CARBAPENEMASES WHAT THEY ARE AND WHAT THEY DO

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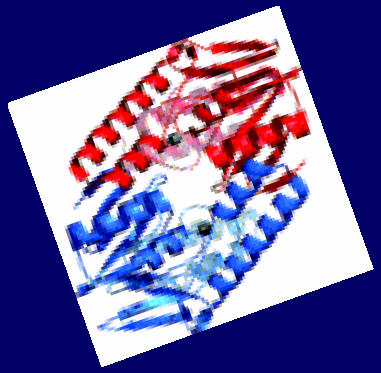

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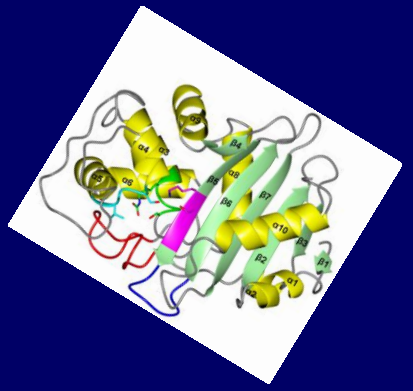


Financial Disclosures

Retirement income

- Johnson & Johnson
- Bristol-Myers Squibb
- Wyeth (Pfizer)

Consultant and research income received

(June 2012 to June 2013)

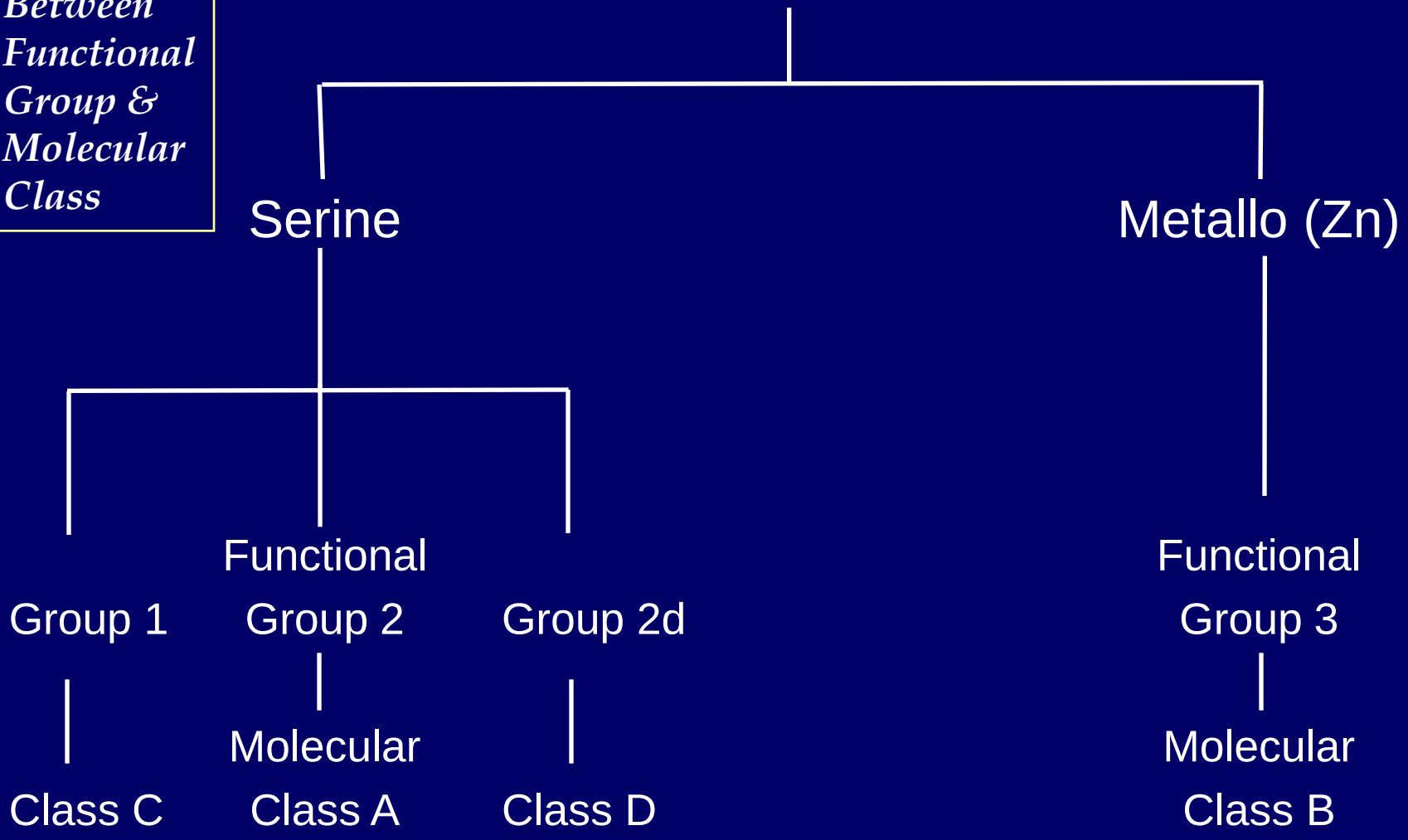
- AstraZeneca
- Cubist
- Fedora
- Forest
- Medivir
- Merck

Carbapenemases

- What are they?
- What are the most common types?
- What molecular characteristics are responsible for their phenotypic behavior?

