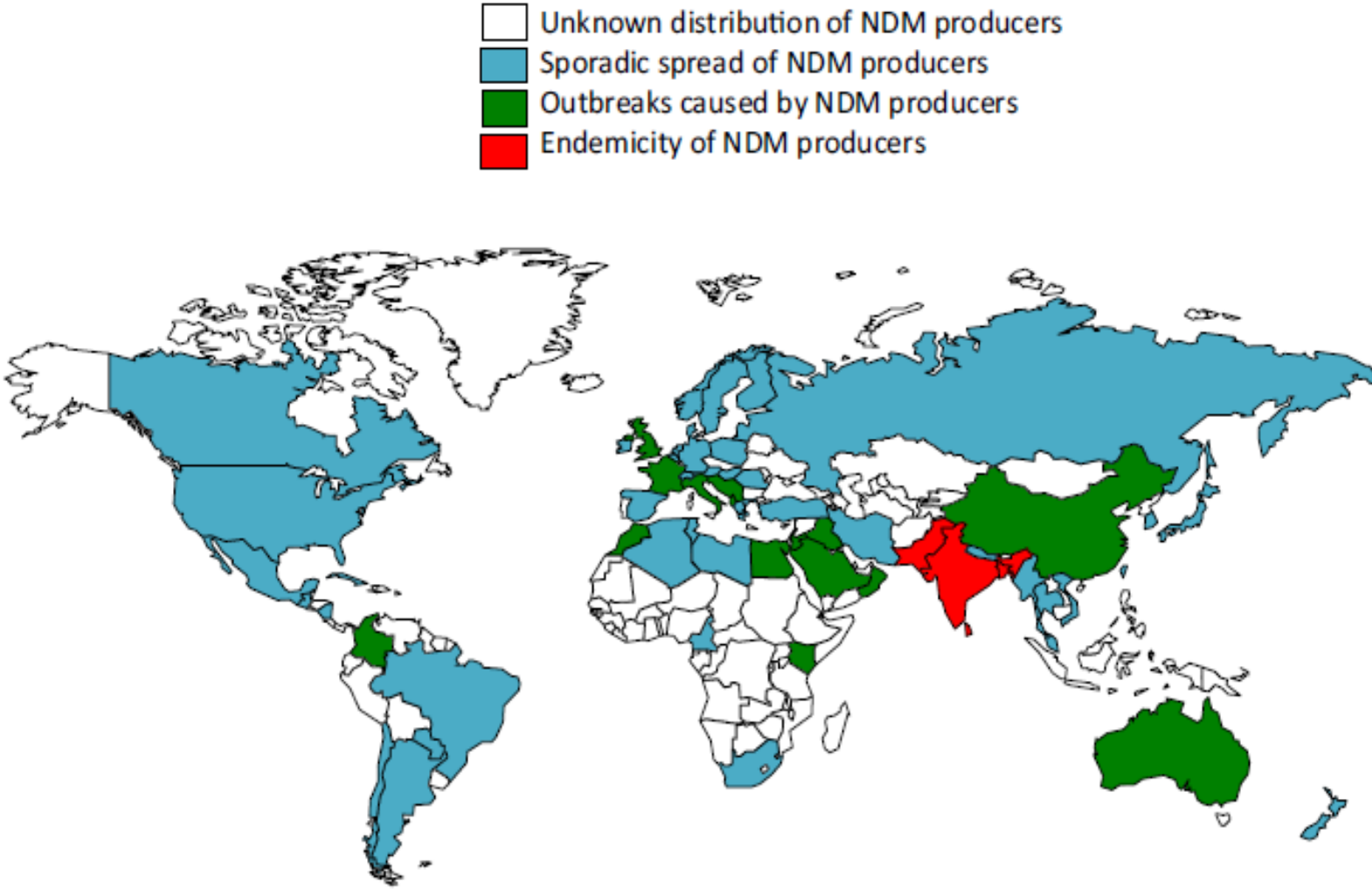


Figure: Epidemiological features of producers of Klebsiella pneumoniae carbapenemases by country of origin
Other carbapenemase types include VIM, OXA-48, or NDM. KPC=Klebsiella pneumoniae carbapenemase.

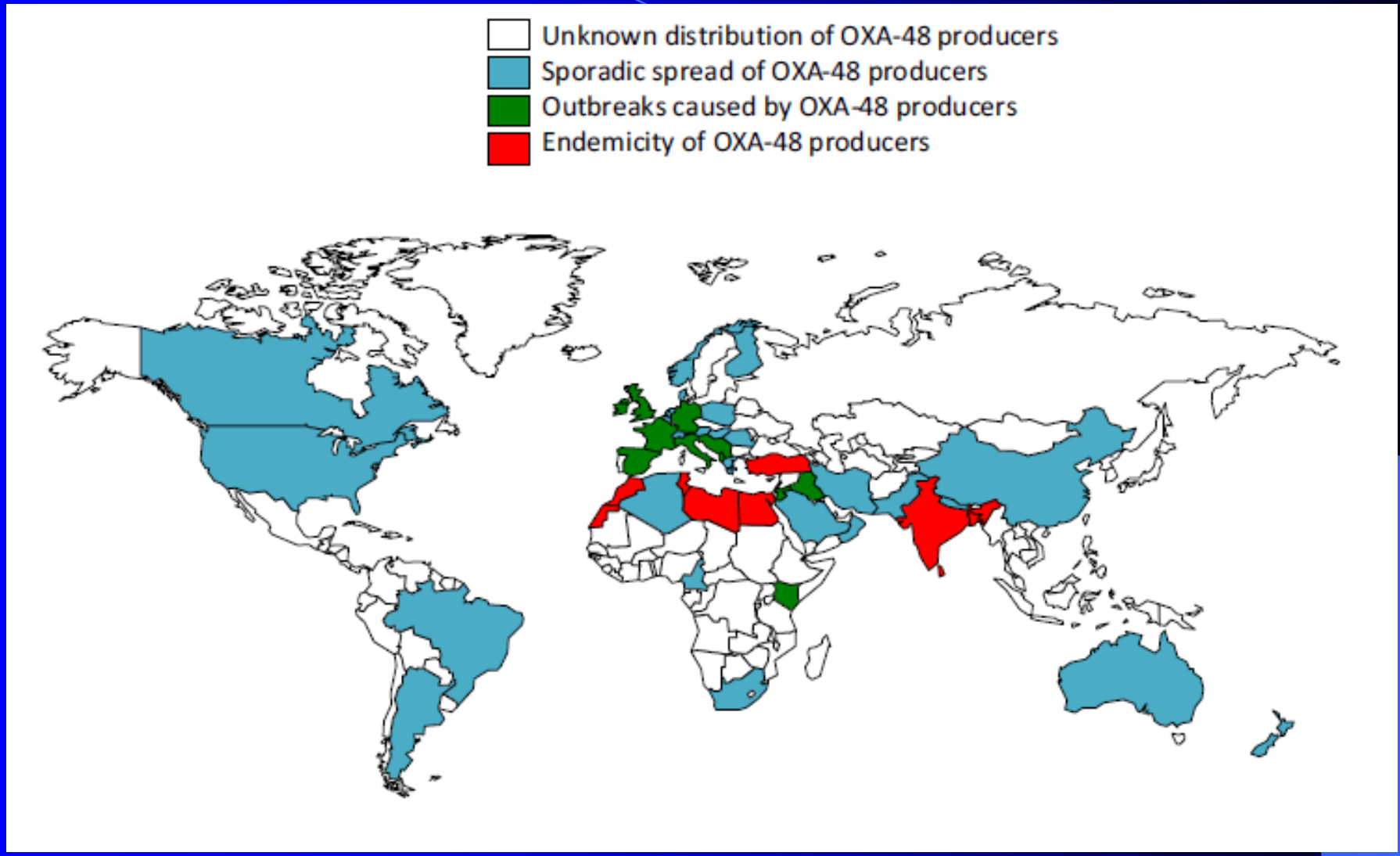
THE LANCET Infectious Diseases
Volume 13, Issue 9, September 2013, Pages 785–796

