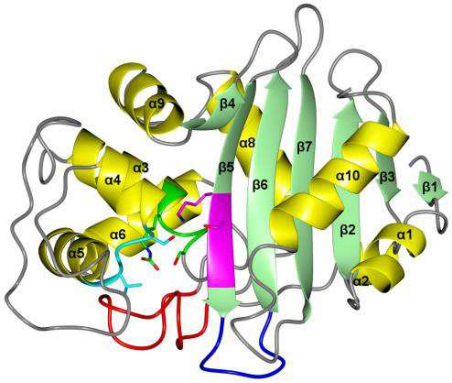
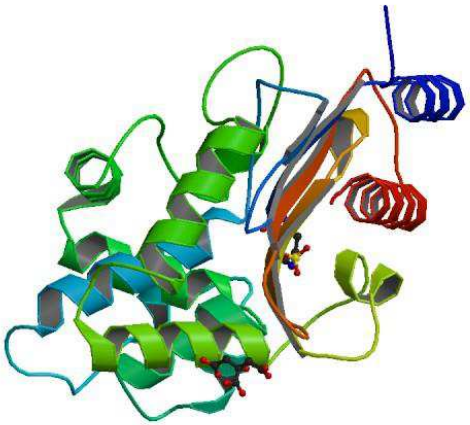
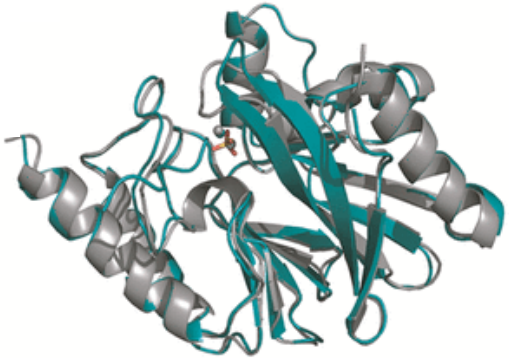
Carbapenemases and More



OXA-48
Docquier
2009



KPC-2
Nguyen
2016



IMP-1
Palzkill
2012

Karen Bush, PhD

January 18, 2018

Texas Medical Center Antimicrobial Resistance & Stewardship Conference, Houston

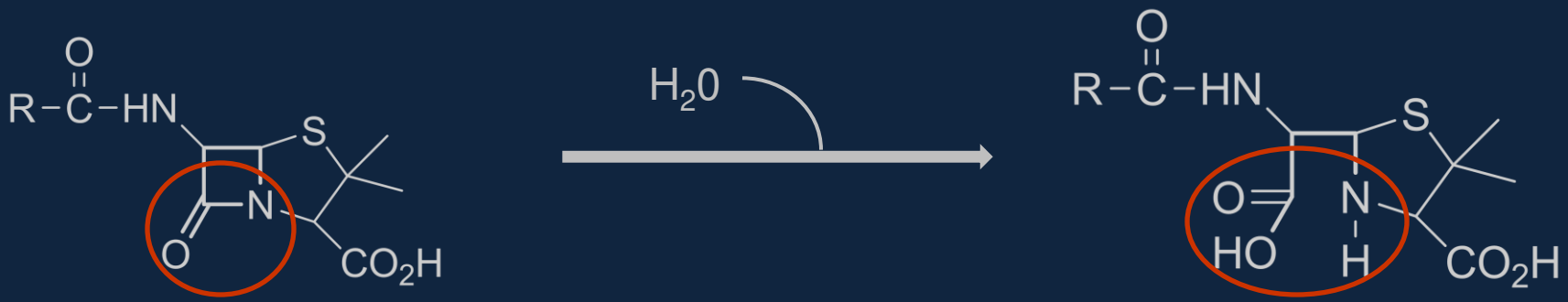
INDIANA UNIVERSITY

Major β-Lactam Resistance Mechanisms

- Gram-Positive Bacteria
  - Penicillin-Binding Proteins (cell wall synthesis)
  - Penicillinases (staphylococci)
- Gram-Negative Bacteria
  - β-Lactamases
  - β-Lactamases + Porin Changes
  - β-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 β-Lactamase Families

Serine

Metallo (Zn)

Classified according to active site configurations and hydrolysis mechanism

Based on Bush, Jacoby & Medeiros AAC:39:1211 (1995)

The First Reported β-Lactamases

- Abraham and Chain, Oxford 1940
  - Described an enzyme that destroyed penicillin from a *Bacillus (Escherichia) coli*
  - Most likely the chromosomal 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-β-lactamase

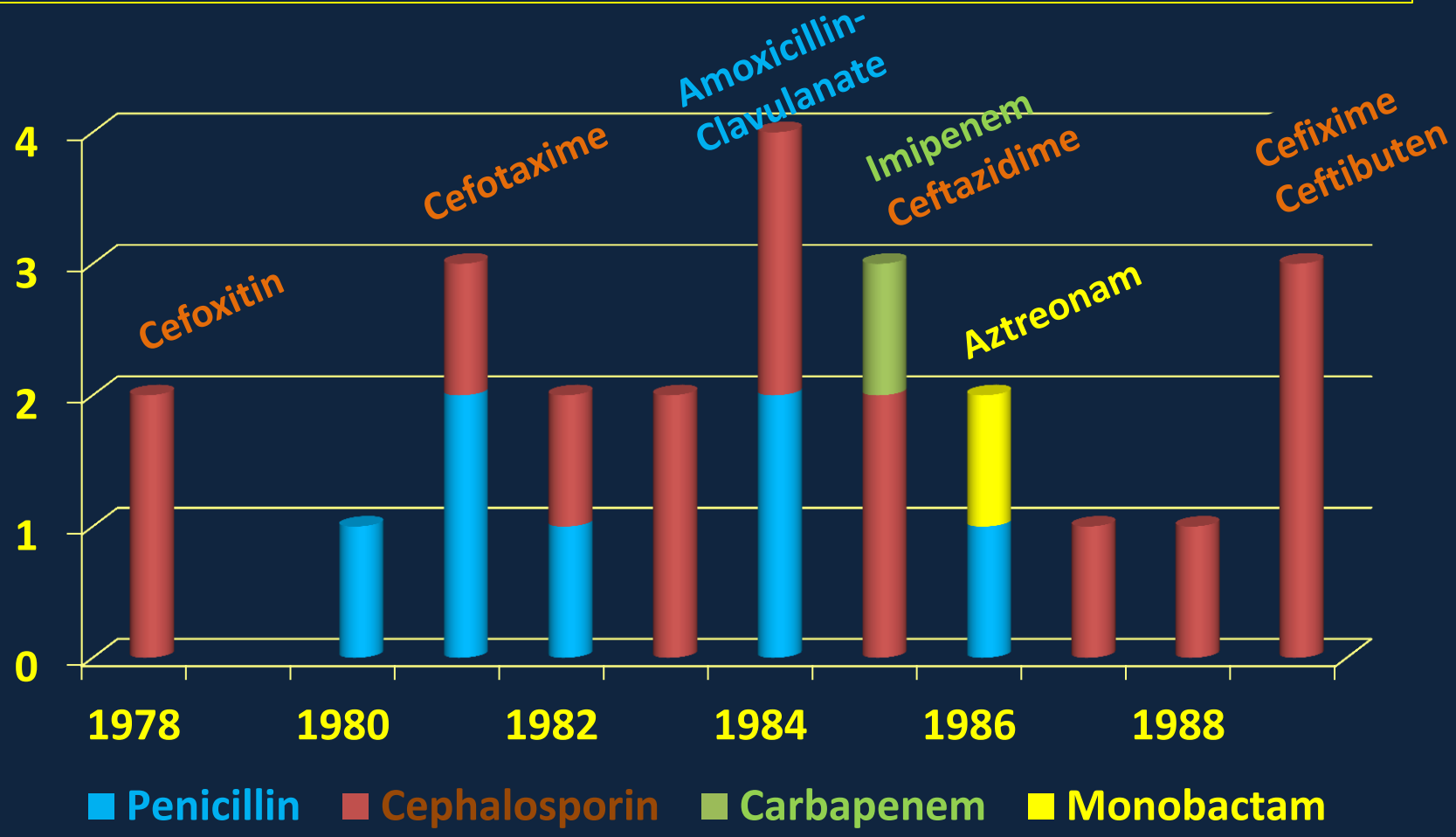
www.oxforddnb.com

Abraham and Chain. Nature 146:837 (1940 Barber, Br. Med. J. 2:565 (1949); Medeiros, Clin. Inf. Dis. 24 (Suppl 1):S19 (1997)