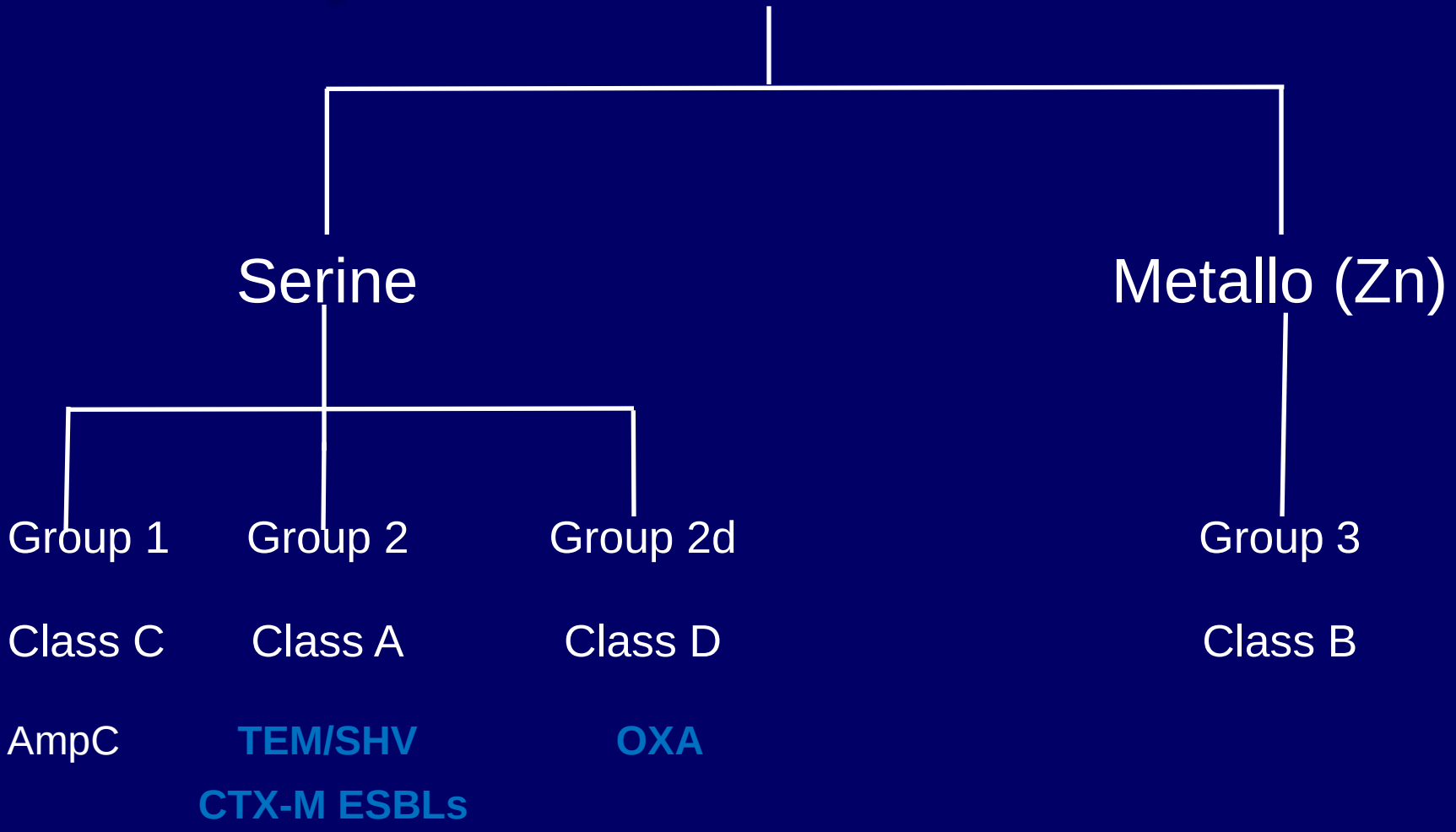
β-Lactamases

Relations Between Functional Group & Molecular Class



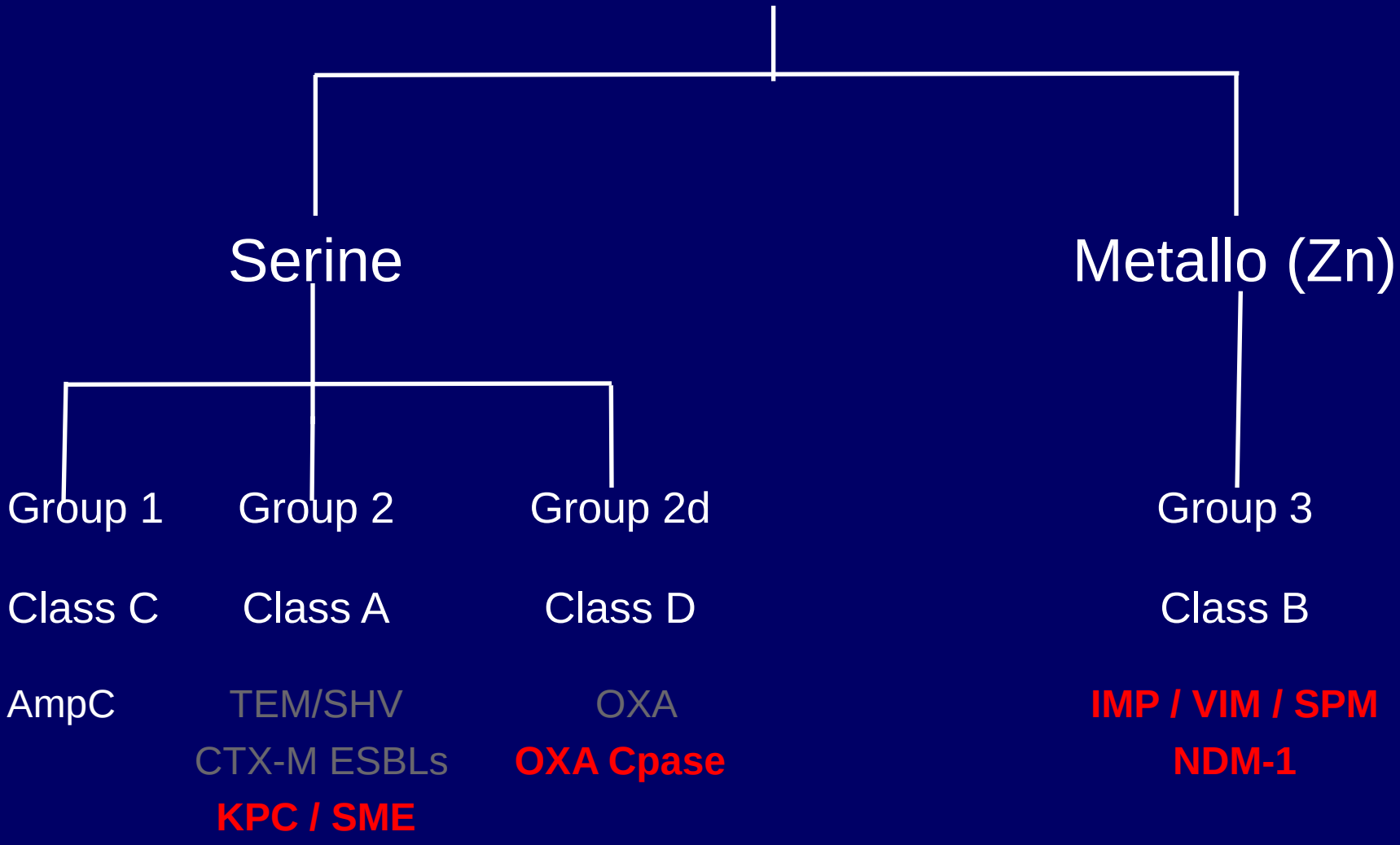
Based on Bush, Jacoby & Medeiros AAC:39:1211 (1995)