NDM Producers



Nordman and Poirel. *Clin Microbiol Infect*, 2014:20-821

OXA-48 Producers



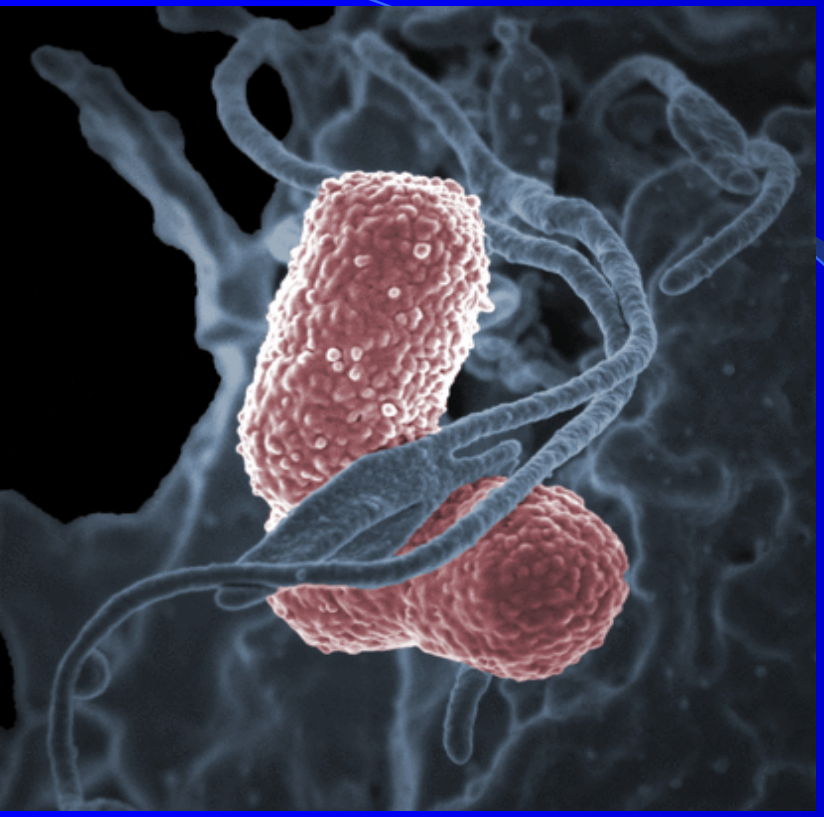
Nordman and Poirel. *Clin Microbiol Infect*, 2014:20-821

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 Tn*4401* harbored on large plasmids
- Plasmids are transmissible in Enterobacterceae
- Strains are approaching pan-resistance
- Molecular epidemiology not defined
- ~50% mortality among transplant patients

Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*

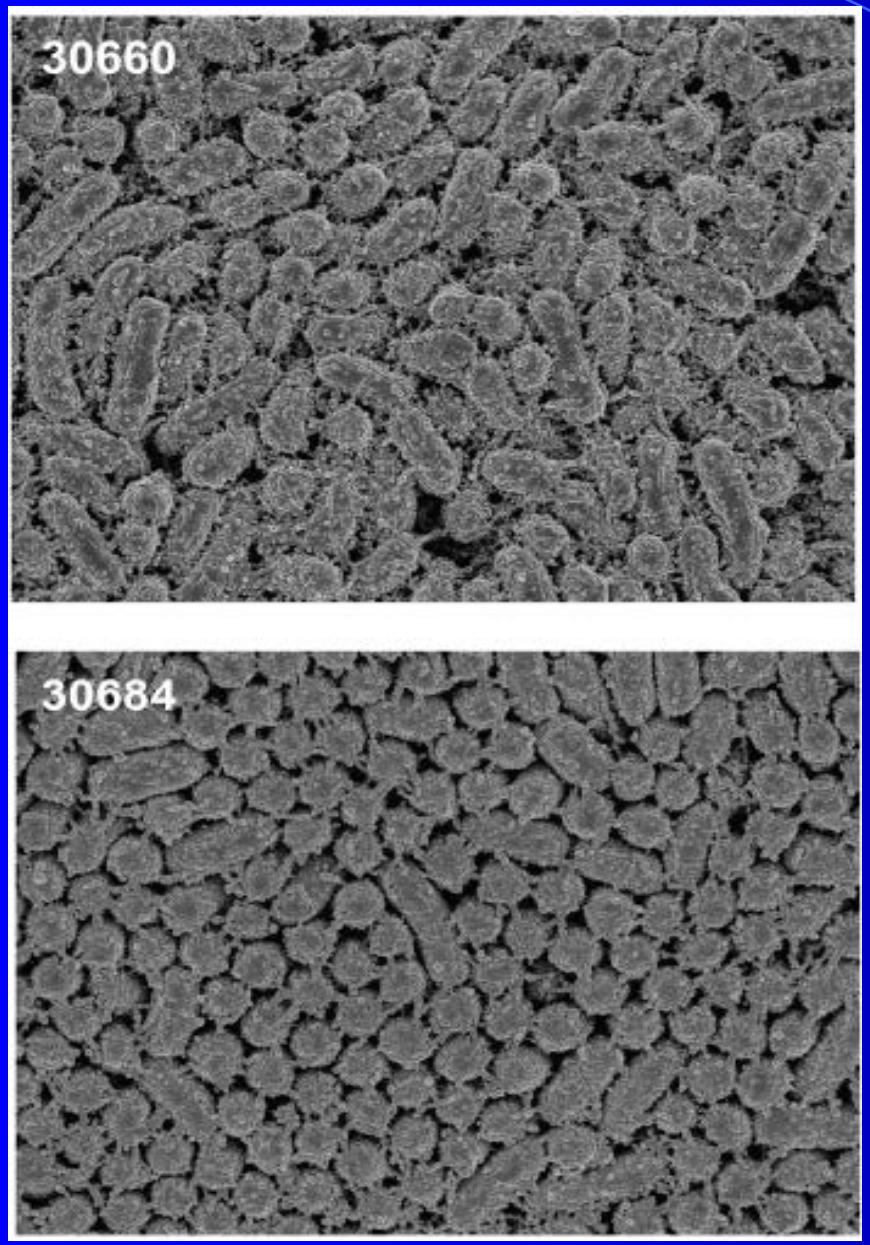
Frank R. DeLeo[a,1], Liang Chen[b,1], Stephen F. Porcella[c], Craig A. Martens[c], Scott D. Kobayashi[a], Adeline R. Porter[a], Kalyan D. Chavda[b], Michael R. Jacobs[d], Barun Mathema[b], Randall J. Olsen[e,f], Robert A. Bonomo[g,h], James M. Musser[e,f], and Barry N. Kreiswirth[b,2]



Is ST258 a single genetic clone that has disseminated world wide?