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

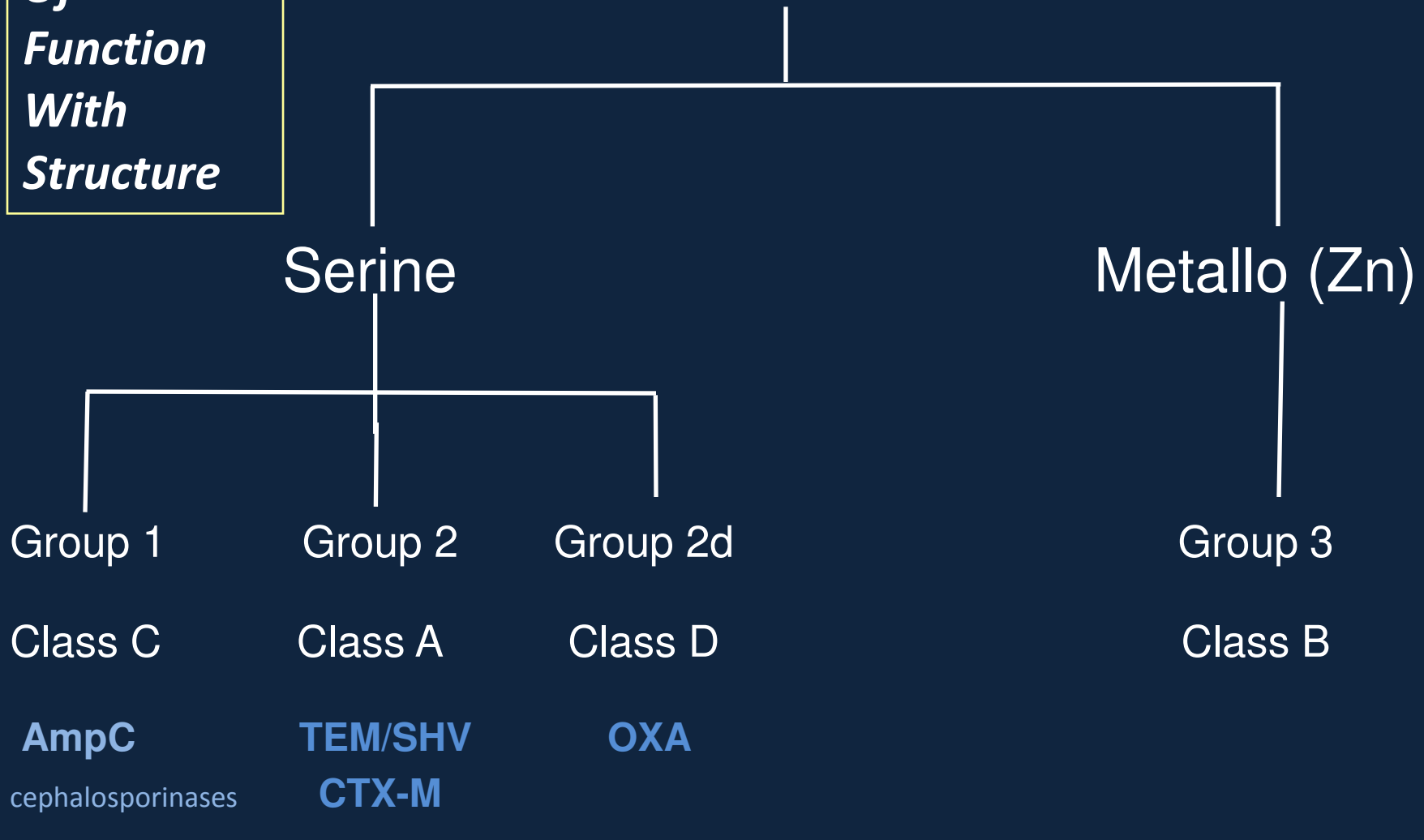
Predicted Resistance Mechanisms (Gram-Negatives)

