Diverse Mechanisms of Resistance in Carbapenem-Resistant Enterobacteriaceae at Stanford Health Care

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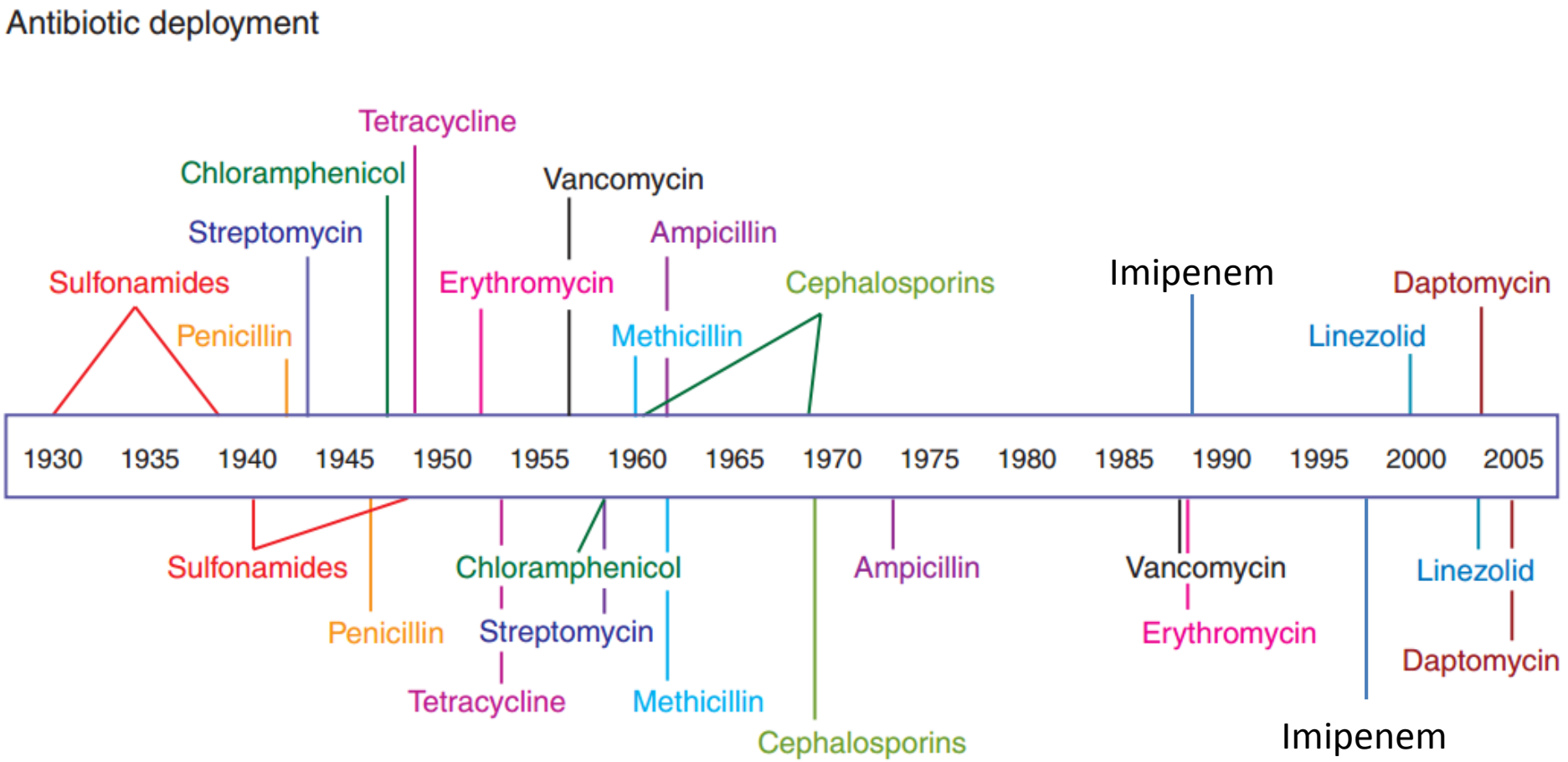
Associate Professor of Pathology and Medicine
Stanford University School of Medicine

Timeline of Microbial Arms Race



Clatworthy et al *Nature Chemical Biology* 2007

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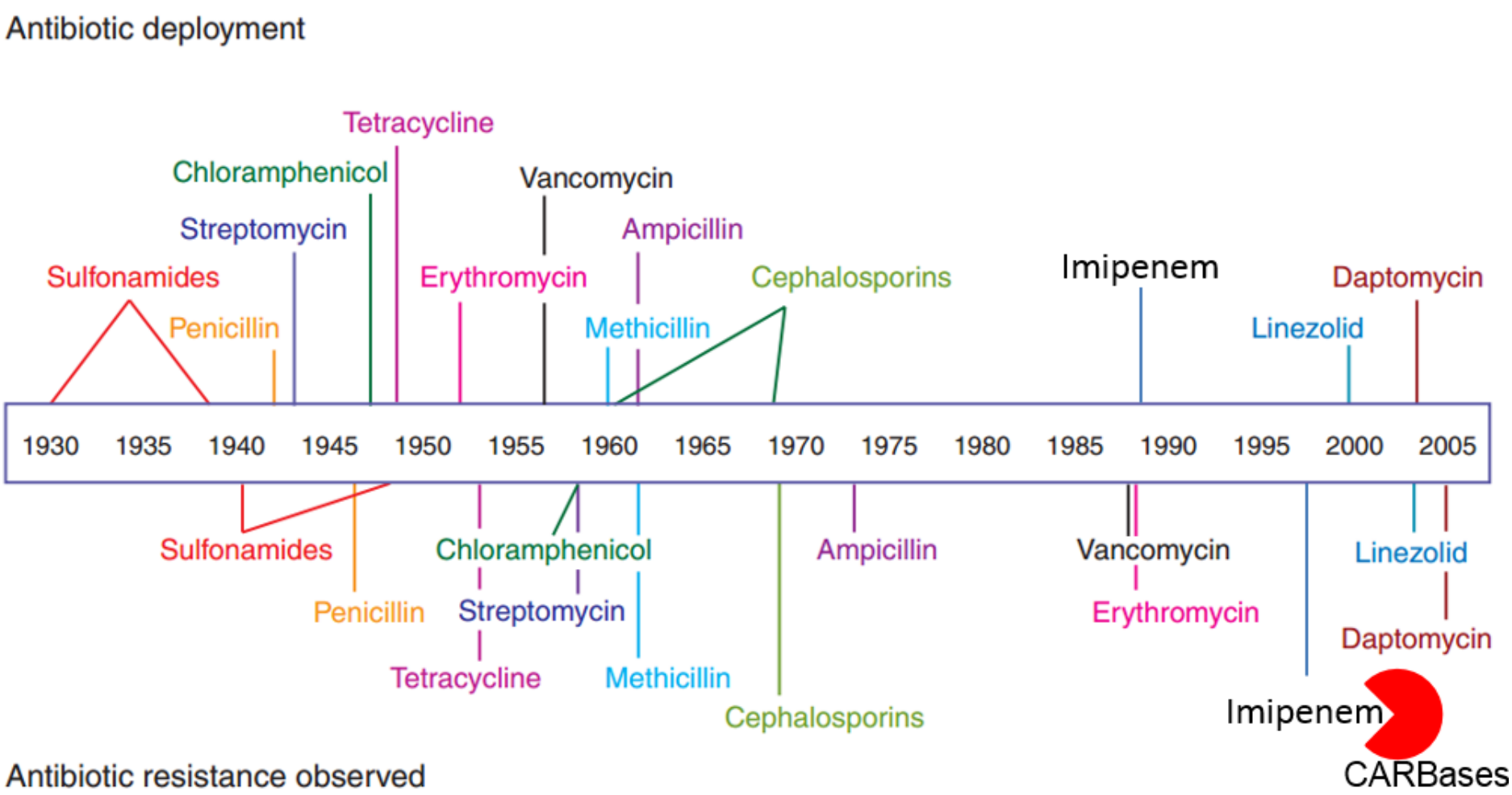
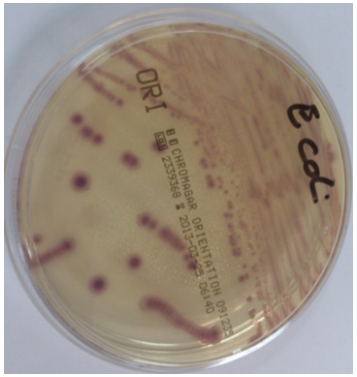