PNAS. 2014:111;4983-93

Comparative Genomic Analysis



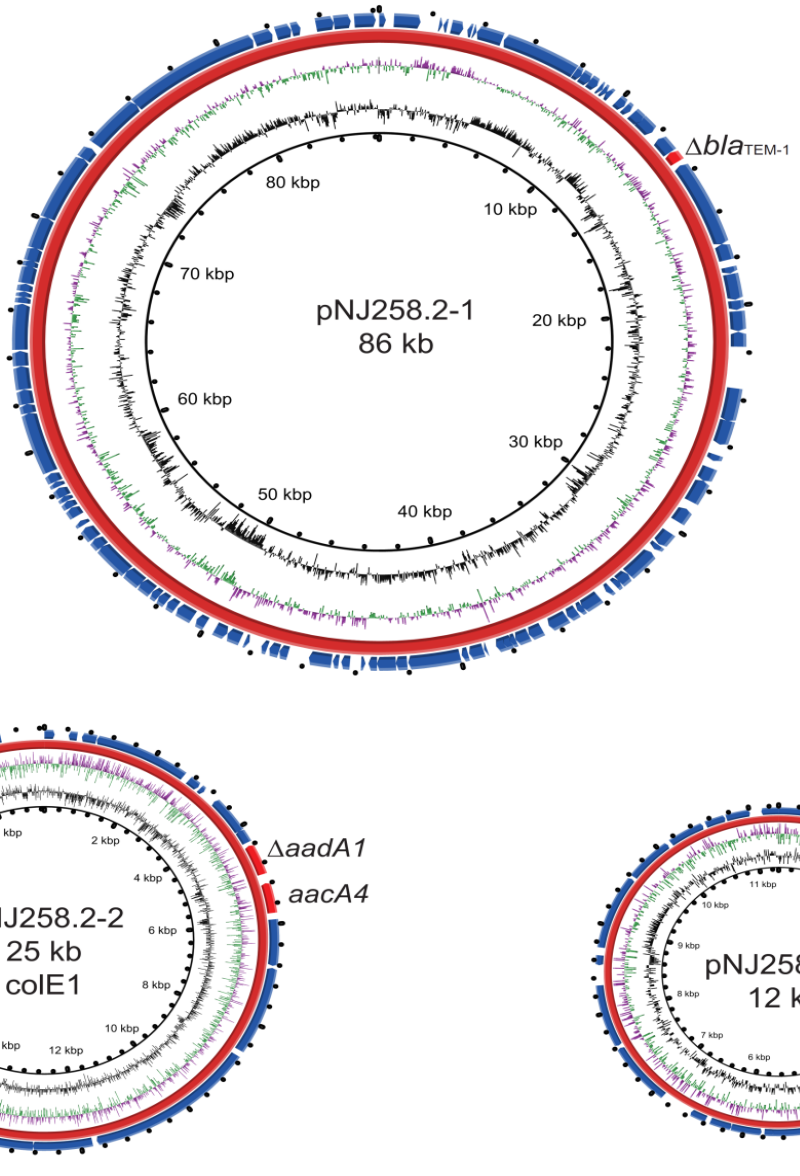
Genome = 5,266,518 bp
Prophages = 8
IS-elements = 22
Conjugative elements = 2

Plasmids = 5
12 – 147 kb

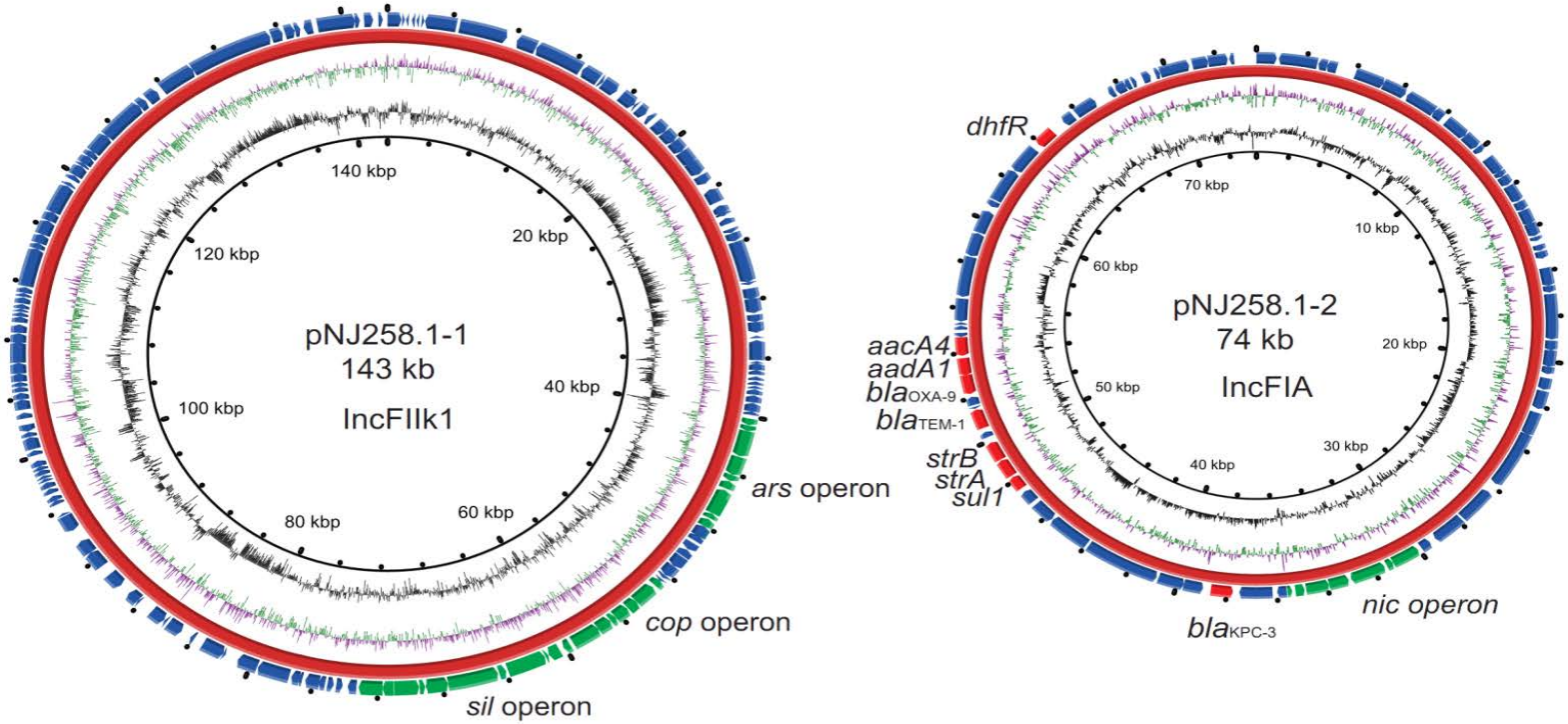
Genome = 5,293,301 bp
Prophages = 7
IS-elements = 19
Conjugative elements = 2