- "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

Nikaido et al. AAC 27: 393 (1985); Bush et al. AAC **39:121 (1995)**

Correlation Of Function With Structure

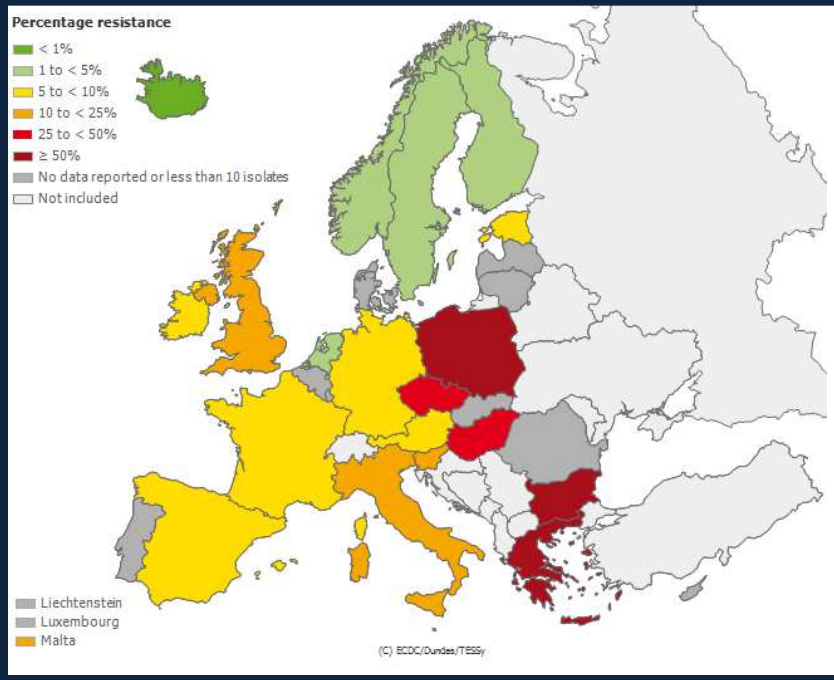
β-Lactamases / ESBLs



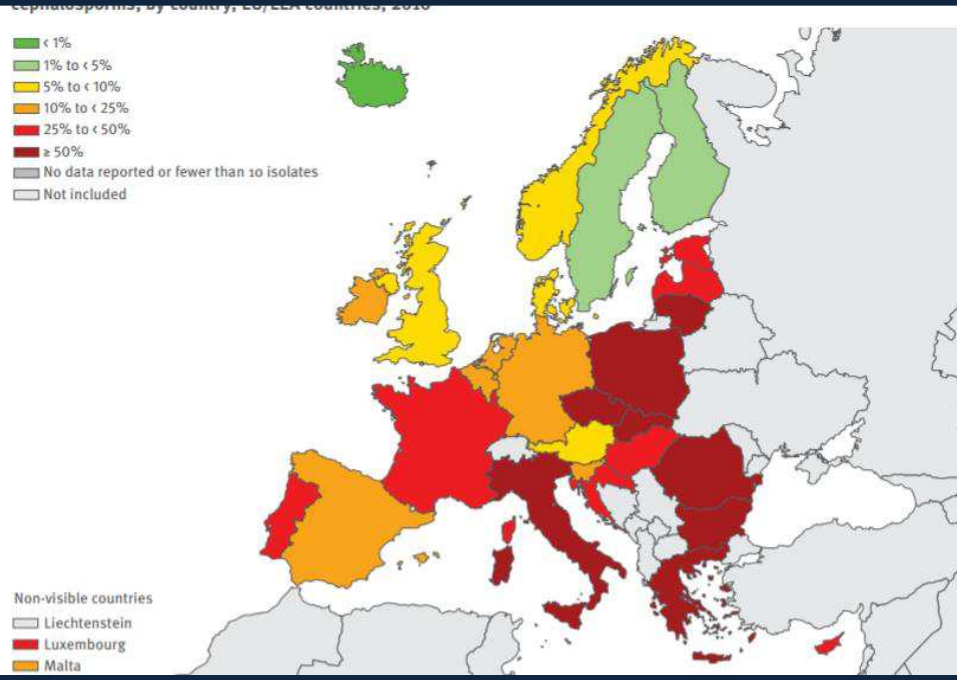
Based on Bush, Jacoby & Medeiros AAC:39:1211 (1995)

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

K. pneumoniae 2005



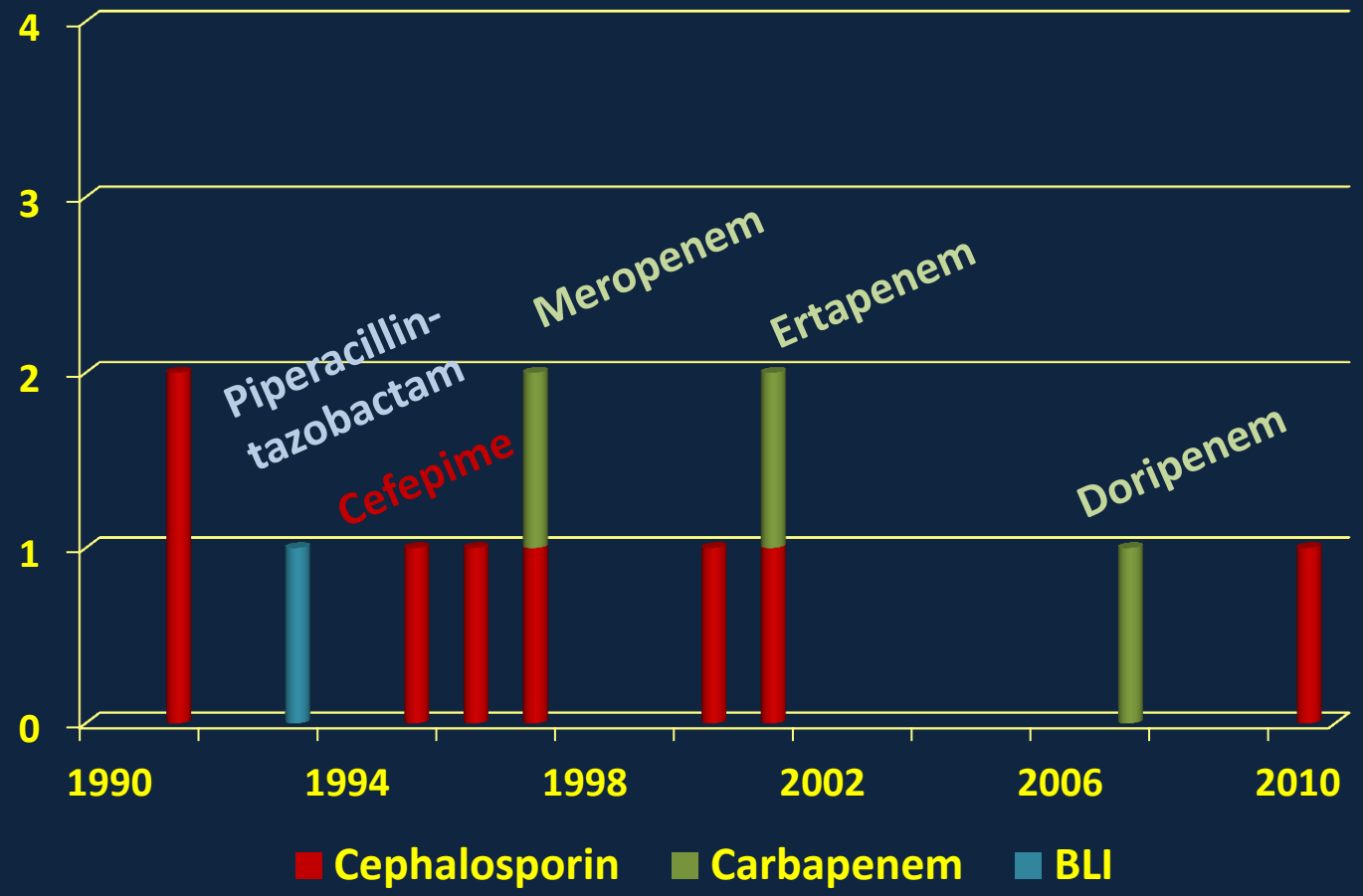
K. pneumoniae 2016



≥50% 25 - <50% 10 - <25% 5 - <10%

Data from EARSS website: http://www.rivm.nl/earss/database/;
http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx

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

Fung-Tomc et al. AAC **33:**498 (1989); Queenan et al. J. Clin. Microbiol. 42:269 (2004)

CARBAPENEM RESISTANCE DUE TO CARBAPENEMASES

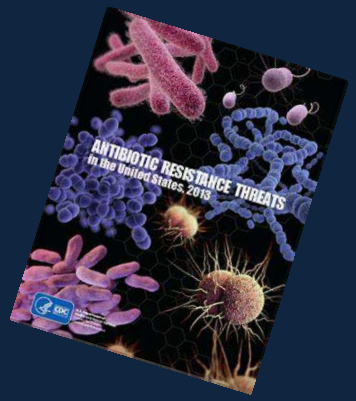
Carbapenemases in Gram-Negative Pathogens

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

Watanabe et al. AAC **35:**147 (1991); Bandoh et al. J Antibiot . **45:**542 (1992); Yigit et al. AAC **45:**1151 (2001)