Clatworthy et al *Nature Chemical Biology* 2007

Carbapenem-Resistant Enterobacteriaceae (CRE)

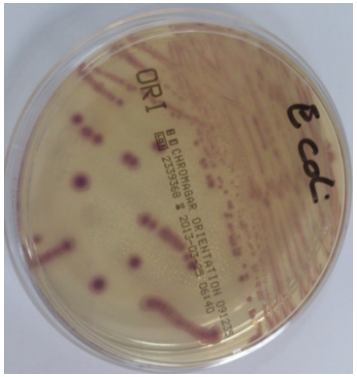
Indian patient with pyelonephritis.
Visiting daughter in Silicon Valley.
Ureteral stent placed in India for kidney stones.
Urine culture: *E. coli* >100,000 cfu/mL



Susceptibility

	MIC by Vitek 2	
Amikacin	>=64 ug/mL ...	RESISTANT
Amoxicillin/Clavulanic Acid	>=32 ug/mL ...	RESISTANT
Ampicillin	>=32 ug/mL ...	RESISTANT
Cefazolin	>=64 ug/mL ...	RESISTANT [1]
Cefepime	>=64 ug/mL ...	RESISTANT
Cefoxitin	>=64 ug/mL ...	RESISTANT
Ceftazidime	>=64 ug/mL ...	RESISTANT
Ceftolozane/Tazobactam		
Ceftriaxone	>=64 ug/mL ...	RESISTANT
Ciprofloxacin	>=4 ug/mL (...	RESISTANT
Doxycycline		
Ertapenem	>=8 ug/mL (...	RESISTANT
FOSFOMYCIN		
Gentamicin	>=16 ug/mL ...	RESISTANT
Imipenem		
Levofloxacin	>=8 ug/mL (...	RESISTANT
Meropenem	8 ug/mL (MIC)	RESISTANT
Nitrofurantoin	64 ug/mL (MIC)	INTERMEDIATE
Piperacillin/Tazobactam	>=128 ug/mL...	RESISTANT
Polymixin B		
Tetracycline	>=16 ug/mL ...	RESISTANT
Tobramycin	>=16 ug/mL ...	RESISTANT
Trimethoprim/Sulfamethoxazole.	>=320 ug/mL...	RESISTANT

Indian patient with pyelonephritis.
Visiting daughter in Silicon Valley.
Ureteral stent placed in India for kidney stones.
Urine culture: *E. coli* >100,000 cfu/mL



Susceptibility

	MIC by Vitek 2			MIC by Etest	
	Not Specified		Disk Diffusion	MIC MCG/ML BY ET	
Amikacin	>=64 ug/mL ...	RESISTANT			
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Ceftazidime	>=64 ug/mL ...	RESISTANT			
Ceftolozane/Tazobactam				>256 ug/mL ...	R
Ceftriaxone	>=64 ug/mL ...	RESISTANT			
Ciprofloxacin	>=4 ug/mL (...	RESISTANT			
Doxycycline				>256 ug/mL ...	R
Ertapenem	>=8 ug/mL (...	RESISTANT	RESISTANT		
FOSFOMYCIN			SUSCEPTIBLE		
Gentamicin	>=16 ug/mL ...	RESISTANT			
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Levofloxacin	>=8 ug/mL (...	RESISTANT			
Meropenem	8 ug/mL (MIC)	RESISTANT	RESISTANT		
Nitrofurantoin	64 ug/mL (MIC)	INTERMEDIATE			
Piperacillin/Tazobactam	>=128 ug/mL...	RESISTANT			
Polymixin B				1 ug/mL (MIC)	
Tetracycline	>=16 ug/mL ...	RESISTANT			
Tobramycin	>=16 ug/mL ...	RESISTANT			
Trimethoprim/Sulfamethoxazole.	>=320 ug/mL...	RESISTANT			

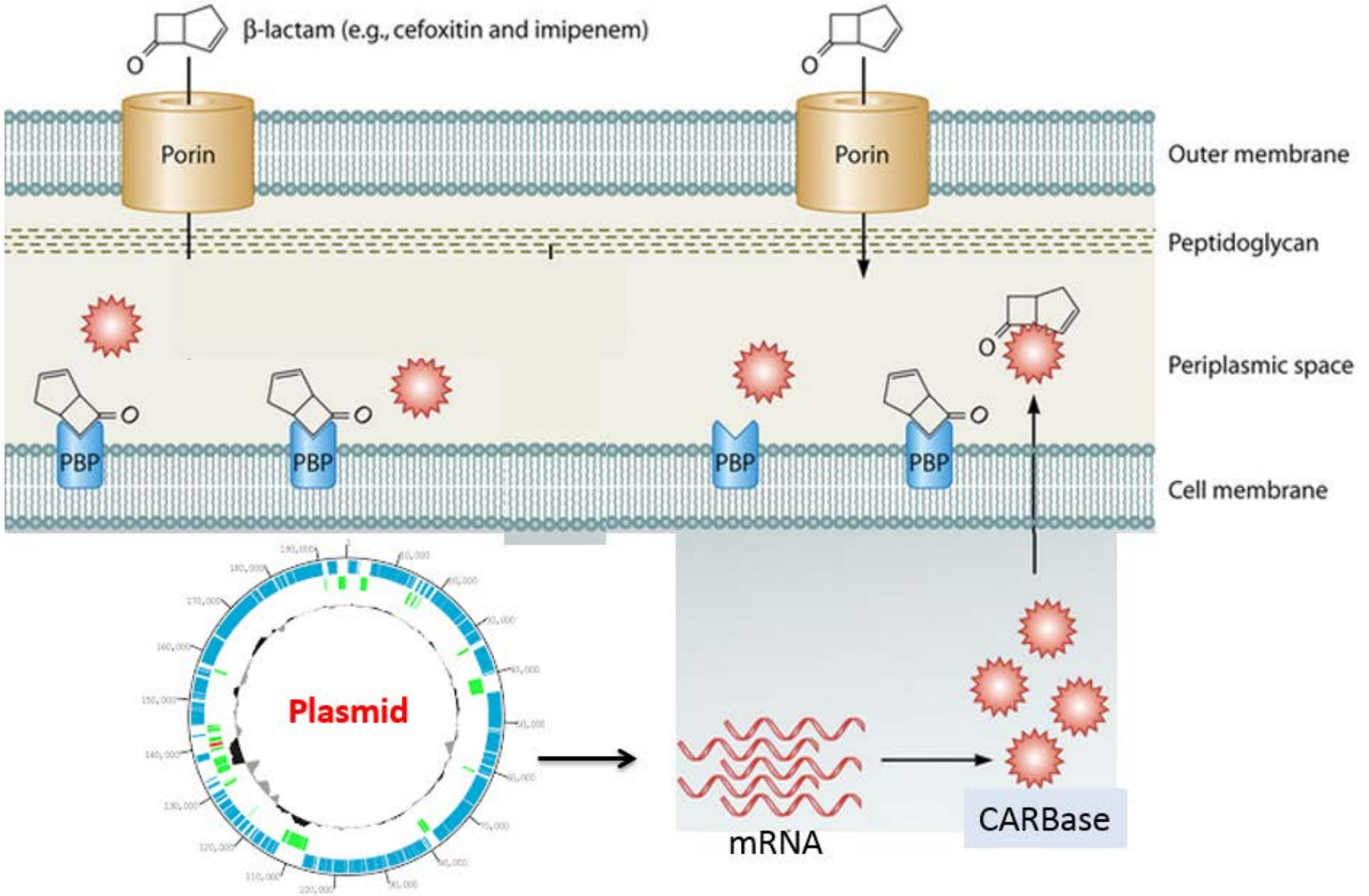
Outline

- Introduction to CRE
- Local Experience
  - Rate
  - Mechanism
  - In vitro susceptibility
  - Transmission
- Future challenges