Plasmids = 3
12 – 86 kb

30684– Plasmid Content



30660 – PLASMID CONTENT

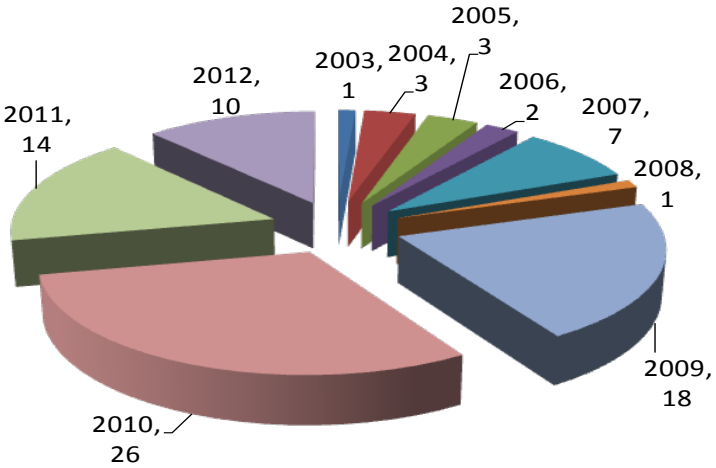


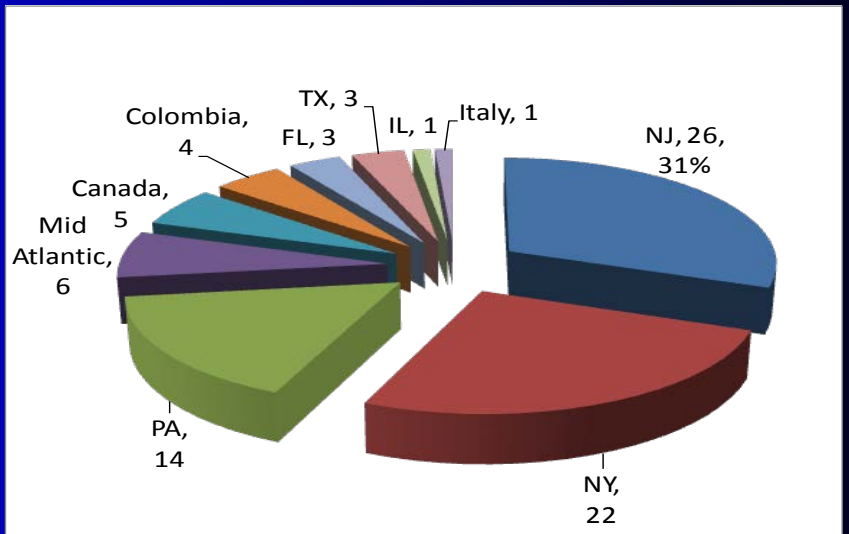
Initial Conclusion

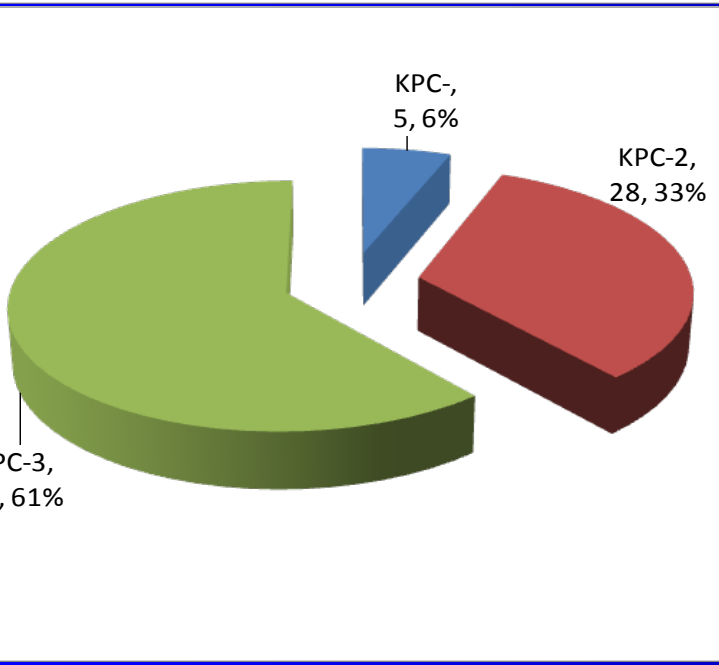
Complete genome analysis suggest that *bla*$_{KPC}$ harboring ST258 are clonal but contain different plasmids

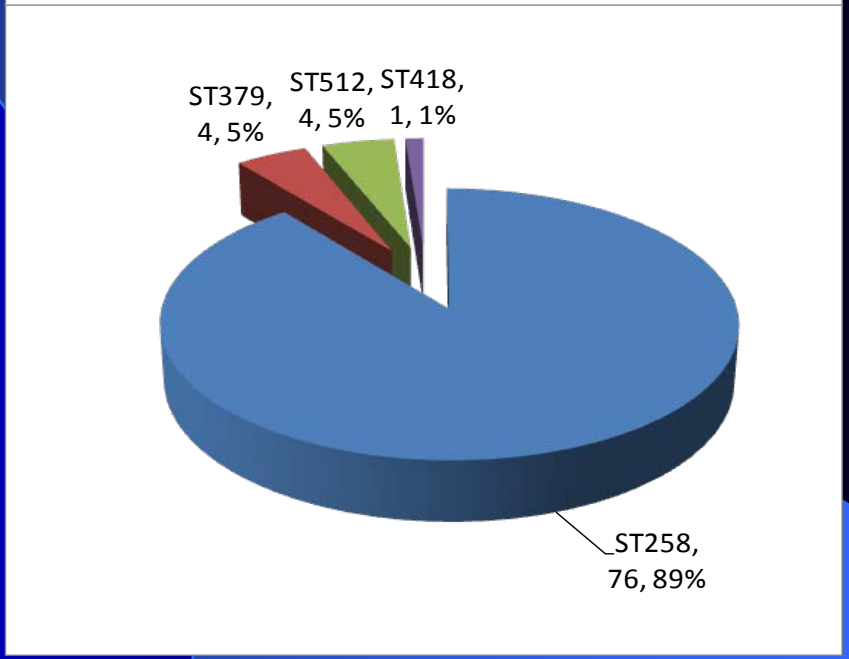
Comparative Analysis on 85 *K. pneumoniae* Genomes