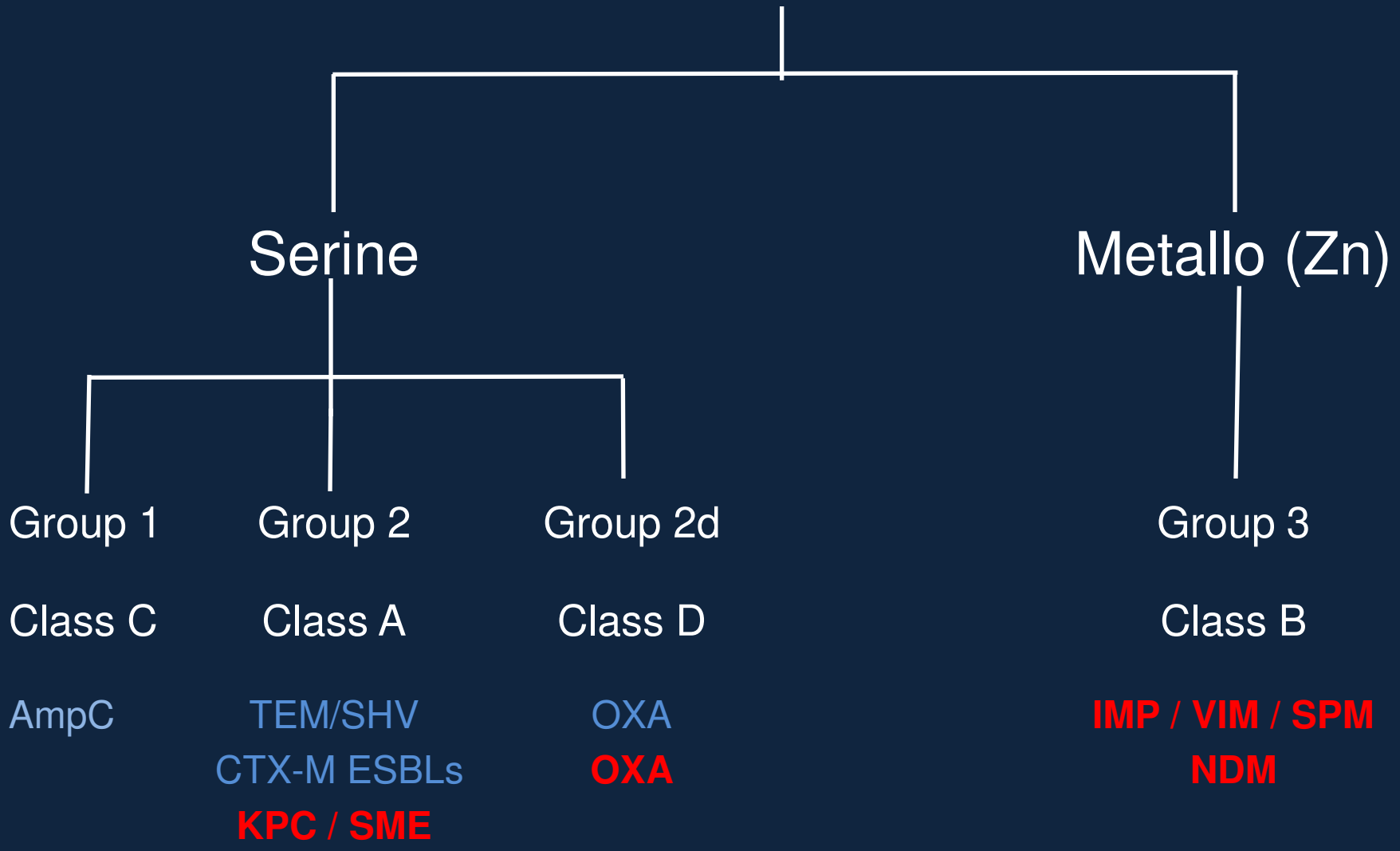
Carbapenem-Resistant *Enterobacteriaceae* (CRE) May be Due to Multiple Factors

- 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)



CDC: ANTIBIOTIC RESISTANCE THREATS in the United States, 2013
http://www.who.int/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1

β-Lactamases / Carbapenemases



Based on Bush, Jacoby & Medeiros AAC:39:1211 (1995); Bush & Jacoby, AAC 54:969 (2010)

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)

Bradford, CID, 39:55 (2004); Bratu, Arch. Int. Med. 165:1430 (2005); Communication - S. Jenkins ,CLSI, June 2007; Mouloudi, Infect. Cont. Hosp. Epidemiol. 31:1250 (2010); Navon-Venezia , AAC *50:3098* (2006); Geffen, JAC 68:719-729; Kitchel, AAC 53: 3365 (2009)

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

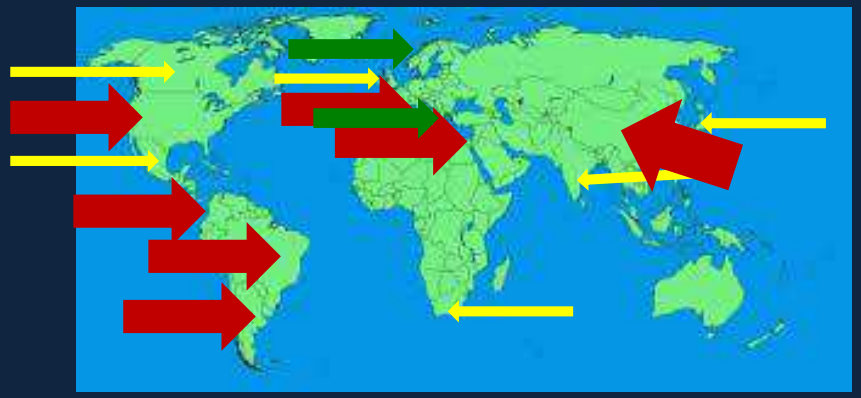
Yong et al. AAC 53:5046 (2009); Walsh et al. Lancet Infect Dis **11:**355 (2011); Kumarasamy & Kalyanasundaram, JAC 67:243 (2012)

Global Prevalence of KPC and NDM Carbapenemases

Sporadic identification

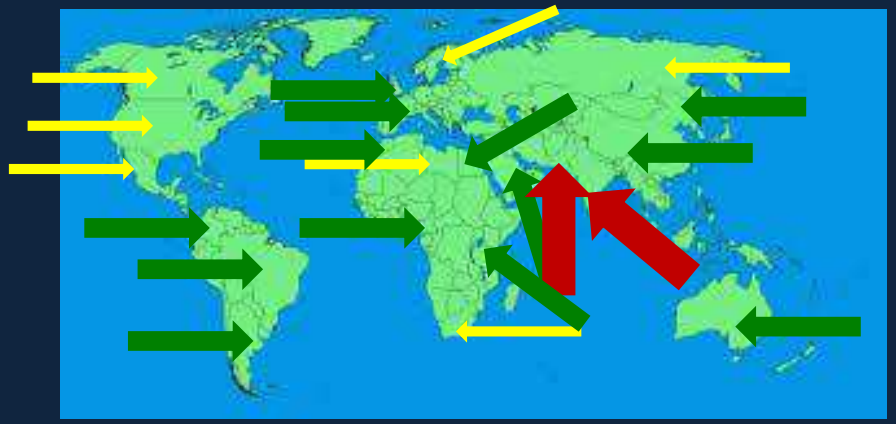
Outbreaks

Endemic production



KPC Production

NDM Production



Drug susceptibility for both KPC- and NDM-producing CRE frequently only

- [Tigecycline]
- Polymyxins /colistin

Based on Munoz-Price et al. The Lancet, 13:785 (2013); Nordmann and Poirel, Clin. Microbiol. Infect. (2014)

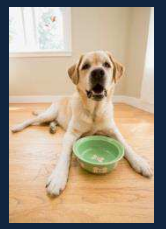
Don't Forget the OXA (Class D) Carbapenemases and Their Impact

- 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

Girlich et al. AAC 48:4217 (2004); Poirel & Nordmann, Clin. Micro Infect. **12:**826 (2006); Poirel et al. , AAC 54:24; Perichon et al. AAC 58:936 (2014); Oteo et al. AAC 57:6344 (2013); Seiffert et al. AAC 58 (2014); Hsieh et al. AAC 58:201(2013)