β-Lactamases / ESBLs



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

β-Lactamases / Carbapenemases



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

Distinguishing Features of Carbapenem-Hydrolyzing β-Lactamases

- Serine carbapenemases
  - Hydrolyze cephalosporins, carbapenems, monobactams
  - Inhibited (*in* vitro) by clavulanic acid, tazobactam and boronic acid
- Metallo-β-lactamases
  - Most can hydrolyze everything except monobactams
  - Not inhibited by clavulanic acid
  - Inhibited by metal chelators such as EDTA
  - Diagnostic Etest with carbapenem/EDTA not always predictable
    - EDTA may permeabilize membranes to allow carbapenem easier access to AmpC hydrolysis, especially in derepressed mutants

Increase in Major Carbapenemase Families

	Enzyme type	Number in class	
		1995	2010
Serine	SME	1	3
	IMI	1	2
	NMC	1	1
	KPC	0	10
	GES	0	16
	OXA	0	37
Metallo-(Plasmid)	IMP	1	28
	VIM	0	26

http://www.lahey.org/Studies ; Bush, Jacoby & Medeiros AAC:39:1211 (1995); Bush & Jacoby, AAC 54:969 (2010)

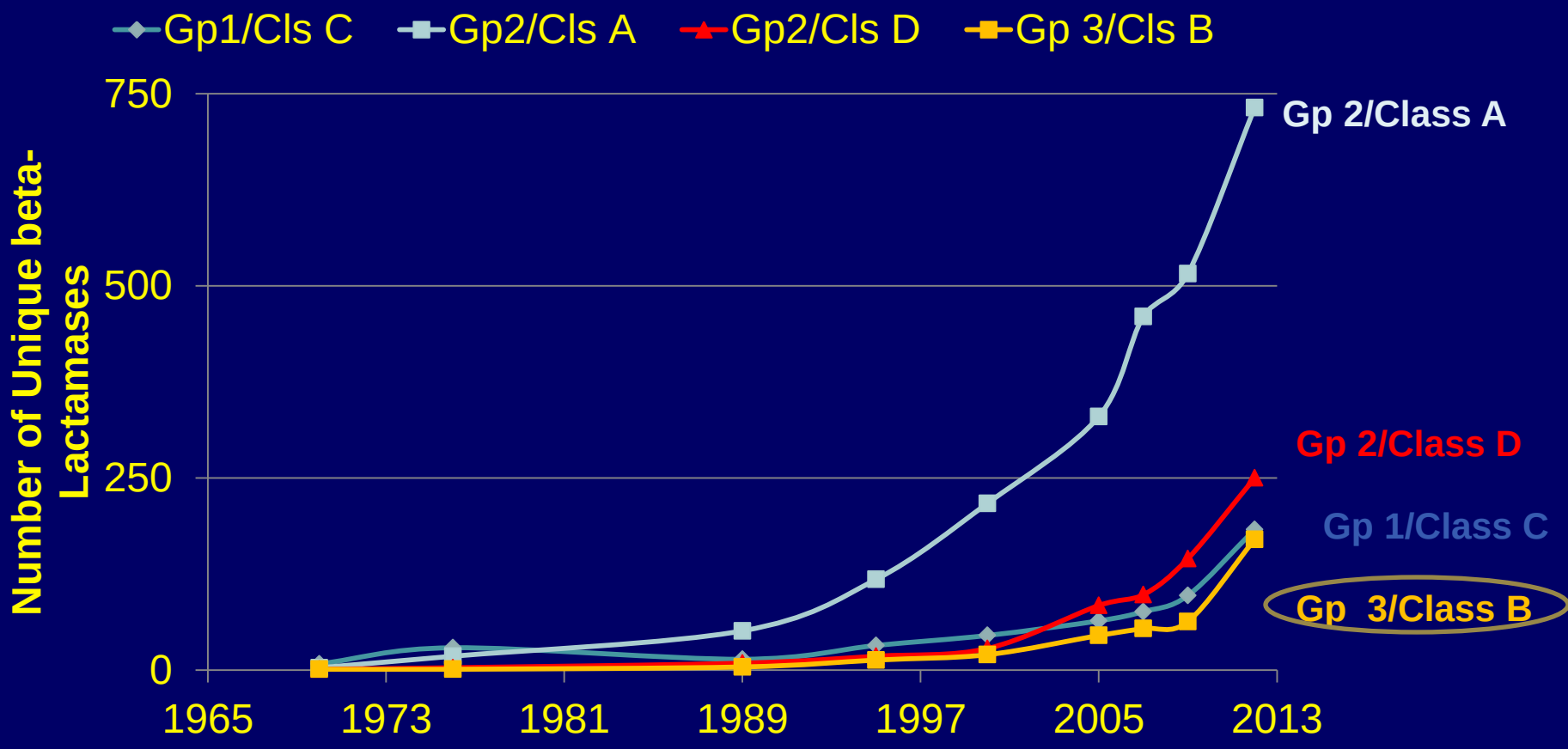
Major Plasmid-Encoded Carbapenemase Families

	Number in Class			
Enzyme type	1961	2000	2011	June 2013
KPC	0	1	10	14
OXA	0	3	40	>60*
IMP	0	3	31	44
VIM	0	2	32	39
NDM	0	0	2	9

*Total number of OXAs = 347, many from *Acinetobacter* spp.

G. A. Jacoby http://www.lahey.org/Studies/ 05JUn2013; Bush & Jacoby. AAC 54:969 (2010)

Increasing Numbers of β-Lactamases by Class



Compilation of Unique β-Lactamase Sequences from Natural Isolates

Based on Bush & Fisher Annu. Rev. Microbiol. 2011. 65:455; Bush, Ann. NY Acad. Sc. (2013)
Jacoby & Bush: http://www.lahey.org/Studies/

Serine Carbapenemases

- **Functional group 2f / Molecular class A**
- **Chromosomal often from environmental sources**
  - **Species-specific**
  - **IMI and NMC isolated from *Enterobacter* spp.**
  - **Most important are SME-1, -2, -3 from *S. marcescens***
- **Plasmid-encoded**
  - **Most common are the KPC enzymes**
    - ***Klebsiella pneumoniae carbapenemase***
  - **Global dissemination**
  - **May occur in clonal outbreaks (ST258)**

Yang et al. AAC 34:755 (1990); Queenan et al. AAC 44:3035 (2000); Yigit et al. AAC 47:3881 (2003)