85 – Selected *K. pneumoniae*

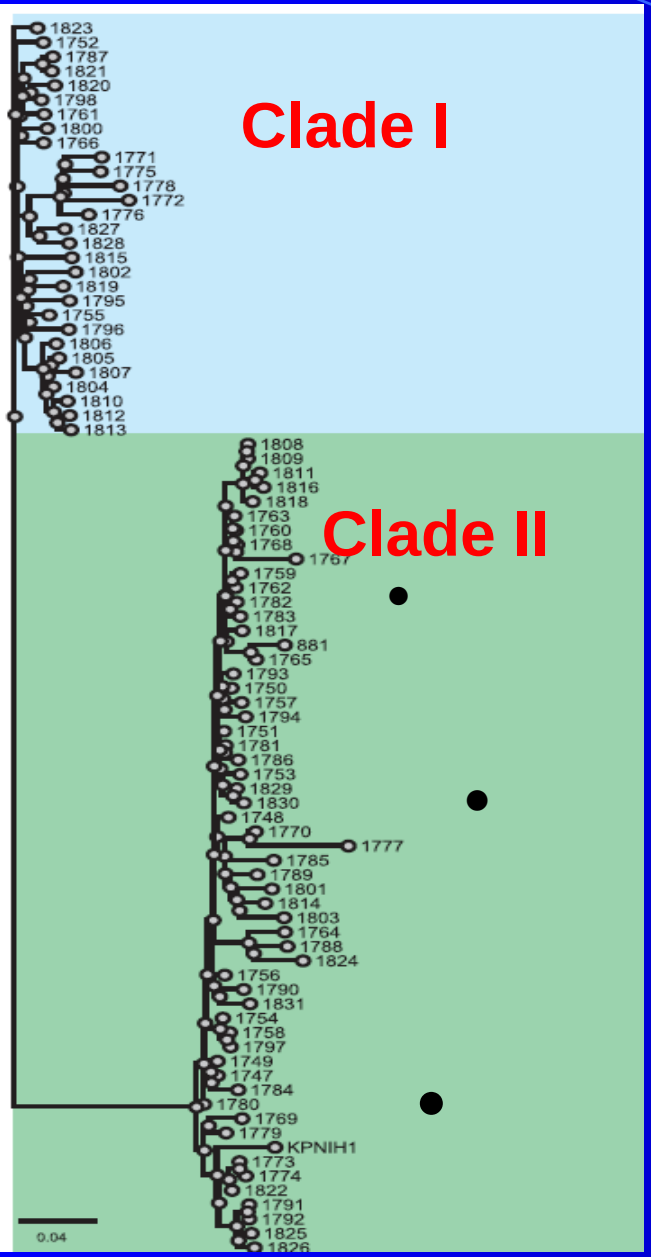




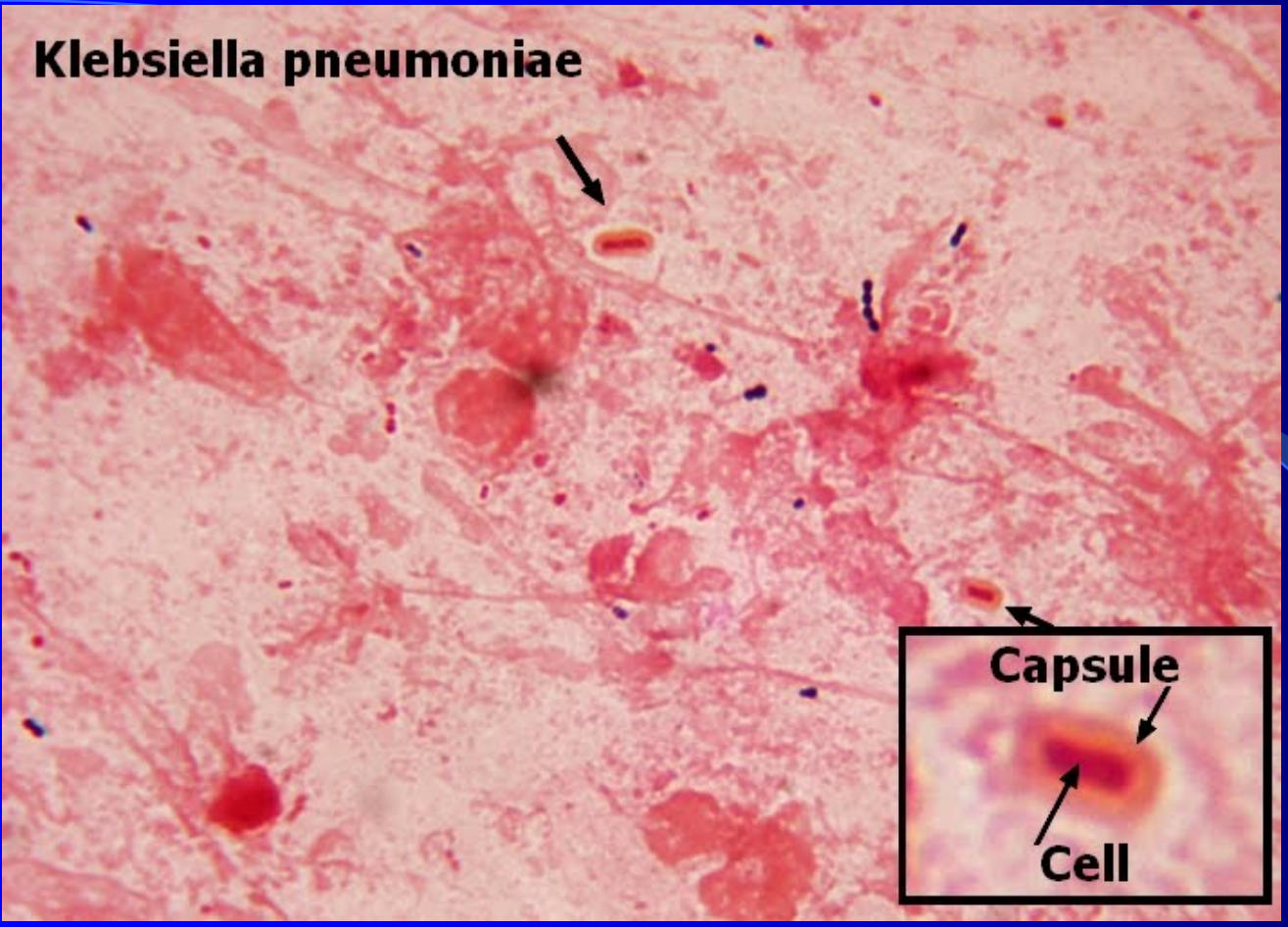




PHYLOGENETIC ANALYSIS OF ST258 *K. pneumoniae*

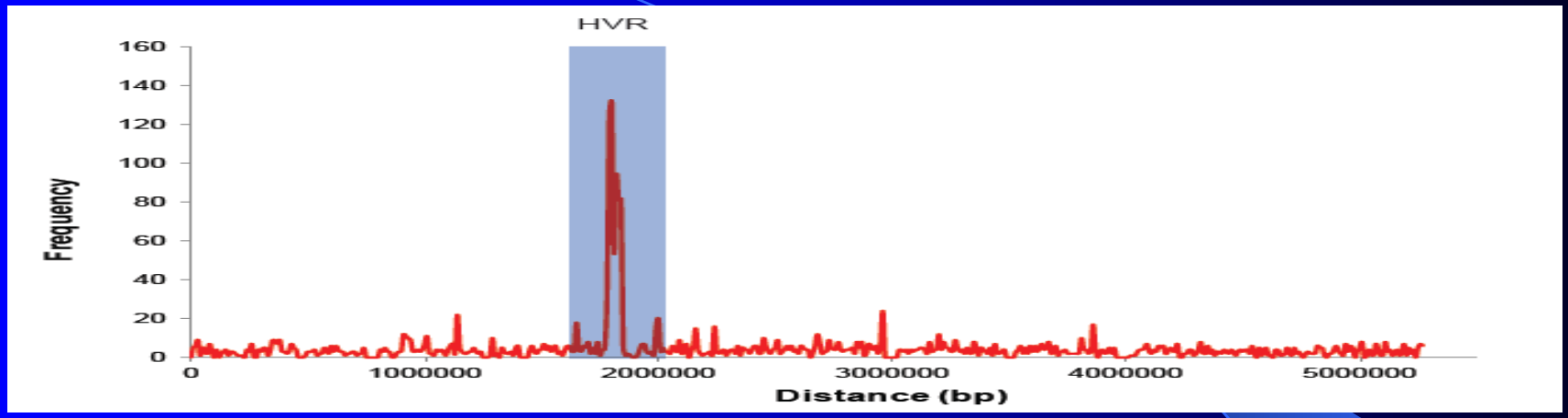


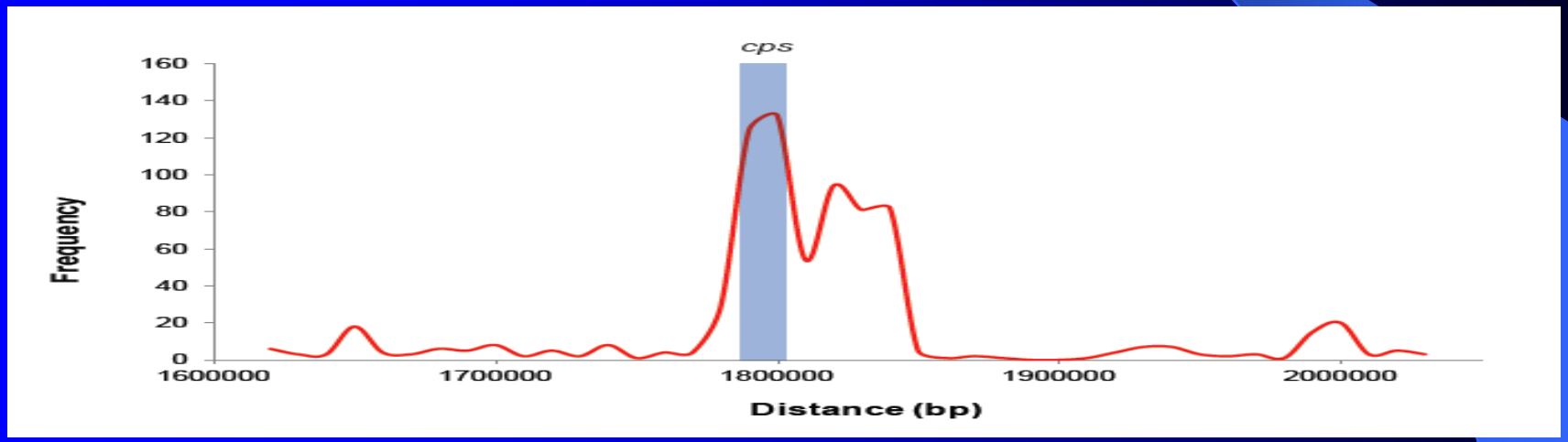
- Comparative whole genome analysis on 85 strains showed that ST258 can be distinguished in two subclones: Clades I and II
- ~350 SNP differences among the strains ranging from 116 to 784 SNPs
- Genetic divergence between the two clades is found in a ~215 kb region of divergence which contains the genes encoding the capsular polysaccharide biosynthesis



Literature indicates that the *K. pneumoniae* capsule polysaccharide (CPS) and the lipopolysaccharide promote resistance to phagocytosis *in vitro* and/or killing by serum complement

SNP Analysis in the Hypervariable Region





The region of differences, including the capsular and lipid polysaccharide pathway genes, are the result of chromosomal replacement from other *K. pneumoniae* STs

Summary of 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 Tn*4401* which is found on numerous conjugative plasmids and four major sub-clones of ST258 harbor: IncI2, IncFIA and IncFIIK2 , IncR

Clinical Dilemma

2017:61;1-13

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Multicenter Clinical and Molecular Epidemiological Analysis of Bacteremia Due to Carbapenem-Resistant *Enterobacteriaceae* (CRE) in the CRE Epicenter of the United States