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

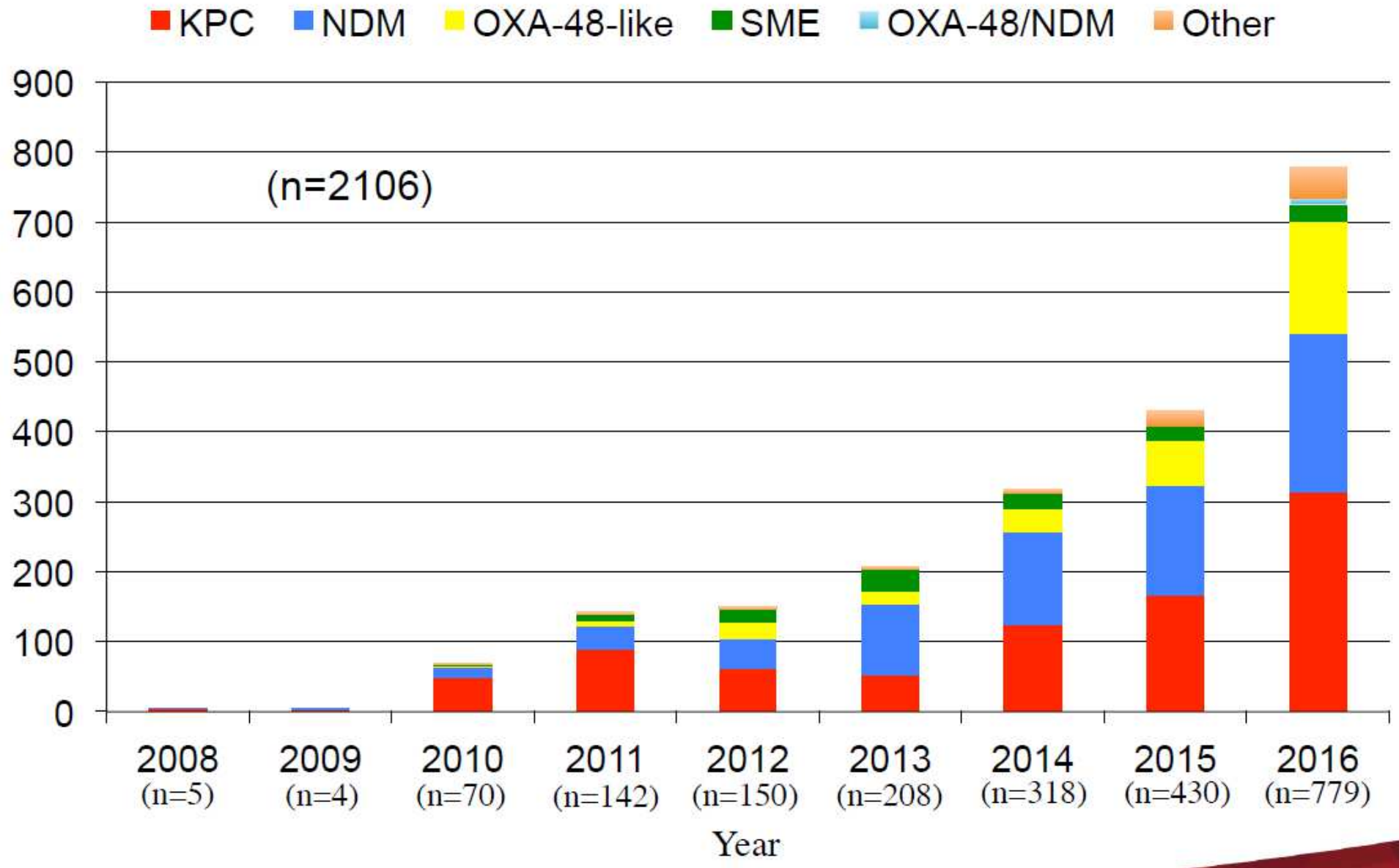


Jones et al. Diagn. Microbiol. Inf. Dis. 78:429 (2011); Ortega et al. JAC 71:2131 (2016)