Resistance Profiles Associated with SME and KPC Serine Carbapenemases

	MIC (μg/mL)	
Antibiotic	*S. marcescens* (SME-1)	*K. oxytoca* (KPC-2)
Ampicillin	>16	>64
Cefuroxime	>16	Not tested
Cefoxitin	>16	>64
Ceftazidime	<=1	>64
Cefotaxime	<=2	>64
Cefepime	<=2	>32
Aztreonam	>16	>64
Imipenem	>8	32
Meropenem	>8	32
Piperacillin-tazobactam	Not tested	>128/4
Tigecycline	<=1	Not tested
Gentamicin	<=1	8

Bush et al. IJAA 41:1-4 (2013); Yigit et al. AAC 47:3881 (2003)

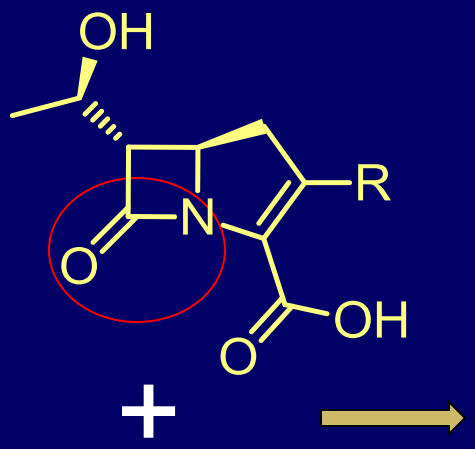
Hydrolysis of Key Substrates by Serine β-Lactamases

	k_{cat} (sec^{-1}) *		
Antibiotic	**TEM-1**	**SME-3**	**KPC-2**
Penicillin G	1600	6.3	530
Cephaloridine	1500	1370	Not tested
Cefotaxime	9	1.5	22
Ceftazidime	0.3	0.14	≤ 0.12
Aztreonam	<1	148	30
Imipenem	<1	322	15
Meropenem	Not tested	3.2	4.0

*Molecules of substrate hydrolyzed per sec per molecule of enzyme

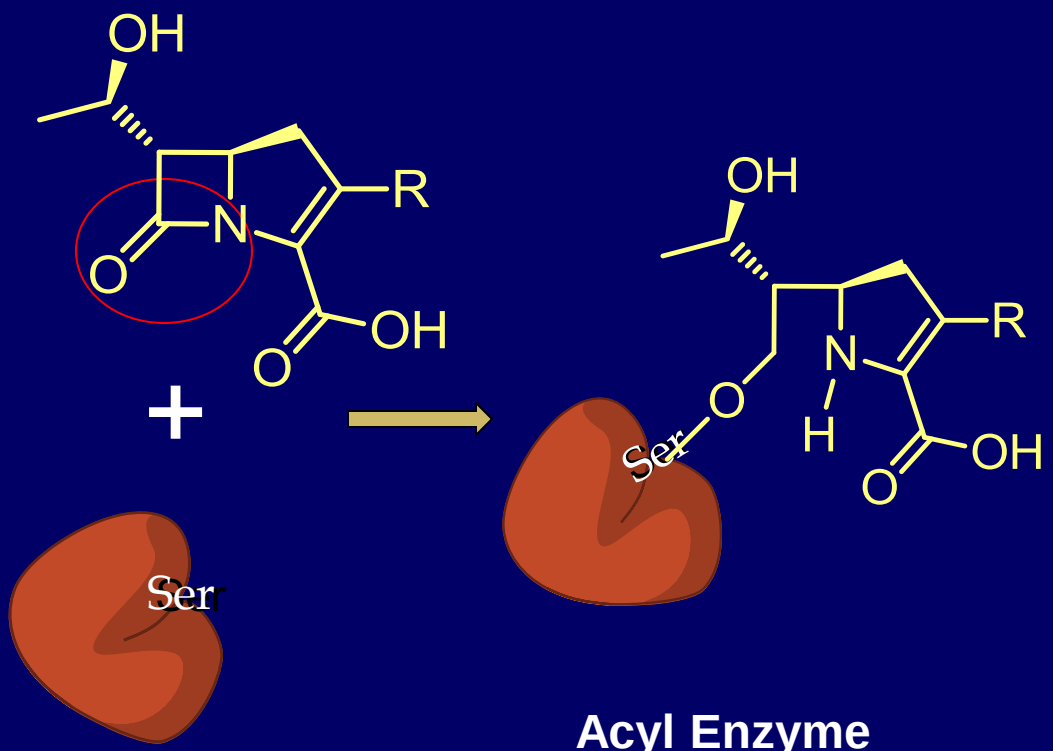
Raquet et al. J. Mol. Biol. 244:625 (1994); Queenan et al. AAC (2006); Yigit et al. AAC 47:3881 (2003)