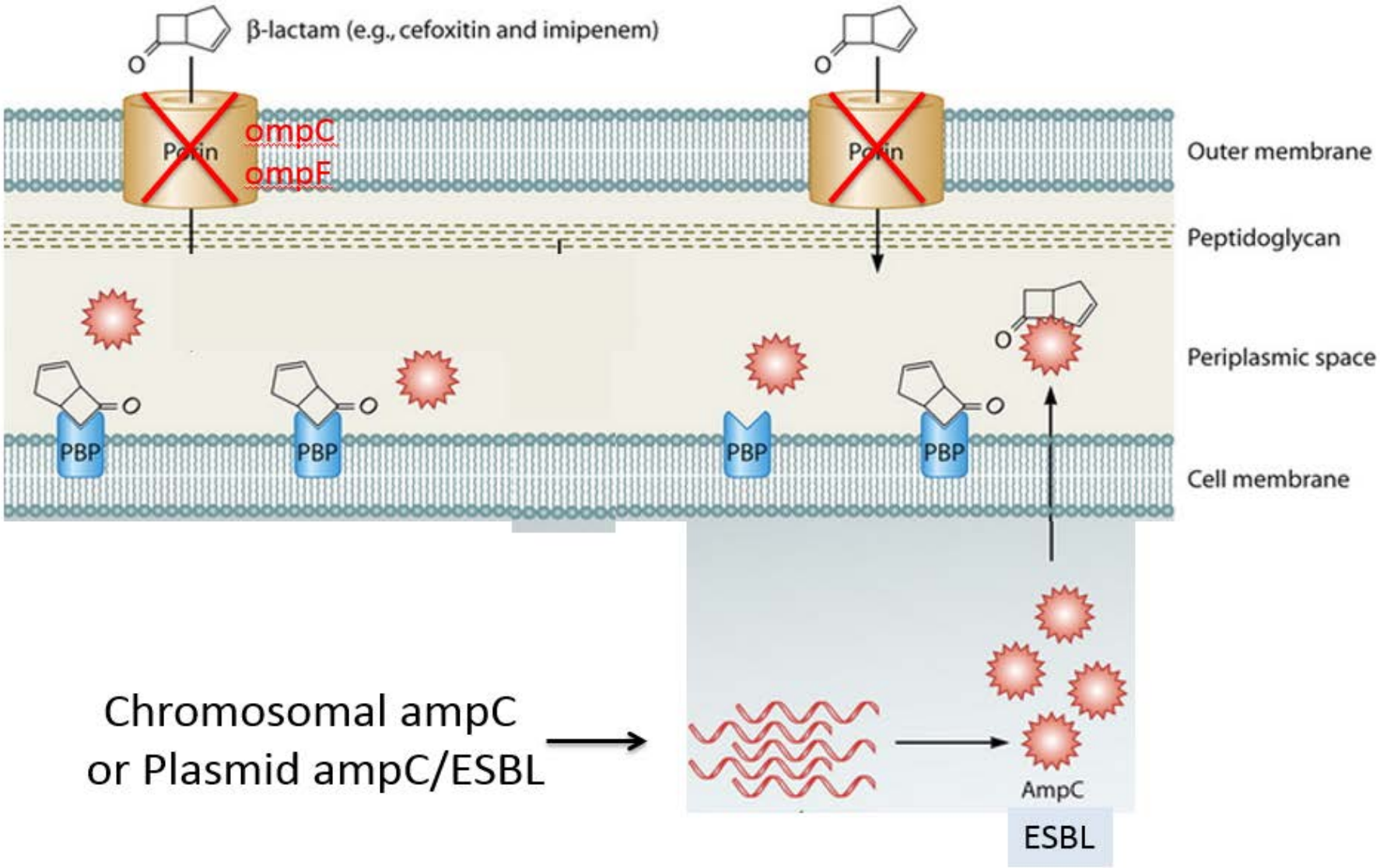
Carbapenem-Resistant Enterobacteriaceae (CRE)

2015 CDC definition: Resistant to imipenem, meropenem, doripenem or ertapenem

Mechanism of Carbapenem Resistance



Mechanism of Carbapenem Resistance



Emergence of Carbapenemases

Enzyme	Class	Location	Year of Isolation	Country of origin
SME	A	Chromosome	1982	London, UK
IMI-1	A	Chromosome	1984	CA, USA
IMP	B	Plasmid	1991	Japan
VIM	B	Plasmid	1996	Verona, Italy
GES	A	Plasmid	2000	French Guiana
OXA-48-like	D	Plasmid	2001	Turkey
KPC	A	Plasmid	2001	NC, USA
GIM	B	Integron	2004	Germany
SIM	B	Integron	2005	Korea
CMY	C	Plasmid	2006	Seoul, Korea
IMI-2	B	Plasmid	2006	China
NDM	B	Plasmid	2008	London, UK

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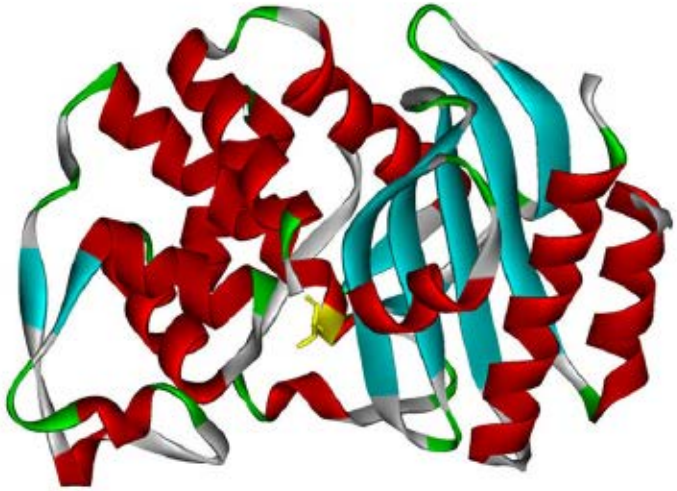
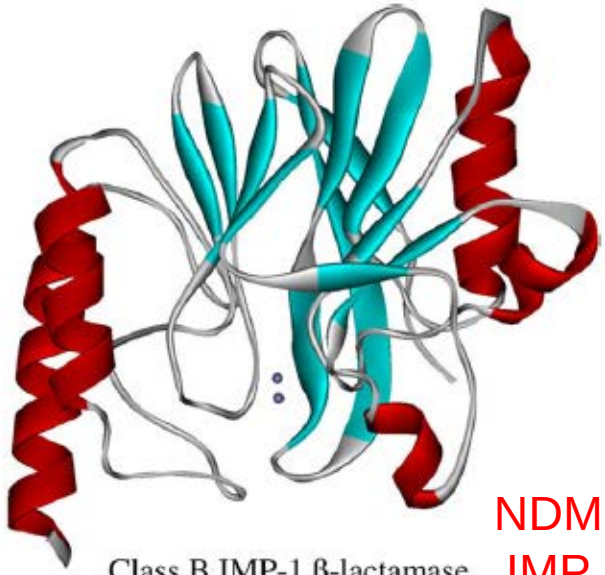
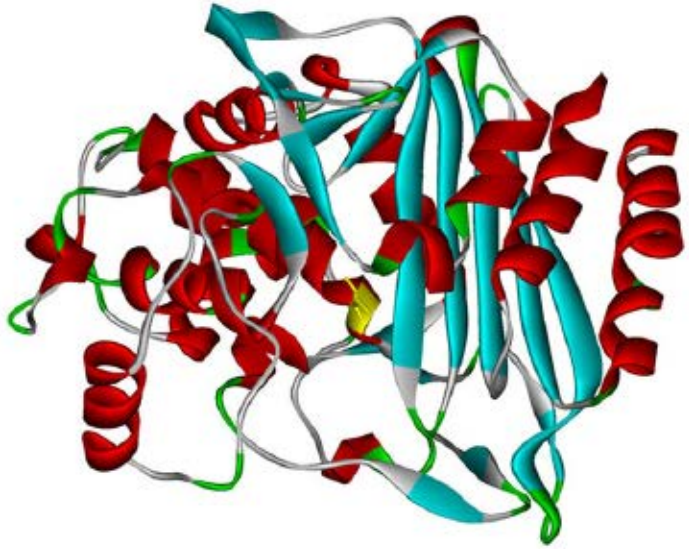
Beta-lactamases