CARBAPENEMASE IDENTITIES HIGHLY DEPENDENT ON COUNTRY OF ISOLATION

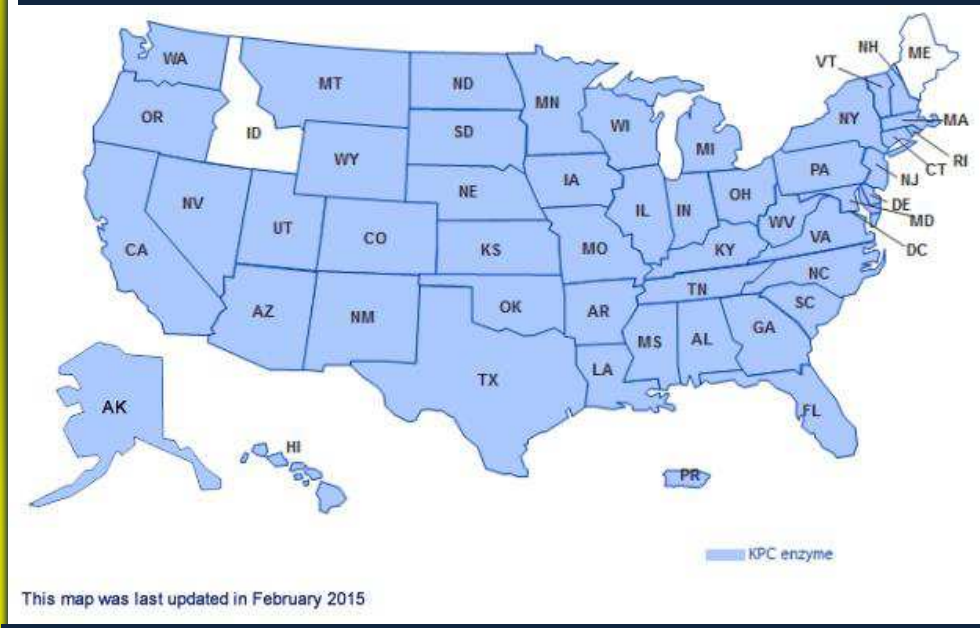
CPE in Canada: CPHLN Data

Courtesy of Mike Mulvey of the Public Health Agency of Canada

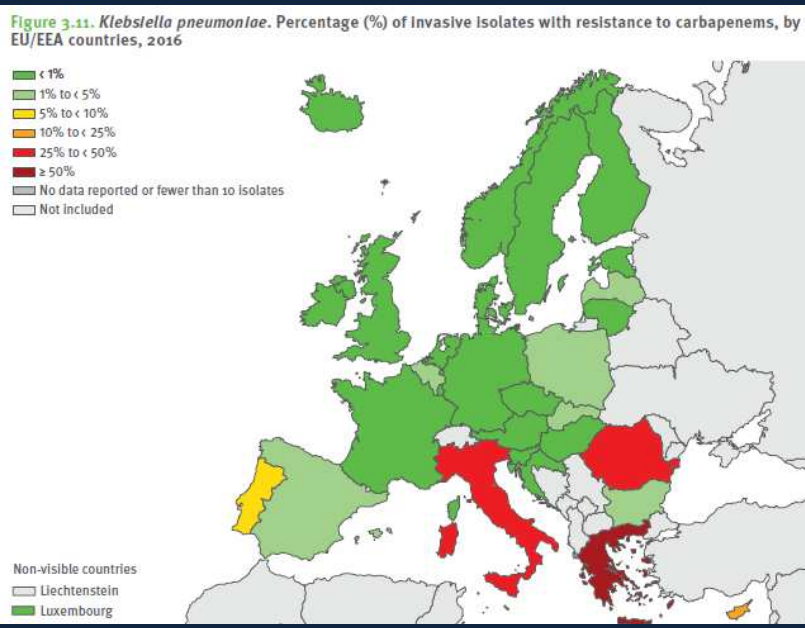


Prevalence of Carbapenem Resistance

KPC carbapenemases reported in the United States



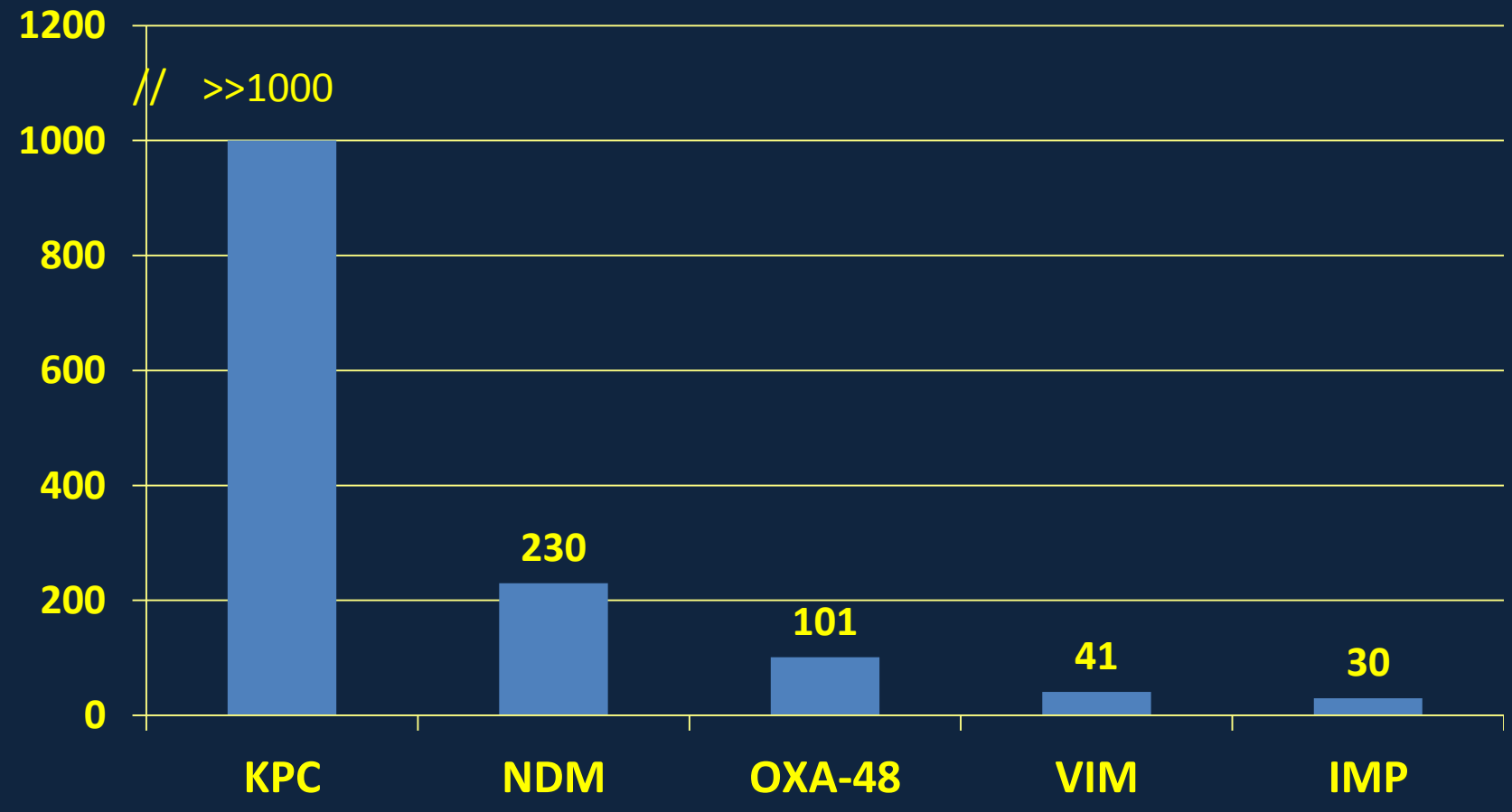
Carbapenem resistance in *Klebsiella* -- Europe (2016)



>50% 25-50% 5-10 %1-5% <1%

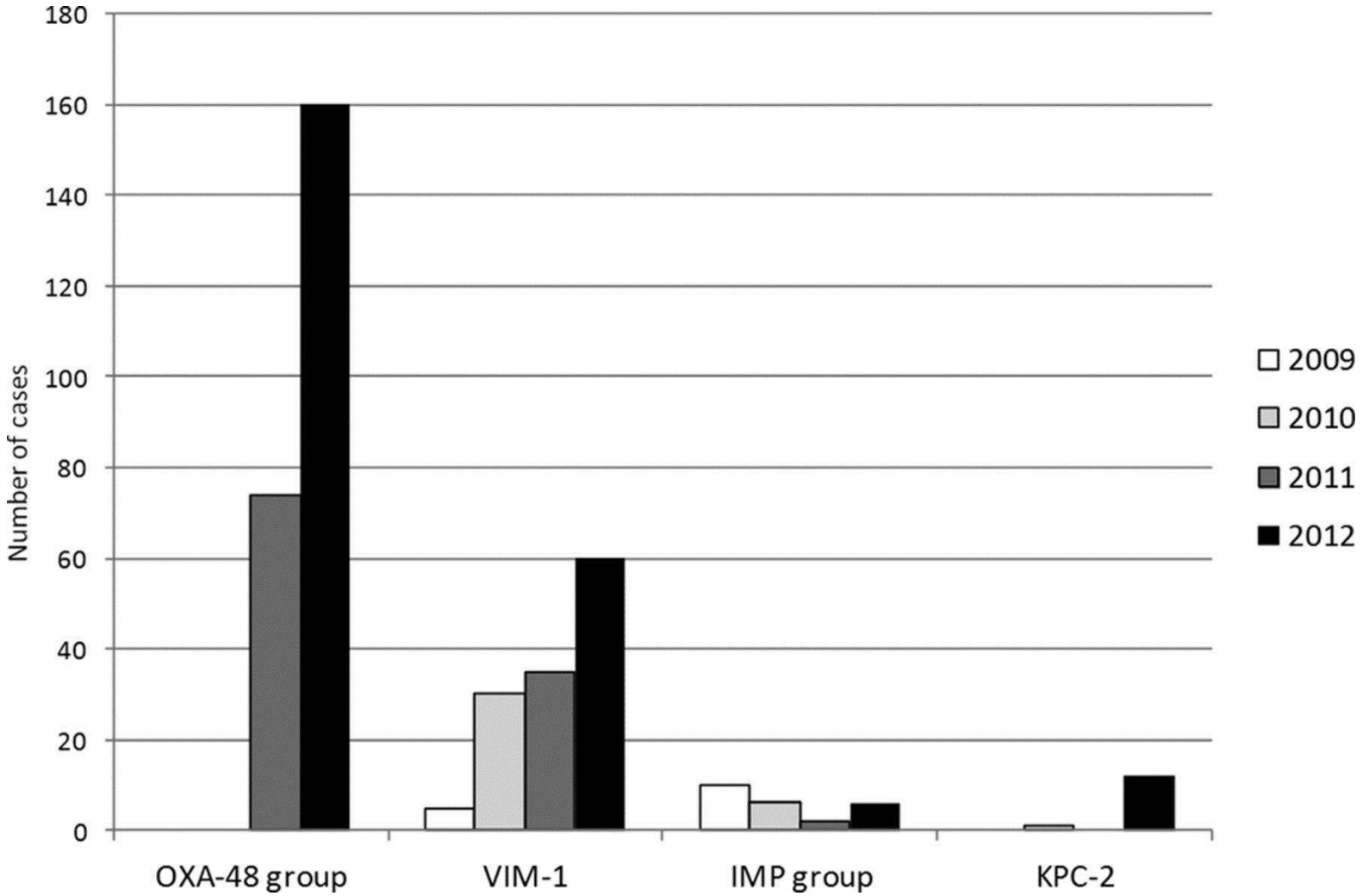
http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html; http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial_resistance/database/Pages/map_reports.aspx