Hydrolysis of a Carbapenem by a Serine β-Lactamase



Carbapenem + β-Lactamase

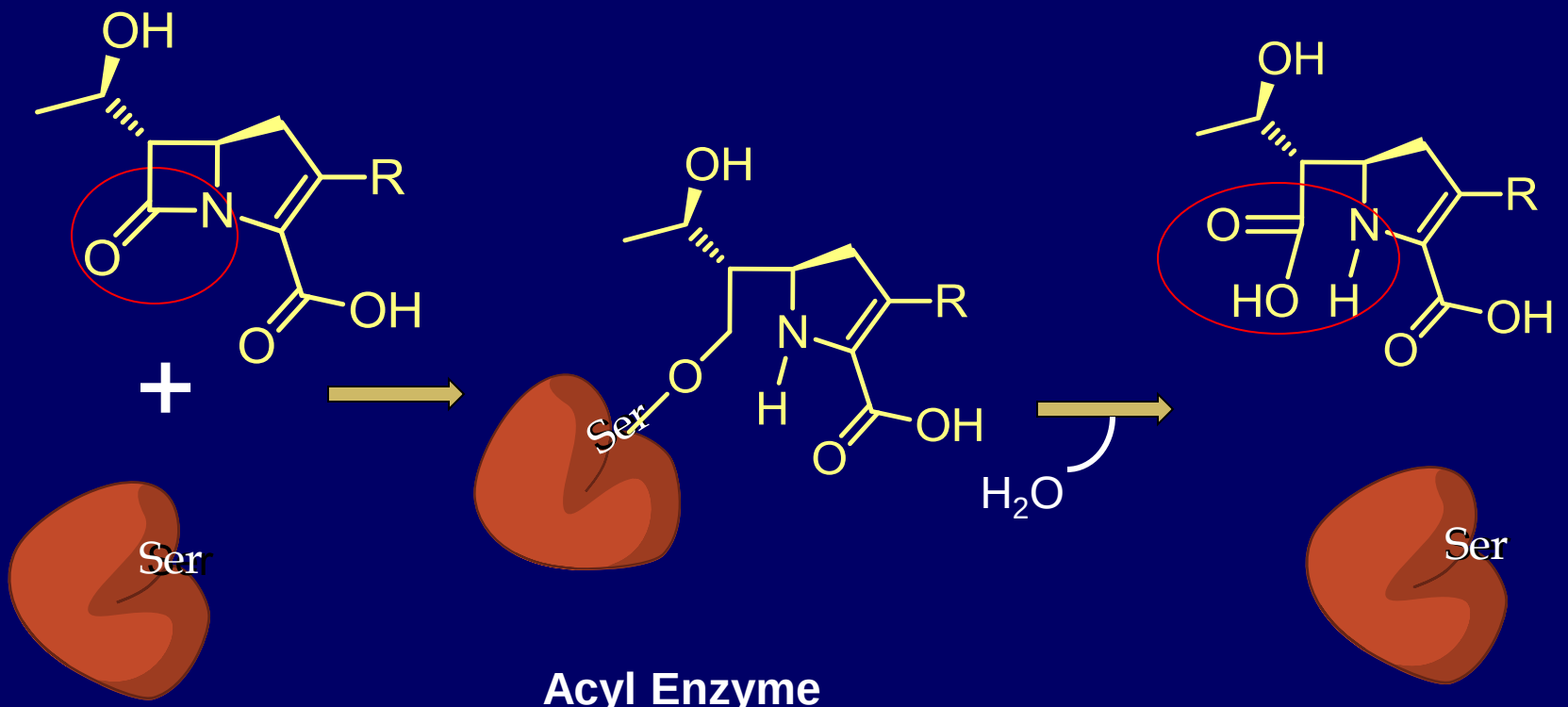
Hydrolysis of a Carbapenem by a Serine β-Lactamase



Carbapenem + β-Lactamase

Acyl Enzyme

Hydrolysis of a Carbapenem by a Serine β-Lactamase



Acyl Enzyme

Carbapenem + β-Lactamase

Hydrolysis product (inactive microbiologically) + β-Lactamase

KPC Carbapenemases (Early History)

- KPC- family of serine carbapenemases (transferable)
  - High level of enzyme production encoded in integrons on plasmids
  - Major resistance problem in Brooklyn/New York City (*K. pneumoniae*)
- Initial KPC-1 isolate from *K. pneumoniae* in North Carolina (1997) now known to be KPC-2 (Yigit et al., AAC45:1151, 2001, + Erratum))
- KPC-3 identified in New Jersey and New York in early 2000s
- Each enzyme differs by one amino acid
- Generally appear with multiple enzymes per isolate
- Marketed β-lactamase inhibitors are not effective *in vivo*
  - Promising inhibitory activity by avibactam and RPX7009
  - Boronic acid inhibition used as a diagnostic test

Inhibitor Resistance of KPC-2

- KPC-2 is considered to be poorly susceptible to current inhibitors
- Inhibitor hydrolysis occurs before inactivation
- Tazobactam is the most effective of the three inhibitors tested

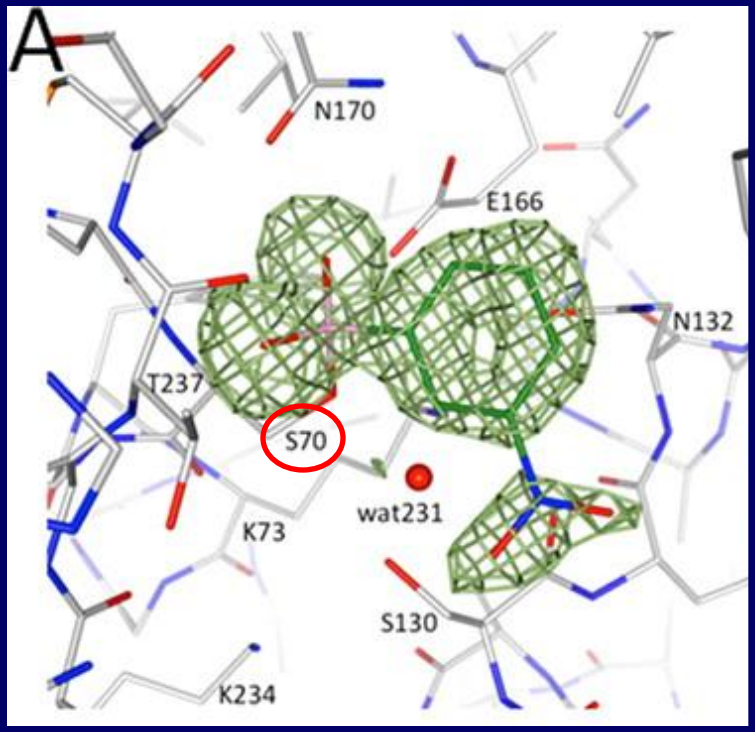
	Partition ratios *(kcat/kinact)*		
Enzyme	Clavulanic acid	Sulbactam	Tazobactam
KPC-2	2,500	1,000	500
TEM-2*	115	7,000	125

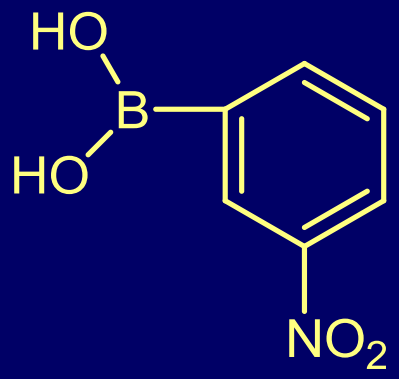
- Turnover of numbers for KPC-2 before inactivation were higher than for TEM-2 for clavulanic acid and tazobactam.

*Turnover numbers before inactivation: Fisher et al. Biochemistry 17: 2180 (1978). Fisher et al. Biochemistry 20: 2726 (1981). Bush et al. AAC 37:851 (1993).