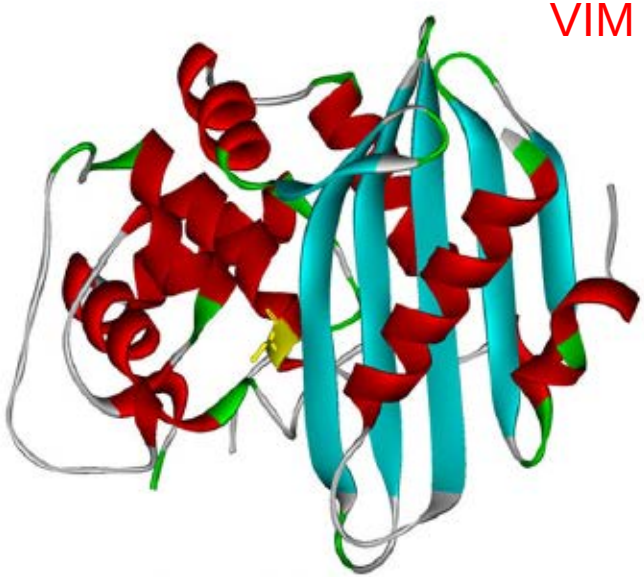
Class A SHV-1 β-lactamase KPC



Class B IMP-1 β-lactamase NDM IMP VIM

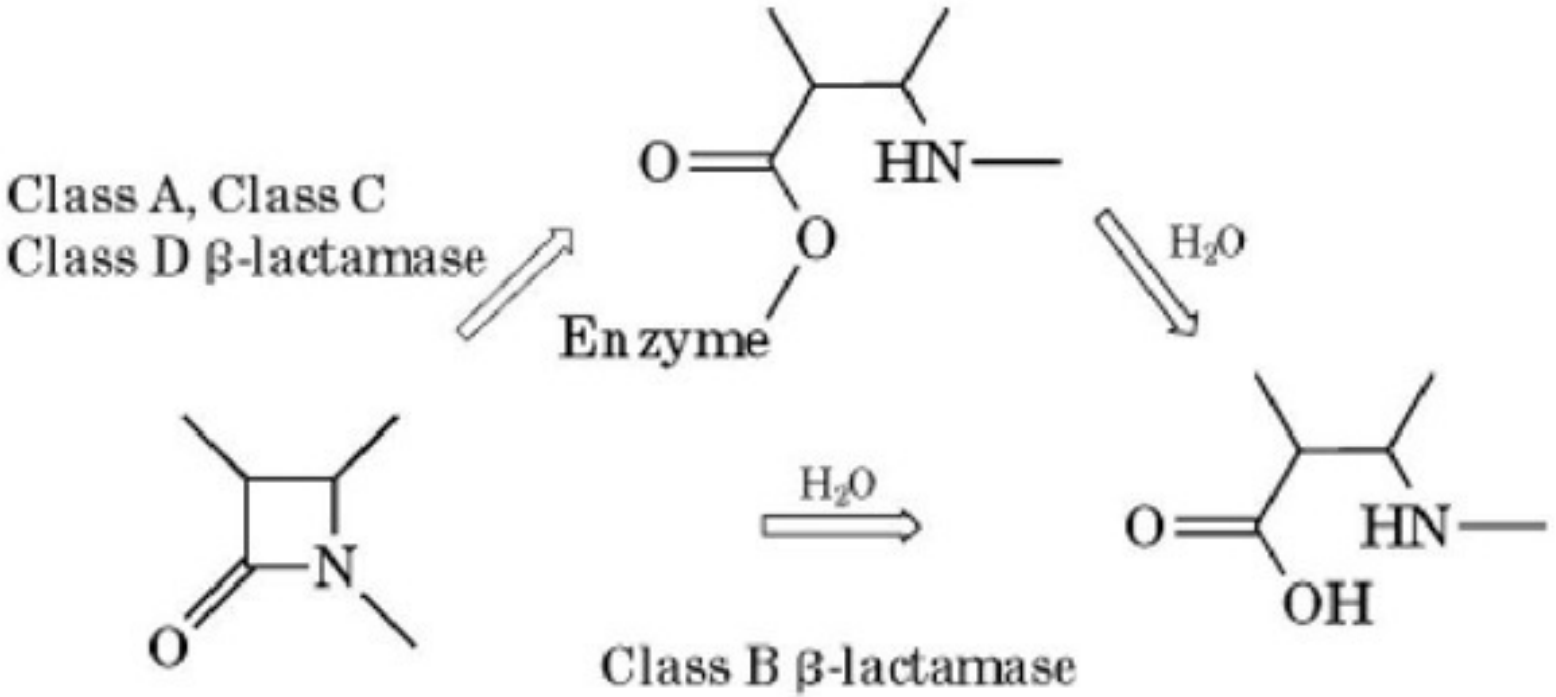


Class C *E.coli* AmpC β-lactamase

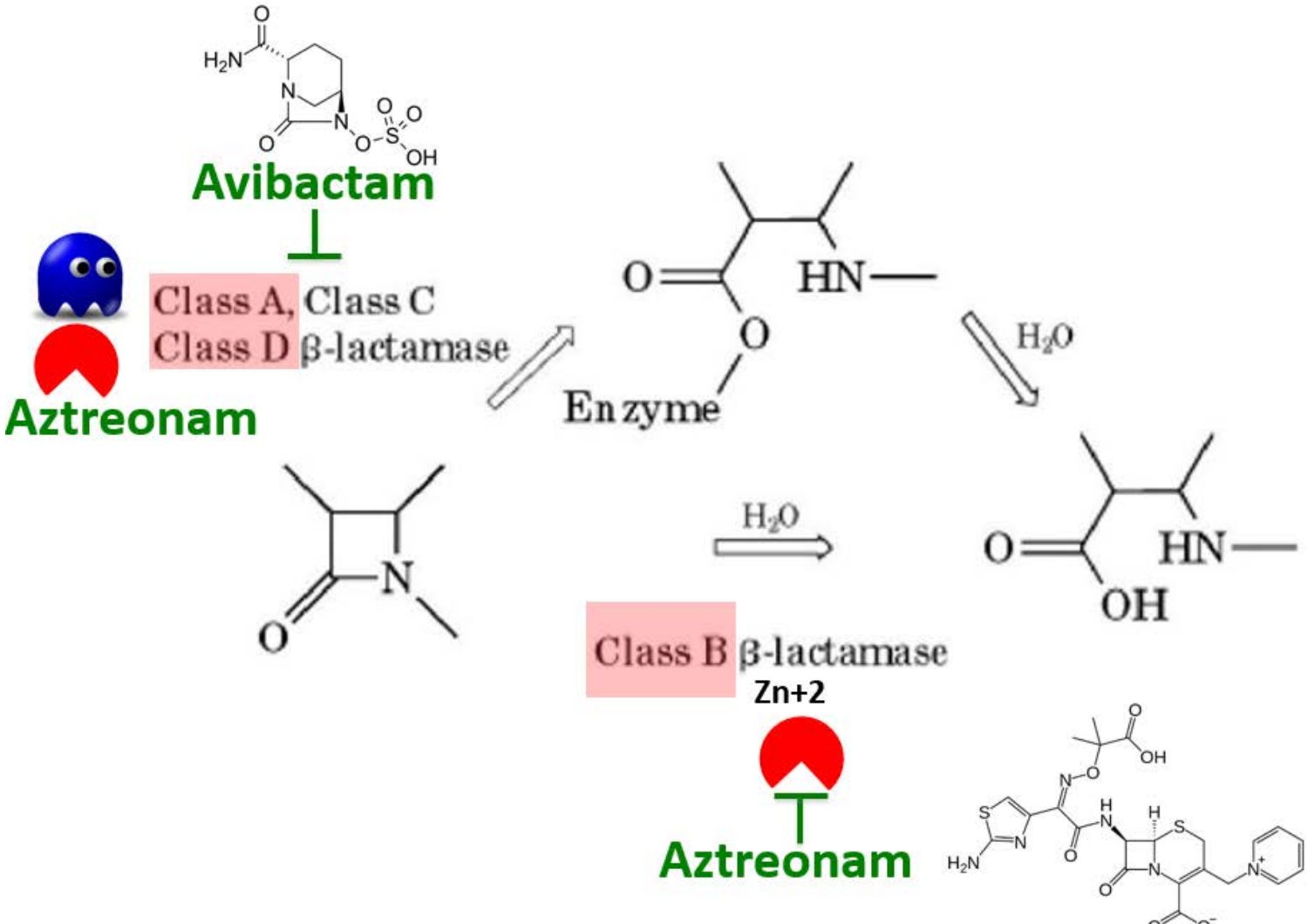


Class D OXA-1 β-lactamase OXA48

Beta-lactamase Mechanisms



Beta-lactamase Molecular Class Predicts Susceptibility to New Inhibitors



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CRE rates at Stanford Health Care

Species	No. of CRE/CRE + non-CRE isolates (%)				
	2013	2014	2015	2016	2013-16
All species	11/5001 (0.2)	14/3550 (0.4)	18/4752 (0.4)	19/5968 (0.3)	62/19271 (0.3)

CRE definition based on pre-2015 CDC definition

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Stanford CRE rate 4-13 fold lower than national rates

- 4.2% per 2011 NHSN data
- 1.4% per 2010 Surveillance Network-USA data
  CDC MMWR 2013 PMID: 23466435

Stanford Rate lower than national rates

- 0.73% per 2011-13 UCLA study
  Pollett et al JCM 2014

Original Investigation

Epidemiology of Carbapenem-Resistant Enterobacteriaceae in 7 US Communities, 2012-2013