Michael J. Satlin,[a] Liang Chen,[d] Gopi Patel,[e] Angela Gomez-Simmonds,[g] Gregory Weston,[h] Angela C. Kim,[k] Susan K. Seo,[m] Marnie E. Rosenthal,[o,w] Steven J. Sperber,[q] Stephen G. Jenkins,[b] Camille L. Hamula,[f] Anne-Catrin Uhlemann,[g] Michael H. Levi,[i] Bettina C. Fries,[h,j] Yi-Wei Tang,[n] Stefan Juretschko,[l] Albert D. Rojtman,[p] Tao Hong,[r] Barun Mathema,[s] Michael R. Jacobs,[u] Thomas J. Walsh,[a,c] Robert A. Bonomo,[t,v] Barry N. Kreiswirth[d]

- 121 Bacteremia patients
- Malignancy: 40%
- Diabetes: 30%
- Congestive heart failure: 17%
- Transplant recipients: 17% (12% solid organ)
- 47 hours until appropriate therapy
- 30 day mortality: 49%
- 84% were ST258

Treatment Regimens

TABLE 5 Comparisons of 30-day mortality rates after CRE bacteremia by a definitive antimicrobial regimen

Definitive antimicrobial regimen	30-day mortality rate (%)
Monotherapy[a] ($n = 55$)	38
Polymyxin ($n = 25$)	40
Polymyxin B ($n = 18$)	39
Colistin ($n = 7$)	43
Tigecycline ($n = 17$)	39
Aminoglycoside ($n = 11$)	43
With a carbapenem ($n = 22$)	50
Without a carbapenem ($n = 33$)	30
Combination therapy[b] ($n = 43$)	
Polymyxin-tigecycline ($n = 18$)	56
Aminoglycoside-tigecycline ($n = 9$)	33
With a carbapenem ($n = 16$)	62
Without a carbapenem ($n = 27$)	44
Regimens with carbapenems ($n = 38$)	55
Regimens without carbapenems ($n = 60$)	37

[a]Use of only one active antimicrobial agent (an agent to which the CRE tested susceptible *in vitro*).
[b]Use of at least two active antimicrobial agents.