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



https://www.cdc.gov/hai/organisms/cre/trackingcre.html

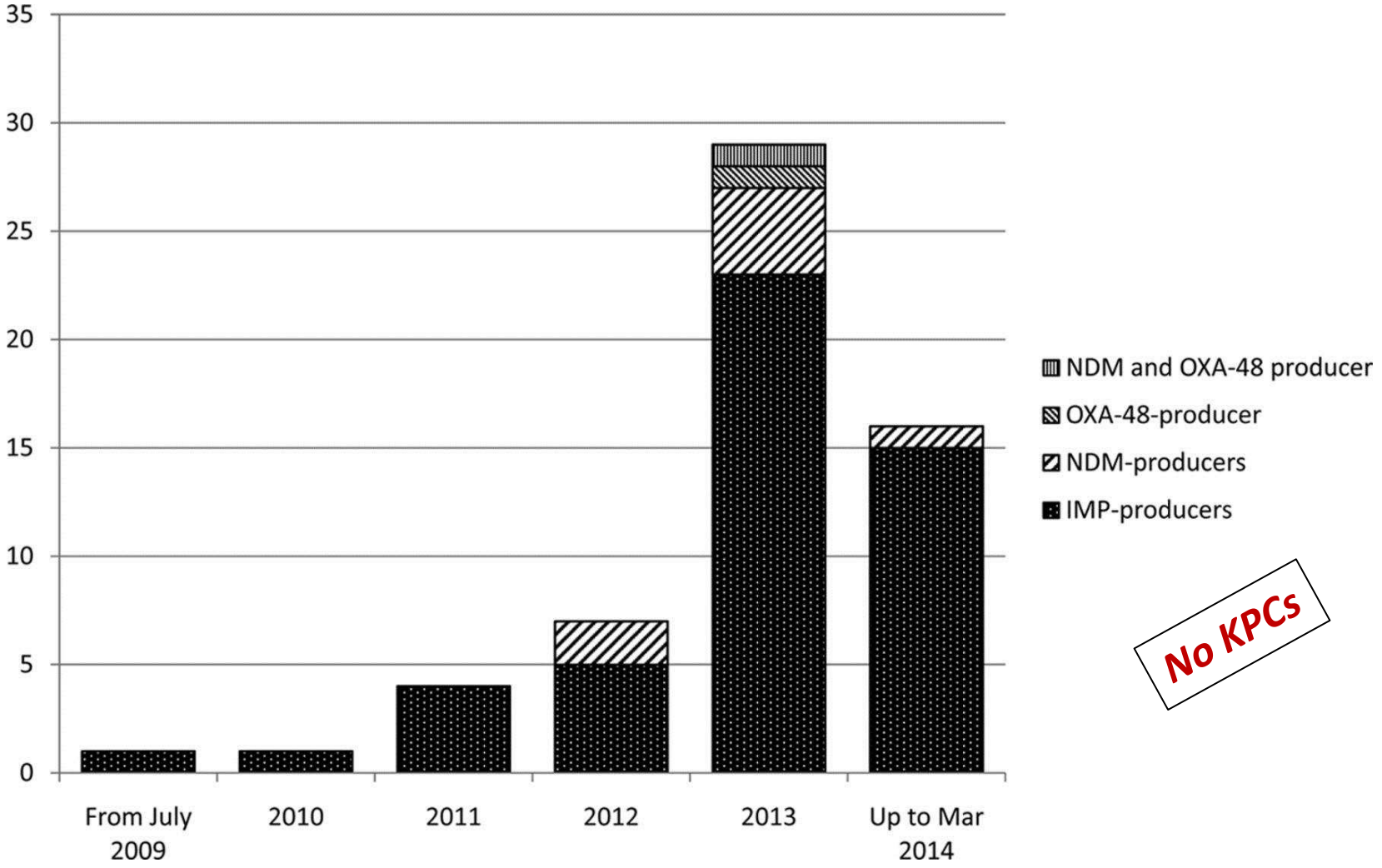
Yearly evolution (2009–2012) of carbapenemase-producing Enterobacteriaceae in Spain and number of individual hospitals reporting cases to the national surveillance program of the Instituto de Salud Carlos III.



Jesús Oteo et al. Antimicrob. Agents Chemother. 2013;57:6344-6347

Antimicrobial Agents and Chemotherapy

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



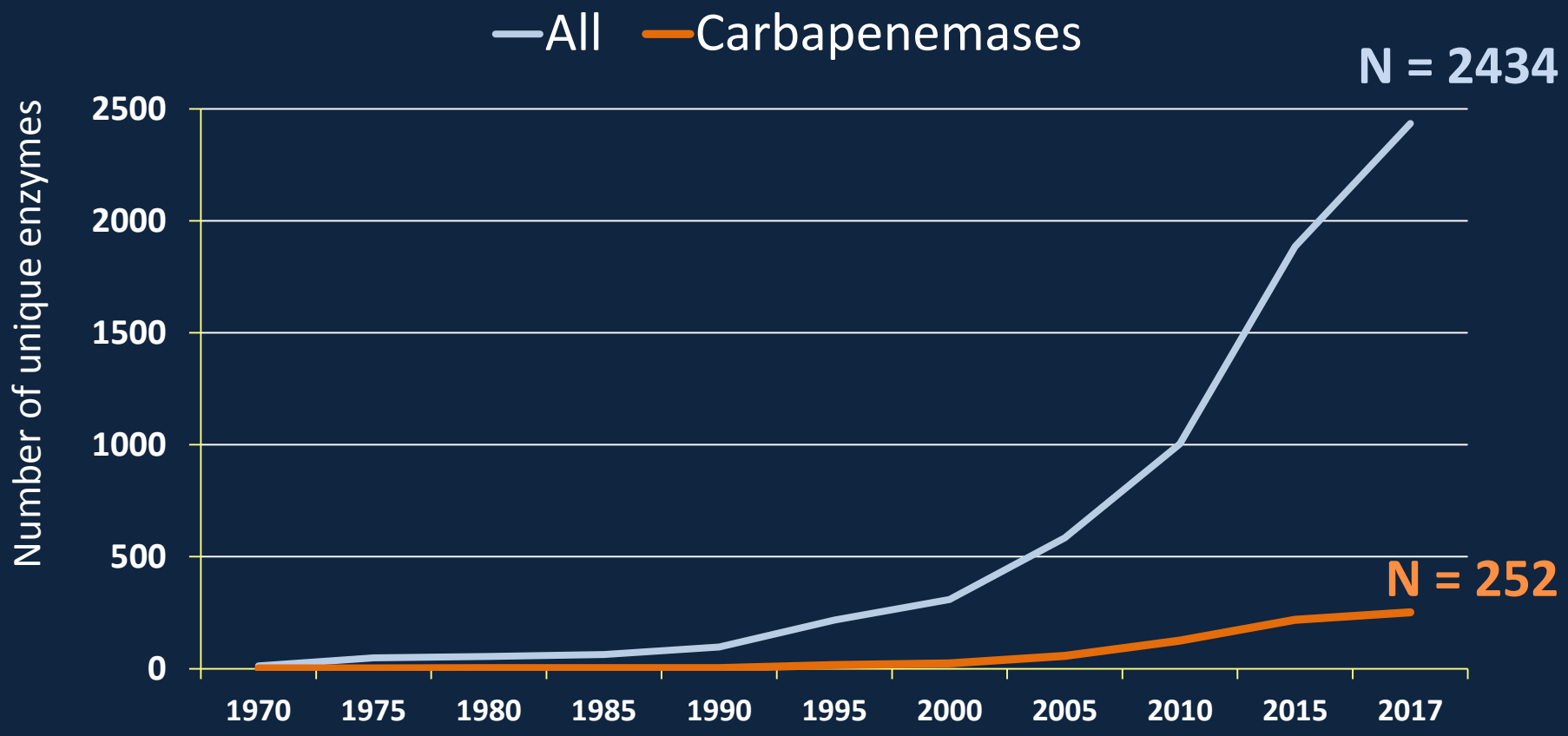
Hanna E. Sidjabat et al. Antimicrob. Agents Chemother. 2015;59:4059-4066