Papp-Wallace KM et al. AAC 54:890-897 (2010)

Electron Density Map of the Serine β-Lactamase Inhibitor 3-Nitrophenyl Boronic Acid in the Active Site of KPC-2





- Reversible covalent interaction with active site serine
- K_m = 1.0 μM

Ke W et al. Antimicrob. Agents Chemother. 2012;56:2713-2718

Antimicrobial Agents and Chemotherapy

Identification of KPC Carbapenemases

KPC	Species	Year	Location
1 / 2	*K. pneumoniae/K. oxytoca*	1996, 1998	East coast USA
3	*K. pneumoniae*	2000	New York
4	*Enterobacter cancerogenus*	2003	Scotland
5	*P. aeruginosa*	2006	Puerto Rico
6	*K. pneumoniae*	2003	Puerto Rico
7	*K. pneumoniae*	2007	Ohio
8	*K. pneumoniae*	2008	Puerto Rico
9	*E. coli*	2009	Israel
10	*A. baumannii*	2009	Puerto Rico
11	*K. pneumoniae*	2010	Greece
12-15	*No data*	2011-2013	No data

Summarized on: http://www.lahey.org/Studies (Jacoby & Bush)

OXA-Carbapenemases

- Most diverse family of carbapenemases
  - 9 remotely related sub-families (36-50% homology common)
- Non-fermenters are frequent hosts
- Plasmid-encoded enzymes
  - Initial OXA carbapenemase in *Acinetobacter baumannii* (1985 Scottish isolate) = ARI-1 or OXA-23
    - Now in England, Brazil, Singapore, Polynesia, Korea and China
  - OXA-58 from central and southern Europe, Argentina, Kuwait
- Chromosomal OXA-51/69-like enzymes in all *A. baumannii*
- OXA-23 outbreak in MDR - *A. baumannii* clones in Rio de Janeiro
  - January 2006-September 2007: 110 isolates; 5 genotypes
- Genomic sequencing of *Acinetobacter* spp. from various labs has added >85 new variants in the past 6 months

Perez et al. AAC 51:3471 (2007); Queenan & Bush, Clin Micro Rev 20:440 (2008); Carvalho, Carvalho-Assef, Peirano, Santos, Pereira, Asensi. Intl. J. Antimicrob. Agents 34:25 (2009).

Metallo-β-lactamases

- Functional group 3, Molecular class B
- Chromosomal
  - *Bacteroides* spp., *Bacillus* spp., *Stenotrophomonas maltophilia*
- Plasmid-encoded MBLs first described in Japan in 1990
  - VIM, IMP, and NDM are major families
  - NDM has become one of the most wide-spread carbapenemases globally
- Multiple β-lactamases of different classes usually found in carbapenemase-producing bacteria

Yong et al. AAC 53:5046 (2009); Tzouvelekis et al. Clin. Micro. Rev. 25:682 (2012)

Chromosomal Metallo-β-Lactamases

- All are co-produced with a more efficient β-lactamase
- *Bacillus cereus* –prototypical MBL in the literature
- *Bacillus anthracis* Bla2 [Materon et al. AAC 47:2040, 2003]
  - 92% identity with *B. cereus* 569H MBL
  - Poor affinity for standard substrates
  - Catalytic efficiency (k_{cat}/K_m) 4-fold lower for imipenem than for cefotaxime; at least one order of magnitude lower than for Bla1 penicillin hydrolysis
- *Stenotrophomonas maltophilia* L1
  - The only tetrameric β-lactamase
- *Aeromonas sobria*
  - Asb1, group 1 cephalosporinase [Rasmussen et al. AAC 38:2078, 1994]
  - Asb2, group 2d oxacillinase
  - AsbM1, group 3 MBL [Yang & Bush, FEMS Microbiol. Lttr. 127:193, 1996]

Occurrence of Transferable Metallo-β-Lactamases

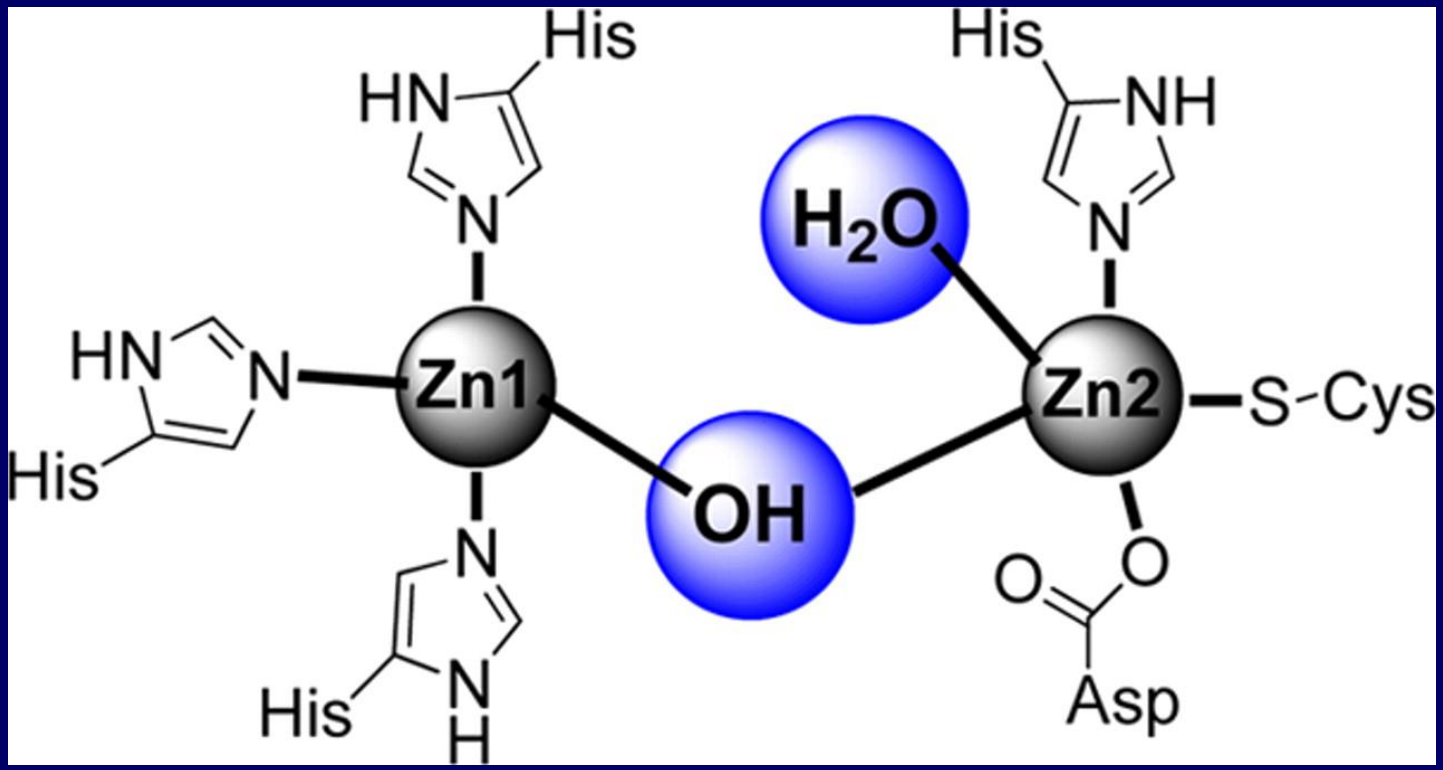
- IMP on transferable element in 1991 in Japan (Bandoh et al., AAC *35:371. 1991)*
- VIM appeared in Italy, France, Greece (1997)
- Eventually, IMP in Europe and VIM in Japan
- SPM-1 appeared to be confined to Brazil or patients associated with Brazil
- Rare in the United States
- MBLs usually are produced in combination with at least one other β-lactamase
  - Plasmids for IMP enzymes may be lost when antibiotic selection pressure removed
  - Metallo-β-lactamases often have lower catalytic efficiencies than other common β-lactamases