Alice Y. Guh, MD, MPH; Sandra N. Bulens, MPH; Yi Mu, PhD; Jesse T. Jacob, MD; Jessica Reno, MPH; Janine Scott, MPH; Lucy E. Wilson, MD, ScM; Elisabeth Vaeth, MPH; Ruth Lynfield, MD; Kristin M. Shaw, MPH; Paula M. Snippes Vagnone, MT(ASCP); Wendy M. Bamberg, MD; Sarah J. Janelle, MPH; Ghinwa Dumyati, MD; Cathleen Concannon, MPH; Zintars Beldavs, MS; Margaret Cunningham, MPH; P. Maureen Cassidy, MPH; Erin C. Phipps, DVM, MPH; Nicole Kenslow, MPH; Tatiana Travis, BS; David Lonsway, MMS; J. Kamile Rasheed, PhD; Brandi M. Limbago, PhD; Alexander J. Kallen, MD, MPH

Emerging Infections Program Site	Incident CRE Cases[a]				
	No. of Cases		Crude Annual Incidence Rate/100 000 Population		Standardized Incidence Ratio (95% CI)[c]
	2012[b]	2013	2012[b]	2013	
Colorado		27		1.05	0.53 (0.39-0.71)
Georgia	175	181	4.58	4.68	1.65 (1.20-2.25)
Maryland		92		4.80	1.44 (1.06-1.96)
Minnesota	31	40	1.82	2.32	0.94 (0.69-1.27)
New Mexico		6		0.89	0.41 (0.30-0.55)
New York		27		3.60	1.42 (1.05-1.92)
Oregon	6	14	0.35	0.82	0.28 (0.21-0.38)
Total	212	387	2.94	2.93	

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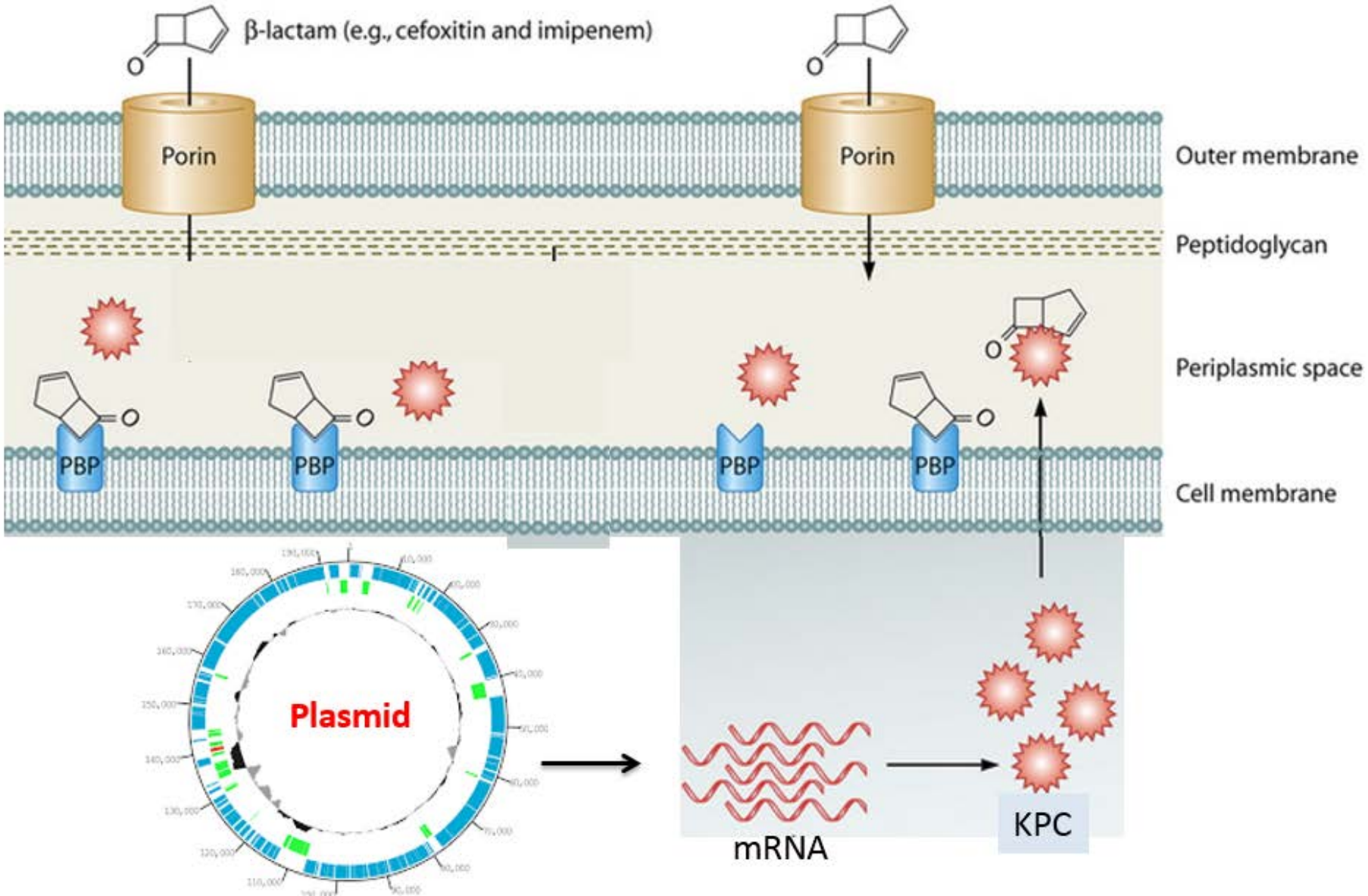
Mechanism of Carbapenem Resistance