Major Clinical Challenge:
New therapies to treat highly drug resistant strains in highly vulnerable immunosuppressed populations

- Soft and solid tissue organ transplant patients
- Cancer patients
- Adult cystic fibrosis patients
- Nosocomial patients

- Combinations with “old” drugs and the repurposing of beta-lactams using inhibitors

AVIBACTAM

AAC
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MINIREVIEW

New β-Lactamase Inhibitors: a Therapeutic Renaissance in an MDR World

Sarah M. Drawz,[a] Krisztina M. Papp-Wallace,[b,c] Robert A. Bonomo[b,c,d,e]

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA[a]; Research Service, Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, Ohio, USA[b]; Departments of Medicine,[c] Pharmacology,[d] and Molecular Biology and Microbiology,[e] Case Western Reserve University, Cleveland, Ohio, USA

TABLE 1 MICs of β-lactam and β-lactam-avibactam combinations against select pathogens[a]

	MIC (μg/ml)[b]					
Pathogen	CAZ	CAZ-AVI	CPT	CPT-AVI	ATM	ATM-AVI
K. pneumoniae with OXA-48	256/512	0.25/0.5				
K. pneumoniae with CTX-M-15	8/64	0.06/0.25				
K. pneumoniae with KPC-2	≥512/≥512	0.25/1			≥512/≥512	≤0.06/≤0.06
E. coli with ESBL	16/64	0.12/0.25				
E. coli with AmpC	16/64	0.12/0.5				
E. coli with OXA-48	4	<0.008				
E. coli with IMP-1	256	64				
Enterobacteriaceae with multiple β-lactamases, including KPC-2			>64/>64	0.5/2		
Enterobacteriaceae with multiple β-lactamases, including AmpC			256/>256	0.5/2		
Enterobacteriaceae with VIM	64–512	64–512			0.25–256	0.12–0.5
P. aeruginosa	8/64	4/8	>64/>64	16/>32	16/32	8/32
P. aeruginosa with ESBL PER-1	128/128	4/16				
A. baumannii			>64/>64	32/>32		
A. baumannii with PER-1, OXA-51, and OXA-58	128/≥512	32/256				
S. aureus			1/2	1/2		

[a] Data were adapted from references 15, 16, 19, 20, 21, and 24. Avibactam was added at 4 μg/ml. Abbreviations: CAZ, ceftazidime; AVI, avibactam; CPT, ceftaroline; ATM, aztreonam.

[b] Numbers separated by a forward slash indicate MIC_{50}/MIC_{90} values. Empty cells indicate that values were not reported.

Ceftazidime-Avibactam

Ceftazidime-avibactam (by Etest): 100/101 isolates tested were susceptible (99%) based on the CLSI ceftazidime breakpoint of 4 µg/mL

- MIC_{50} = 2 µg/mL
- MIC_{90} = 4 µg/mL (susceptible breakpoint for ceftazidime)
- KPC-2 *Kp* (*n*=44): only 1 (2%) with MIC of 4 µg/mL
- KPC-3 *Kp* (*n*=48): 19 (40%) with MIC of 4 µg/mL
- 1 isolate had an MIC of 8 µg/mL

Transmission

Major Infection Control Challenge:

Carbapenem resistant strains of Enterobacteriaceae: *K. pneumoniae*, *Enterobacter spp.* and *E. coli*

- Spread as CRE resistant clones
- Spread their plasmids and create new CRE clones
- To investigate a suspected outbreak, resolution at the whole genome sequencing level and plasmid analysis is necessary

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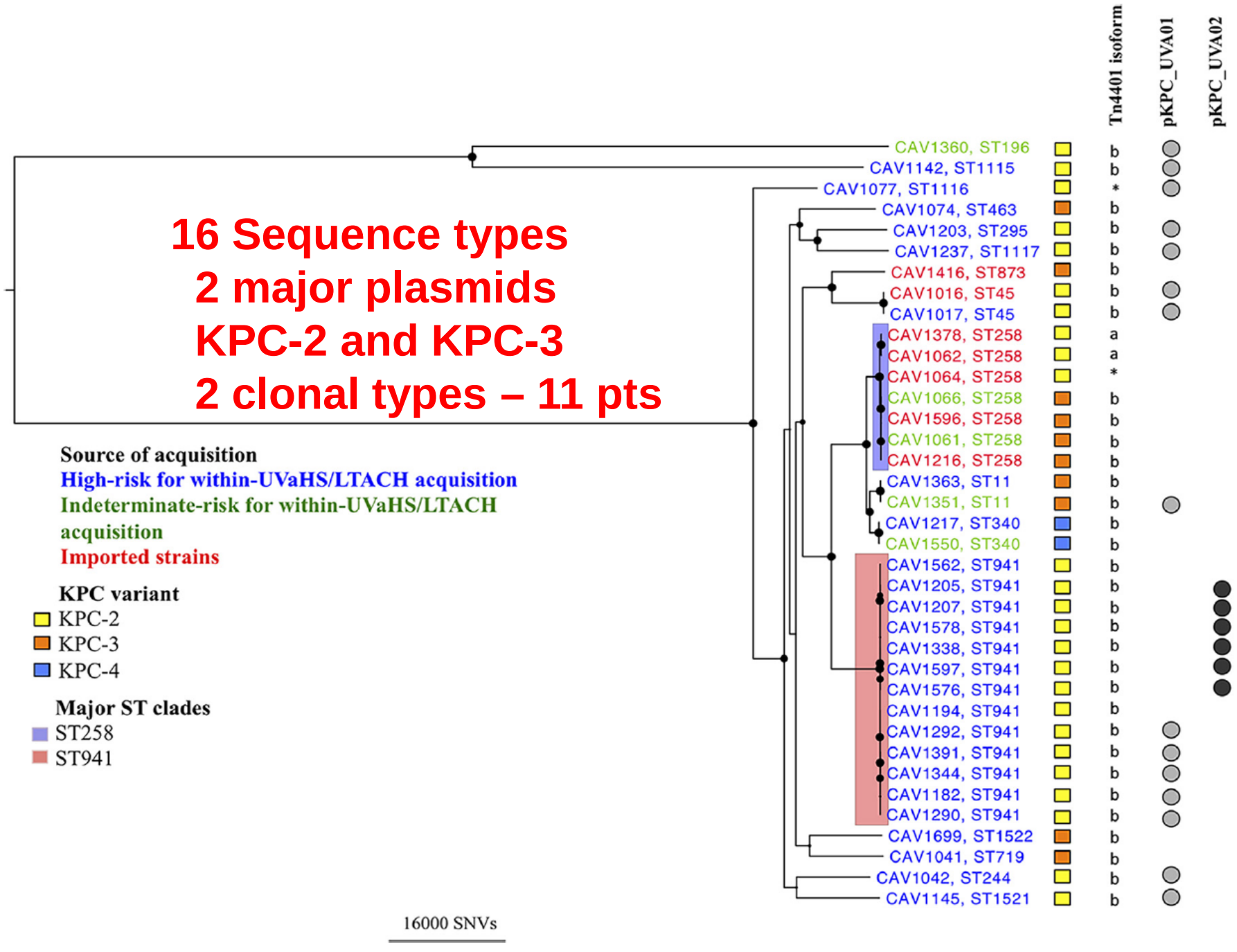
Klebsiella pneumoniae Carbapenemase (KPC)-Producing *K. pneumoniae* at a Single Institution: Insights into Endemicity from Whole-Genome Sequencing