Molecular Diversity of MBLs

Molecular Subclasses	Typical enzymes	Substrate Preference	Number of active site Zn^{2+} atoms
B1a	BCII, IMP family, VIM family, SPM-1, CcrA	All β-lactams except monobactams	2
B1b	NDM family	All β-lactams except monobactams	2
B2	CphA, Sfh-1	Carbapenems	1
B3	L1, FEZ-1, CAU-1	All β-lactams except monobactams	2

Bush, J. Infect. Chemother. 2013

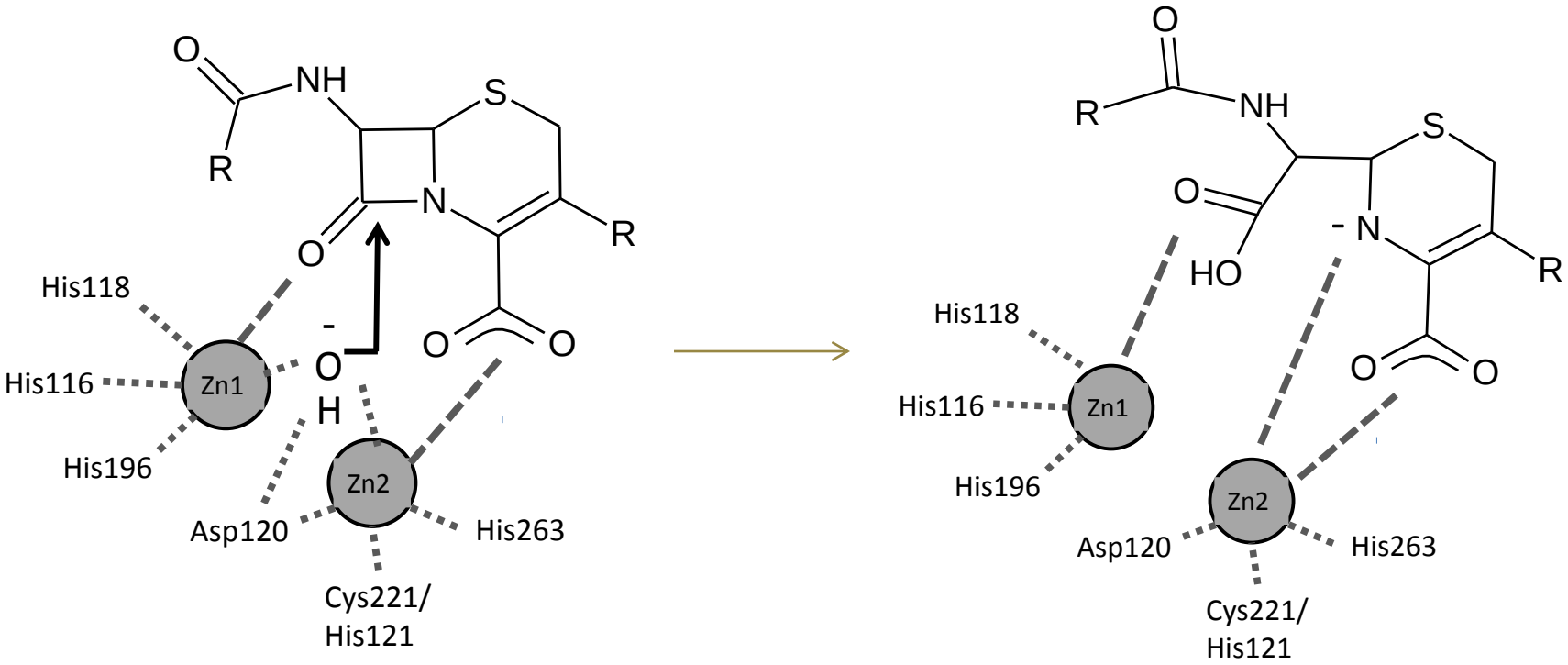
Schematic representation of the Zn2+-binding site of dinuclear subclass B1 metallo-β-lactamases such as *B. cereus* BcII.