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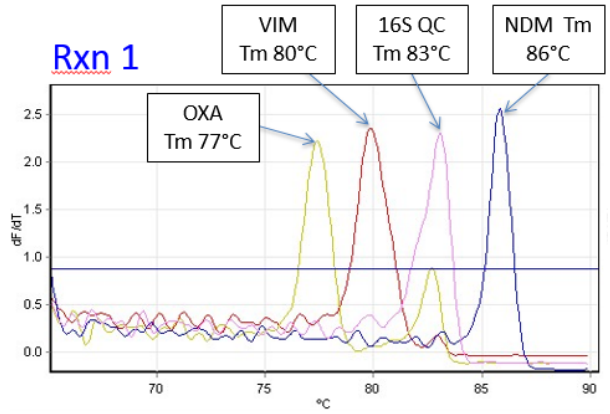
Genotypic Detection of Known Carbapenemases

Stanford LDTs



Carbases:

bla$_{KPC}$
bla$_{NDM}$
bla$_{IMP}$
bla$_{VIM}$
bla$_{OXA-48\ like}$
bla$_{SPM}$
bla$_{GES}$
bla$_{GIM}$
bla$_{SME}$
bla$_{SIM}$
bla$_{IMI}$
bla$_{NMC-A}$

Carbases:

bla$_{KPC}$
bla$_{NDM}$
bla$_{IMP}$
bla$_{VIM}$
bla$_{OXA-48\ like}$
bla$_{SPM}$
bla$_{GES}$
bla$_{GIM}$
bla$_{OXA-23\ like}$
bla$_{OXA-58\ like}$

ESBLs:

bla$_{CTX-M-1\ group}$
bla$_{CTX-M-1-like}$
bla$_{CTX-M-15-like}$
bla$_{CTX-M-32-like}$
bla$_{CTX-M-2\ group}$
bla$_{CTX-M-9\ group}$
bla$_{CTX-M-8,\ \&-25\ group}$
bla$_{TEM-types}$
bla$_{SHV-types}$
bla$_{VEB}$, *bla*$_{PER}$
bla$_{BEL}$
bla$_{GES}$

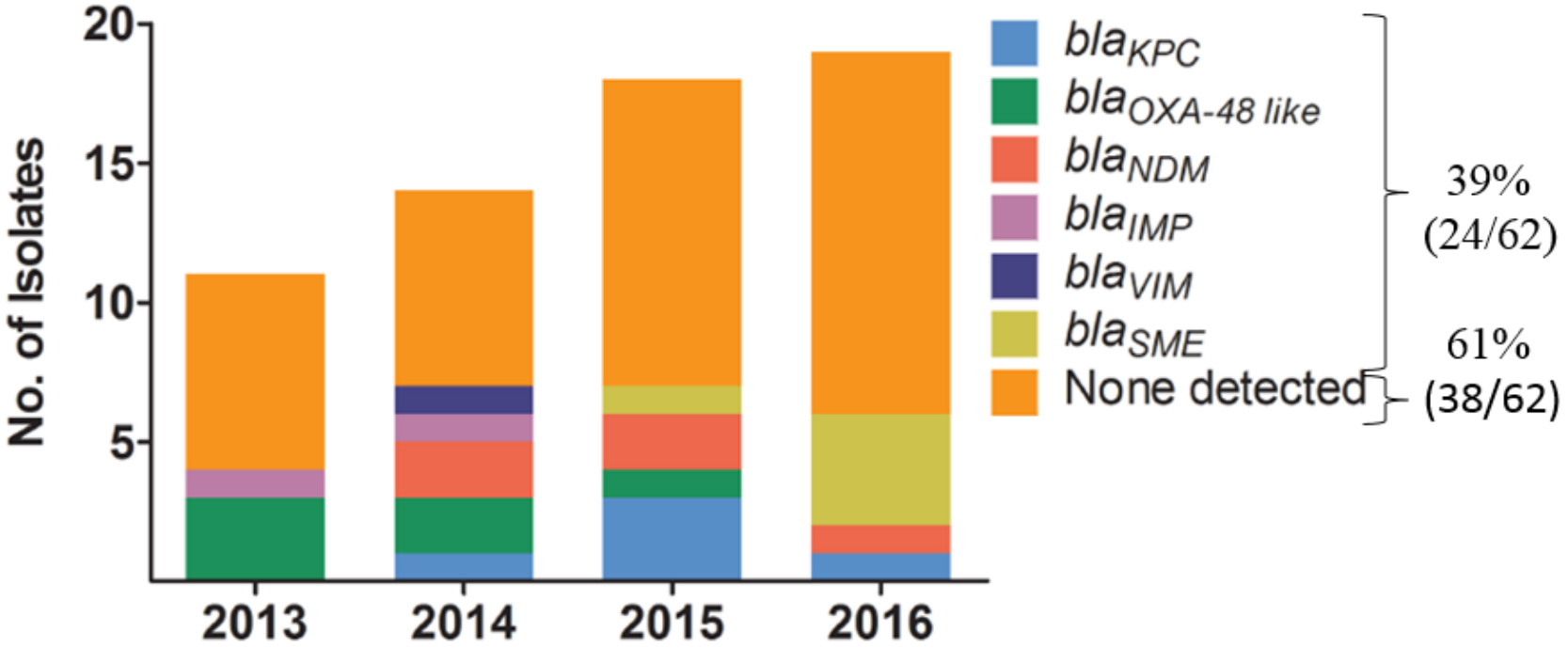
pAmpCs:

bla$_{CMY\ I/MOX}$
bla$_{ACC}$
bla$_{DHA}$
bla$_{ACT/MIR}$
bla$_{CMY\ II}$
bla$_{FOX}$

Carbases:

bla$_{KPC}$
bla$_{NDM}$
bla$_{IMP}$
bla$_{VIM}$
bla$_{OXA-48\ like}$

CRE Mechanisms at Stanford Health Care



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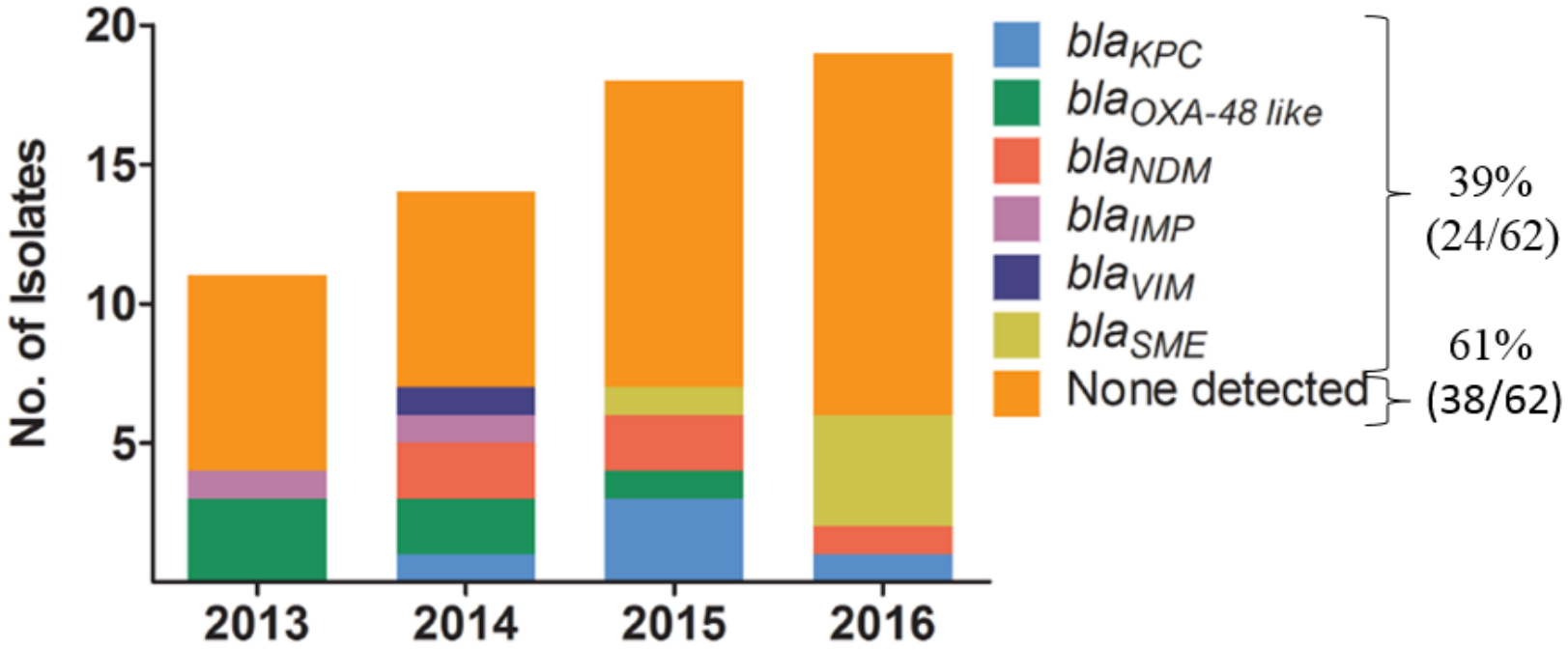
Emerging Infections Program Site	Total No.	CRE Organism or Isolate, No. (%)						No. of Carbapenemase-Producing Isolates/Total No. of Isolates Submitted forTesting (%)[a]
		Enterobacter aerogenes	*Enterobacter cloacae* Complex	*Escherichia coli*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	Isolates Submitted for Carbapenemase Testing	
Colorado[b]	27	7 (25.9)	10 (37.0)	3 (11.1)	7 (25.9)	0	16 (59.3)	5/16 (31.3)
Georgia	356	22 (6.2)	38 (10.7)	56 (15.7)	235 (66.0)	5 (1.4)	75 (21.1)	48/75 (64.0)
Maryland[b]	92	8 (8.7)	6 (6.5)	9 (9.8)	69 (75.0)	0	17 (18.5)	13/17 (76.5)
Minnesota	71	29 (40.8)	16 (22.5)	10 (14.1)	16 (22.5)	0	58 (81.7)	17/58 (29.3)
New Mexico[b]	6	2 (33.3)	0	3 (50.0)	1 (16.7)	0	[c]	[c]
New York[b]	27	3 (11.1)	2 (7.4)	5 (18.5)	17 (63.0)	0	9 (33.3)	5/9 (55.6)
Oregon	20	4 (20.0)	7 (35.0)	3 (15.0)	6 (30.0)	0	13 (65.0)	2/13 (15.4)
Total	599	75 (12.5)	79 (13.2)	89 (14.9)	351 (58.6)	5 (0.8)	188 (31.4)	90/188 (47.9)