Amy J. Mathers,[a,b] Nicole Stoesser,[c] Anna E. Sheppard,[c] Louise Pankhurst,[c] Adam Giess,[c] Anthony J. Yeh,[a*] Xavier Didelot,[d] Stephen D. Turner,[e] Robert Sebra,[f] Andrew Kasarskis,[f] Tim Peto,[c] Derrick Crook,[c,g] Costi D. Sifri[a,h]

Antimicrobial Agents and Chemotherapy. 2015:59;1656

5 year surveillance, one hospital
37 patients with KPC producing *K. pneumoniae*
Whole Genome Sequencing of each strain


16 Sequence types
2 major plasmids
KPC-2 and KPC-3
2 clonal types – 11 pts
Source of acquisition
High-risk for within-UVaHS/LTACH acquisition
Indeterminate-risk for within-UVaHS/LTACH acquisition
Imported strains
KPC variant
KPC-2
KPC-3
KPC-4
Major ST clades
ST258
ST941
Tn4401 isoform
pKPC_UVA01
pKPC_UVA02
CAV1360, ST196 b
CAV1142, ST1115 b
CAV1077, ST1116 *
CAV1074, ST463 b
CAV1203, ST295 b
CAV1237, ST1117 b
CAV1416, ST873 b
CAV1016, ST45 b
CAV1017, ST45 b
CAV1378, ST258 a
CAV1062, ST258 a
CAV1064, ST258 *
CAV1066, ST258 b
CAV1596, ST258 b
CAV1061, ST258 b
CAV1216, ST258 b
CAV1363, ST11 b
CAV1351, ST11 b
CAV1217, ST340 b
CAV1550, ST340 b
CAV1562, ST941 b
CAV1205, ST941 b
CAV1207, ST941 b
CAV1578, ST941 b
CAV1338, ST941 b
CAV1597, ST941 b
CAV1576, ST941 b
CAV1194, ST941 b
CAV1292, ST941 b
CAV1391, ST941 b
CAV1344, ST941 b
CAV1182, ST941 b
CAV1290, ST941 b
CAV1699, ST1522 b
CAV1041, ST719 b
CAV1042, ST244 b
CAV1145, ST1521 b
16000 SNVs

Plasmid Evolution in a Single Patient

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RESEARCH ARTICLE

Plasmid Dynamics in KPC-Positive *Klebsiella pneumoniae* during Long-Term Patient Colonization

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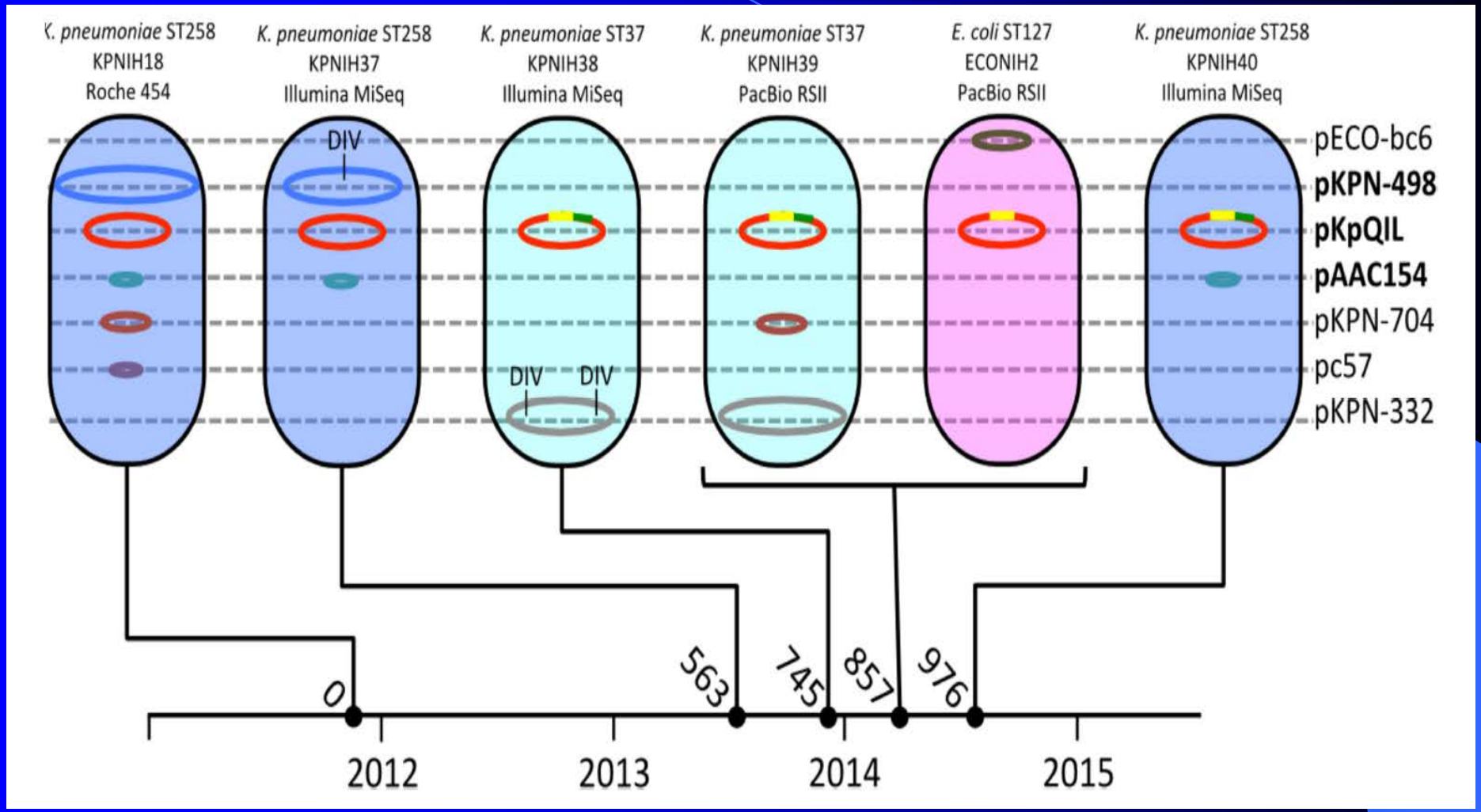
Conlan et al. *M Bio*, 2016:7;e00742-16

Plasmid Evolution in a Single Patient



Conlan et al. *M Bio*, 2016:7;e00742-16

IN SUMMARY

- CRE epidemic is global and the US is currently only experiencing the spread of *bla*$_{KPC}$ plasmids
- There are limited drugs to treat CRE and the infected populations are high risk patients
- β-lactam inhibitors, avibactam, new paradigm
- Spread of CRE is both the movement of resistant clones and the continual creation and spread of resistance plasmids
- Controlling this epidemic will require better diagnostics and improved therapy