Antimicrobial Agents and Chemotherapy

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 β-Lactamases



Updated by Bush. From Bush, Annals, NYAS 1277: 84 (2013) and NCBI compilations (Oct. 2017)

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

- Carbapenemases almost always are produced together with at least one other β-lactamase
- Eight β-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)

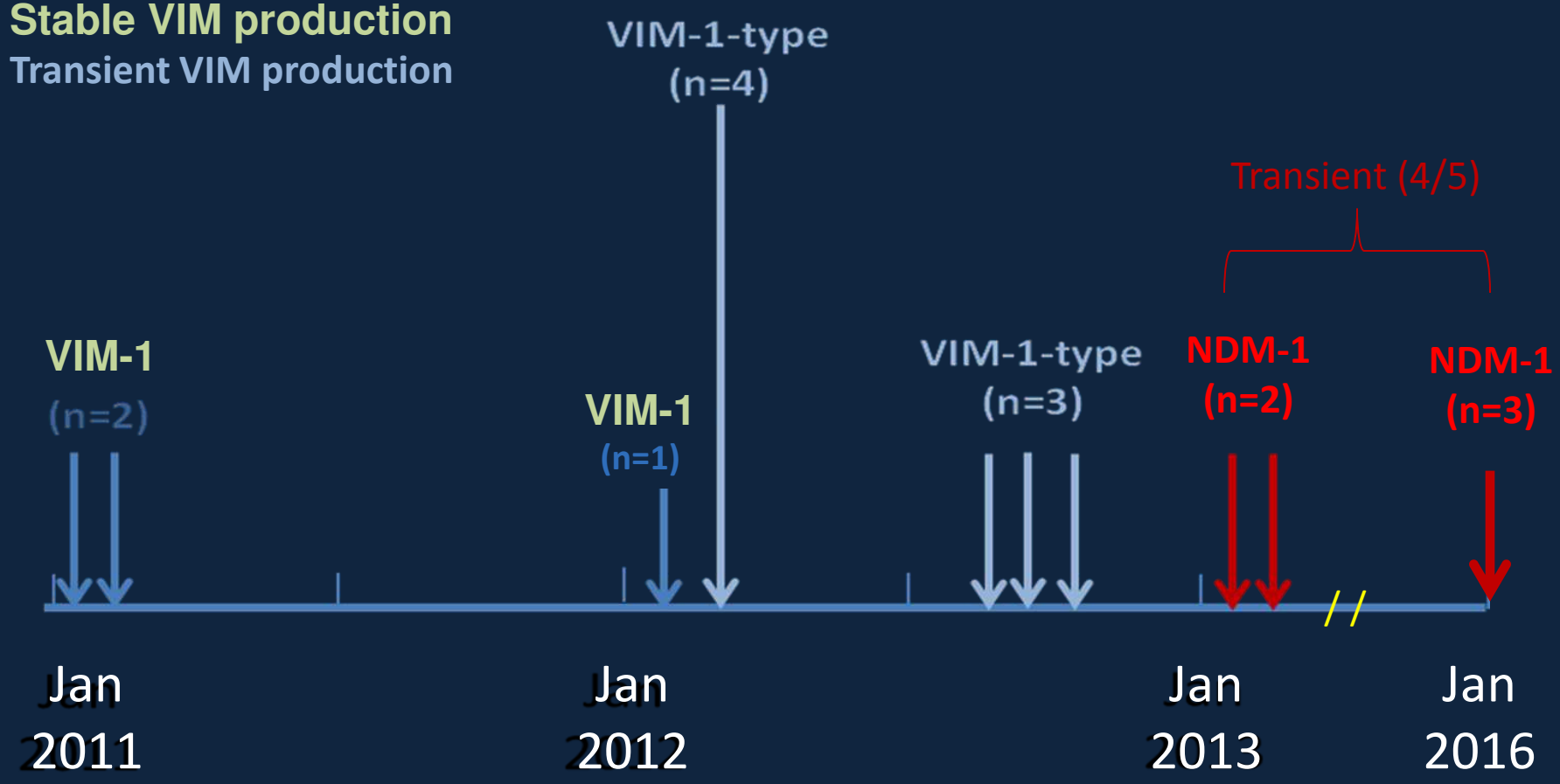
Co-Production of Carbapenemases with Other Plasmid-encoded β-Lactamases (Central Indiana)

β-Lactamase	*E.. cloacae* (n=3)	*E. coli* (n=5)	*K. pneumoniae* (n=96)	*S. marcescens* (n=6)
KPC-2	0	1	15	0
KPC-3	3	4	80	3
KPC-3 + VIM-1	3	0	(4)*	0
KPC-3 + NDM-1	0	0	2	0
SME-1	0	0	0	3
KPC + SHV (plasmid)	2	4	70	2
KPC + TEM	3	5	90	3
KPC + CTX-M-15	0	4	5	0
KPC + TEM + SHV + CTX-M-15	0	4	2	0
KPC + TEM + SHV + OXA	3	4	21	0

*VIM-encoding plasmids lost on storage

Zhang et al., AAC 61:e00389-17 (2017)

Timeline for MBLs in Indianapolis



Bush lab: Kashikar et al. ICAAC 2015; Tulpule ASM Microbe 2017

Characterization of *K. pneumoniae* Isolates that Originally Produced both KPC-3 and MBL

KP#	MBL	TEM	SHV	OXA	ST	PFGE	Plasmids
85	VIM	X			258	KPA1	8, 50
86	VIM	X			258	KPA1	12, 50, >50
49	VIM	X	X	X	258	KPA2	4, 9, 50
83	VIM	X	X		258	KPA2	4, 5, 10, 50, >50
80	VIM	X			258	KPA3	50
84	VIM	X	X		258	KPA3	6, 9, 50
88	NDM		X		674	KPB	2, 3, 5, 6, 50

All isolates were from different health care centers in central Indiana, except for 85 and 86.

A. Tulpule et al. , 2017 ASM Microbe

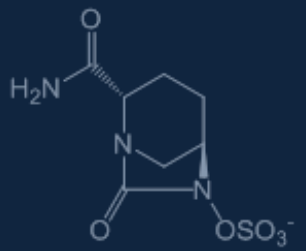
WHERE DOES THIS LEAVE US?

End Result is What We See Today

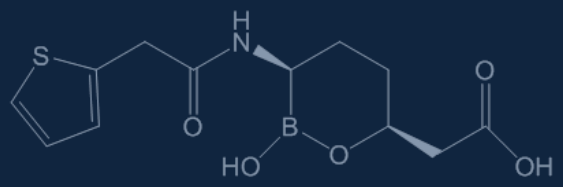
- Environmental *bla* genes in animals and water sources that are highly mutable and transferable among species
- β-lactam pressure in community and hospitals, with carbapenems selecting for multidrug resistance in the most fit pathogens
- Patients with few therapeutic options

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?



Bush, ACS Infect. Dis. 2017

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.