Drawz S M , and Bonomo R A Clin. Microbiol. Rev. 2010;23:160-201

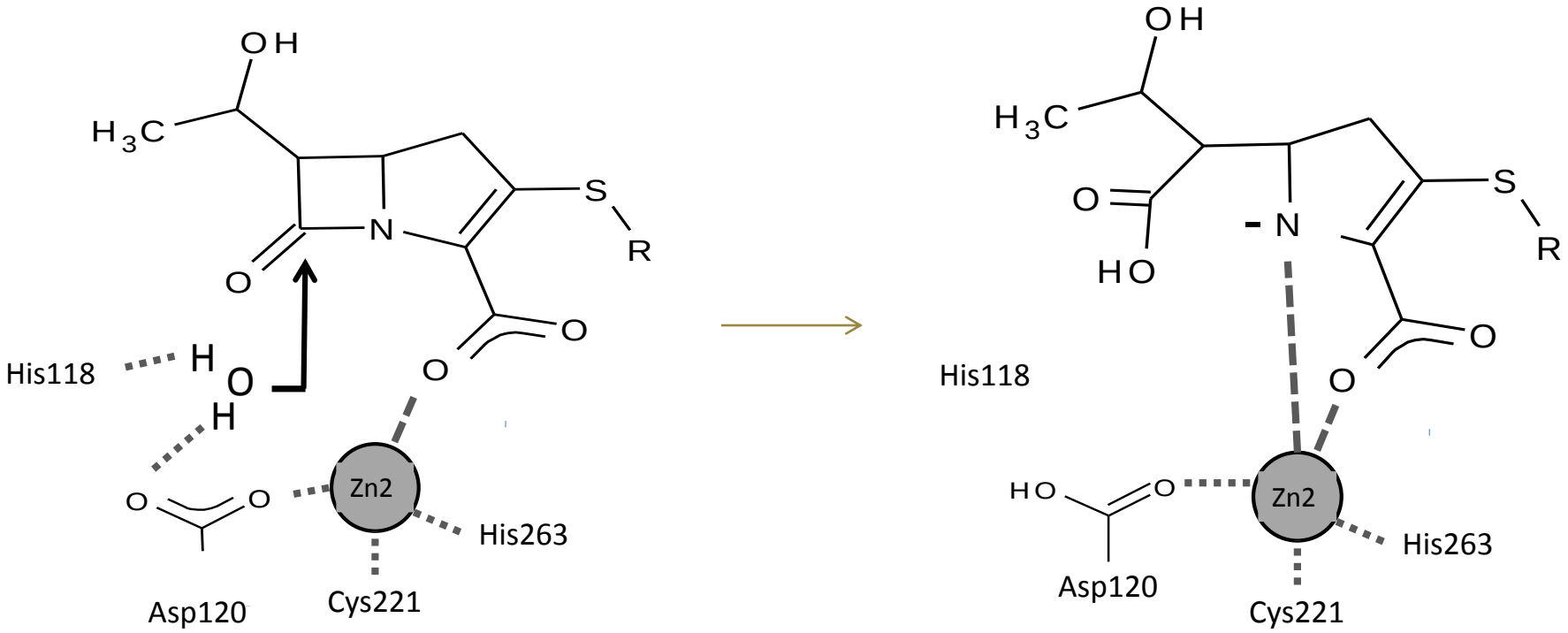
Clinical Microbiology Reviews

Schematic Illustration of Cephalosporin Binding to Di-zinc Metallo-β-Lactamase Active Site (subclasses B1 and B3)



Adapted from T. Palzkill Annals NY Acad Sci. 1277:91-104 (2013)

Carbapenem Substrate and Anionic Intermediate Binding to Mono-zinc Metallo-β-lactamase Active Site (subclass B2).



Adapted from T. Palzkill Annals NY Acad Sci. 1277:91-104 (2013)

Microbiological Impact of Plasmid-Encoded Metallo-β-Lactamases

		MIC in μg/ml				
Organism	Bla	CAZ	CTX	FEP	IMP	MER
P. aeruginosa	None	8	2	2	2	0.5
P. aeruginosa	VIM-1	>128	>128	>128	>128	128
P. aeruginosa	VIM-2	16	256	16	16	8
P. aeruginosa	VIM-4	>512	128	256	256	256
P. aeruginosa	IMP-1	--	--	8	16	--
P. aeruginosa	IMP-9	256	256	--	32	8
P. aeruginosa	IMP-16	--	--	>32	16	
P. aeruginosa	SPM-1	--	--	>32	>32	--

Franceschini et al. AAC 44:3003 (2000); Xiong et al. AAC 50:355 (2006); Marra et al. AAC 50:368 (2006); Fiett et al. AAC 50:880 (2006)

NDM – 1 : The Newest Star in the β-Lactamase Arena

- Swedish patient returning from India where he had been in a surgical ward
- Urinary isolate grew a carbapenem-R *Klebsiella pneumoniae* strain – not responsible for infection
  - ST14 single variant locus of ST15
  - ST15 associated with CTX-M and KPC *Klebsiellae*
- MBL named NDM-1 (New Delhi Metallo-β-lactamase)
- Low identity with other MBLs
  - Most similar MBLs to VIM-1/VIM-2, with 32.4% identity
- Susceptibility profile
  - MICs >32 μg/ml for all β-lactams including carbapenems, also ciprofloxacin
  - Colistin MIC 0.75 μg/ml

Yong et al. Antimicrob. Agents Chemother. 53:5046 (2009)

Unimpressive Hydrolysis Profile of NDM-1

- Hydrolysis profile similar to IMP-1 & VIM-2
  - Lower hydrolysis rates for most substrates
    - NDM-1 : Penicillin /cephalosporin k_{cat} values 1 - 15 sec $^{-1}$
    - IMP/VIM: Penicillin/cephalosporin k_{cat} values 3 - 950 sec $^{-1}$
    - Less efficient hydrolysis of imipenem than IMP-1 and VIM-2 ; MEM similar

	Imipenem			Meropenem		
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m
NDM-1	94	20	0.21	49	12	0.25
IMP-1	39	46	1.2	10	50	0.12
VIM-2	10	10	1.0	5	1	0.28

Yong et al. Antimicrob. Agents Chemother. 53:5046 (2009)

Multiple β-Lactamases are Common in Enterobacteriaceae

- KPC enzymes are frequently co-produced with weak ESBLs or strong penicillinases
  - SHV-11 or SHV-12; TEM-1; OXA-9
- Metallo- β-lactamases, chromosomal and plasmid-encoded, are always produced with at least one other β-lactamase
- Greek isolate of *K. pneumoniae*
  - Plasmid-encoded TEM-1 and CMY-2 cephalosporinase
  - ESBL CTXM-15
  - Two carbapenemases
    - VIM-19 and the serine carbapenemase KPC-2

Bush, Curr. Opin. Microbiol. 13:558 (2010); Pournaras et al.. J. Anti. Chemo. 65:1604 (2010)

Two *Enterobacter cloacae* Isolates Produced KPC-3 and VIM-1 in Indianapolis

- Two patients admitted 11 months apart
  - Isolates collected 33 days apart in same ward in same hospital (2011)

MICs (μg/ml)	Patient #1	Patient #2
Imipenem	4	8
Meropenem	4	1

- Full sequencing confirmed KPC-3 and VIM-1 in both isolates
- MLST and plasmid profiles indicated non-clonal origins
- In 2012 an additional 41 CRE isolates were found positive for bla_{KPC} by PCR.
  - 7 isolates were also positive for bla_{VIM}

Bush et al. ICAAC 2012 Abstract C2-1228; Li et al. ASM 2013 Abstract 1543

Summary

- Carbapenemase production is increasing in Gram-negative bacteria
- Serine carbapenemases of clinical significance include the KPC enzymes and the OXA family of enzymes
- The most important metallo-β-lactamases are in the IMP, VIM and NDM families
- Carbapenemases are often less efficient at hydrolyzing β-lactams than other β-lactamases
- Multiple β-lactamase production in a single organism must be considered in the development of new agents