100% (90/90) were KPC

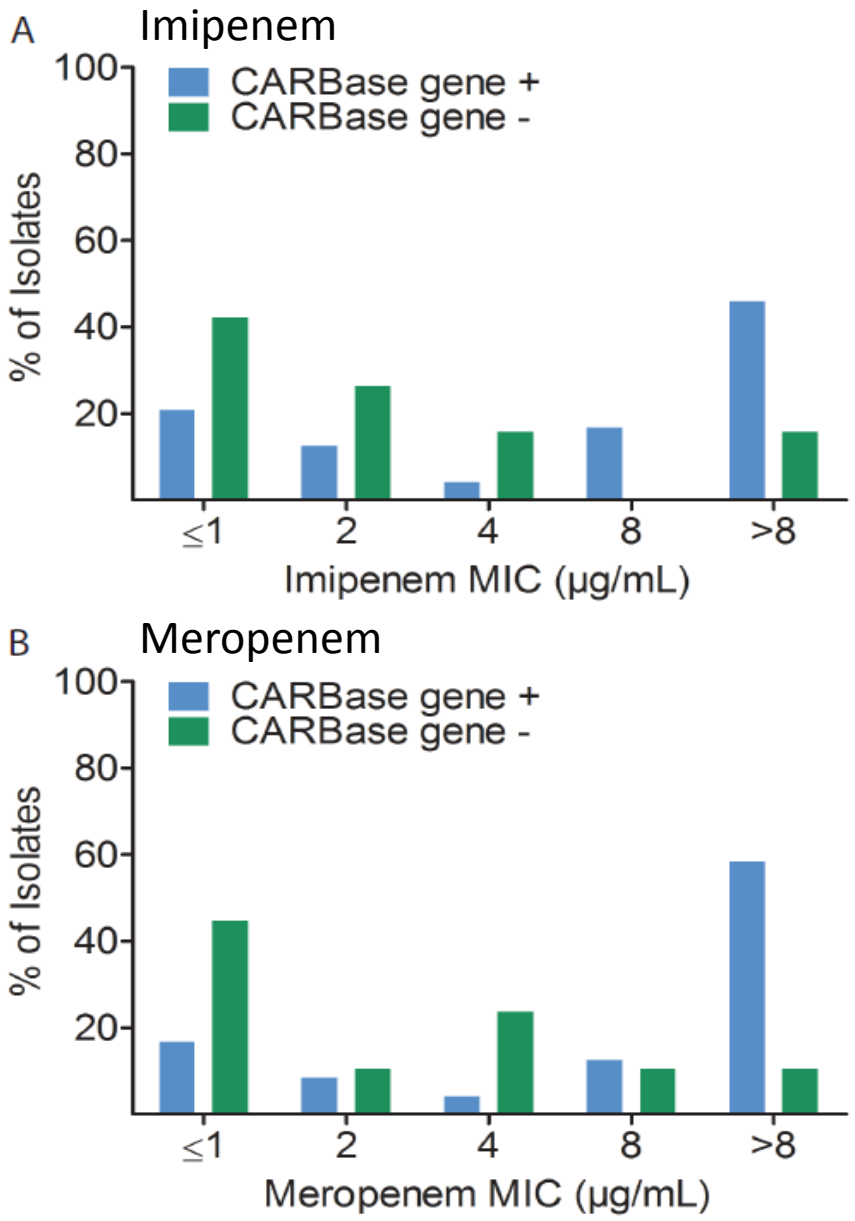
JAMA 2015

CRE Mechanisms at Stanford Health Care

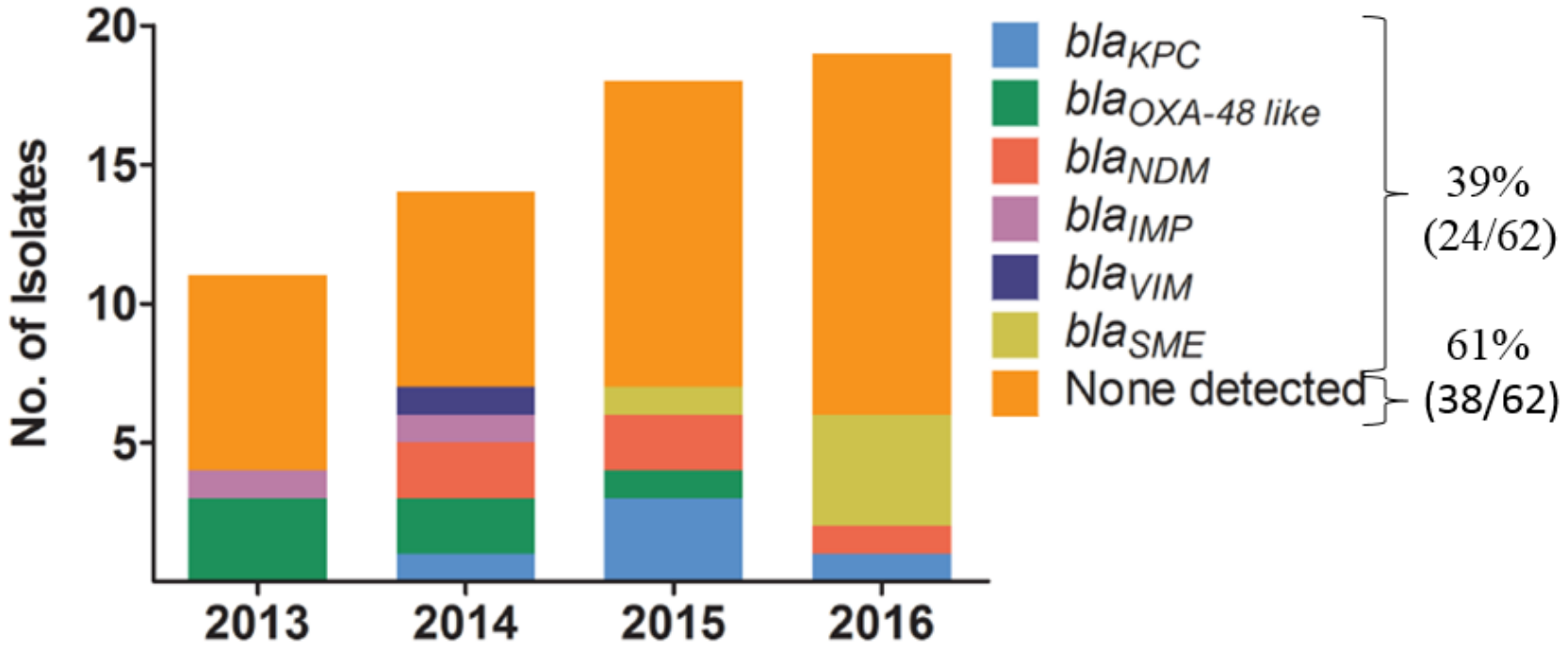


CRE definition based on pre-2015 CDC definition

Higher Carbapenem MIC in Carbapenemase+ CRE



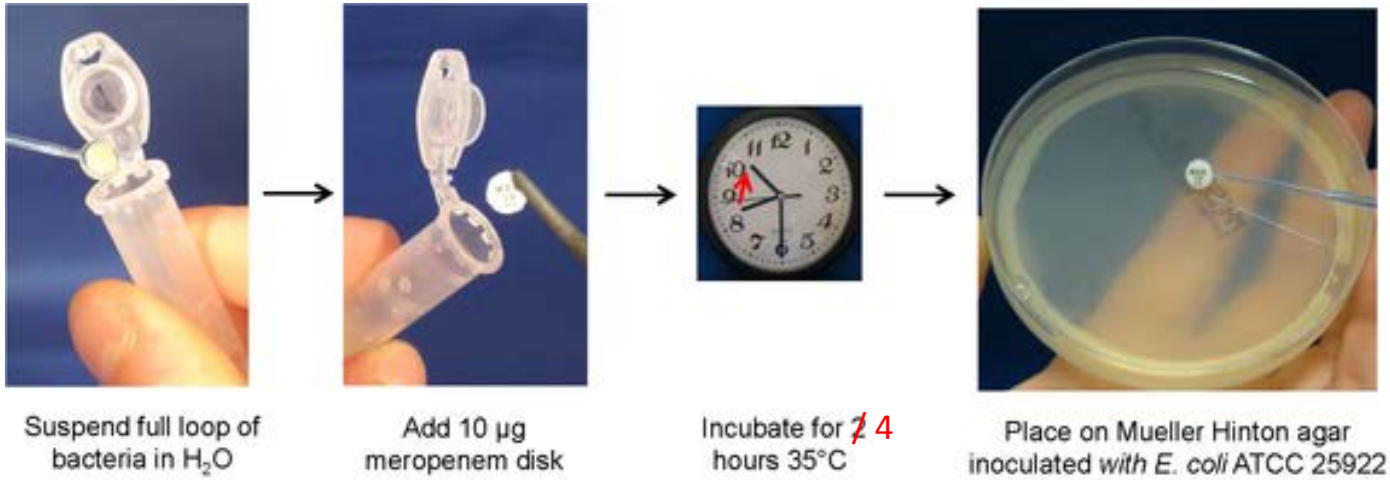
CRE Mechanisms in Carbapenemase Gene-Negative Isolates at Stanford Health Care



"SPACE" organisms made up 63.2% of CRE isolates lacking a carbapenemase gene compared with 33.3% (p=0.04) of isolates harboring a carbapenemase gene.

CRE definition based on pre-2015 CDC definition

Phenotypic Detection of Carbapenemases: Carbapenem Inactivation Method (CIM)



Suspend full loop of bacteria in H_2O

Add 10 µg meropenem disk

Incubate for ~~2~~ 4 hours 35°C

Place on Mueller Hinton agar inoculated *with E. coli* ATCC 25922

Incubate for at least 18 ~~6~~ hours 35°C

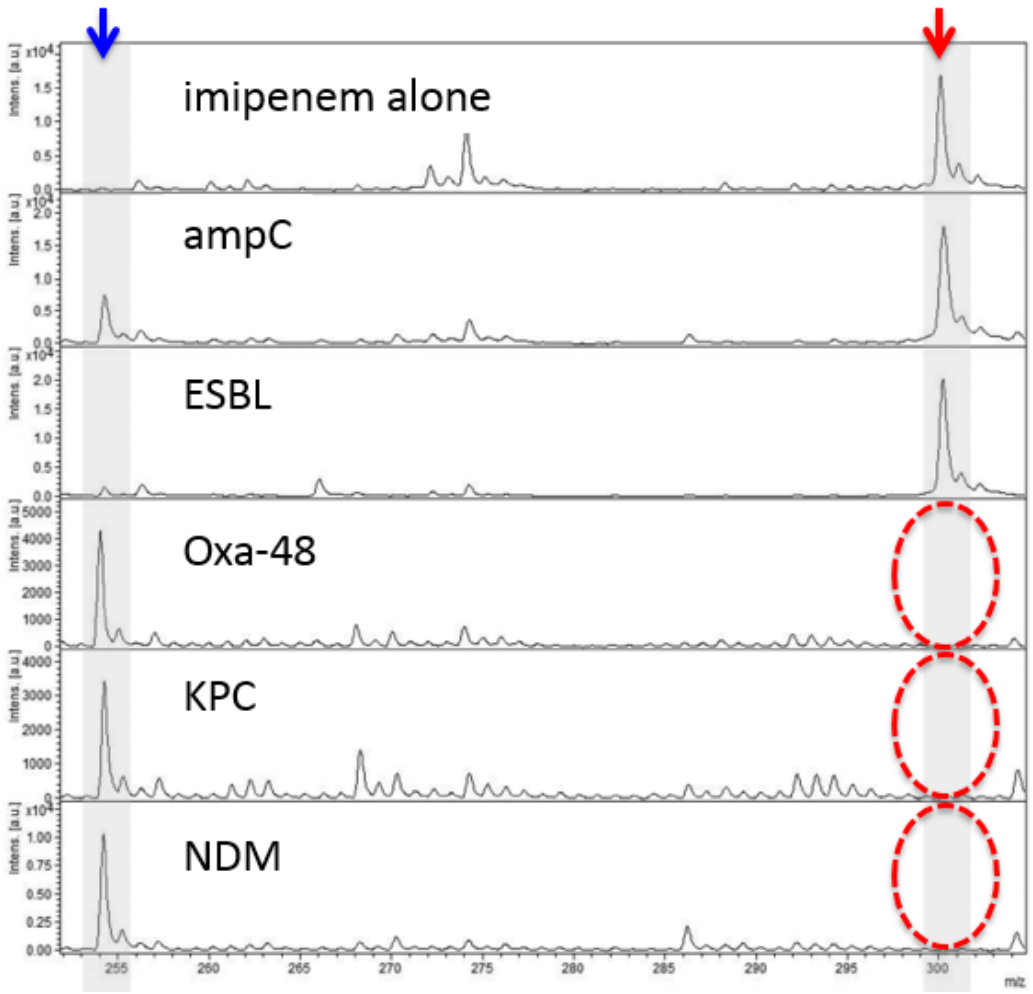
Read presence or absence of inhibition zone

\+ Carbapenemase activity

\- No carbapenemase activity

van der Zwaluw et al PLOS ONE 2015

Phenotypic Detection of Carbapenemases: MALDI-TOF Mas Spectrometry



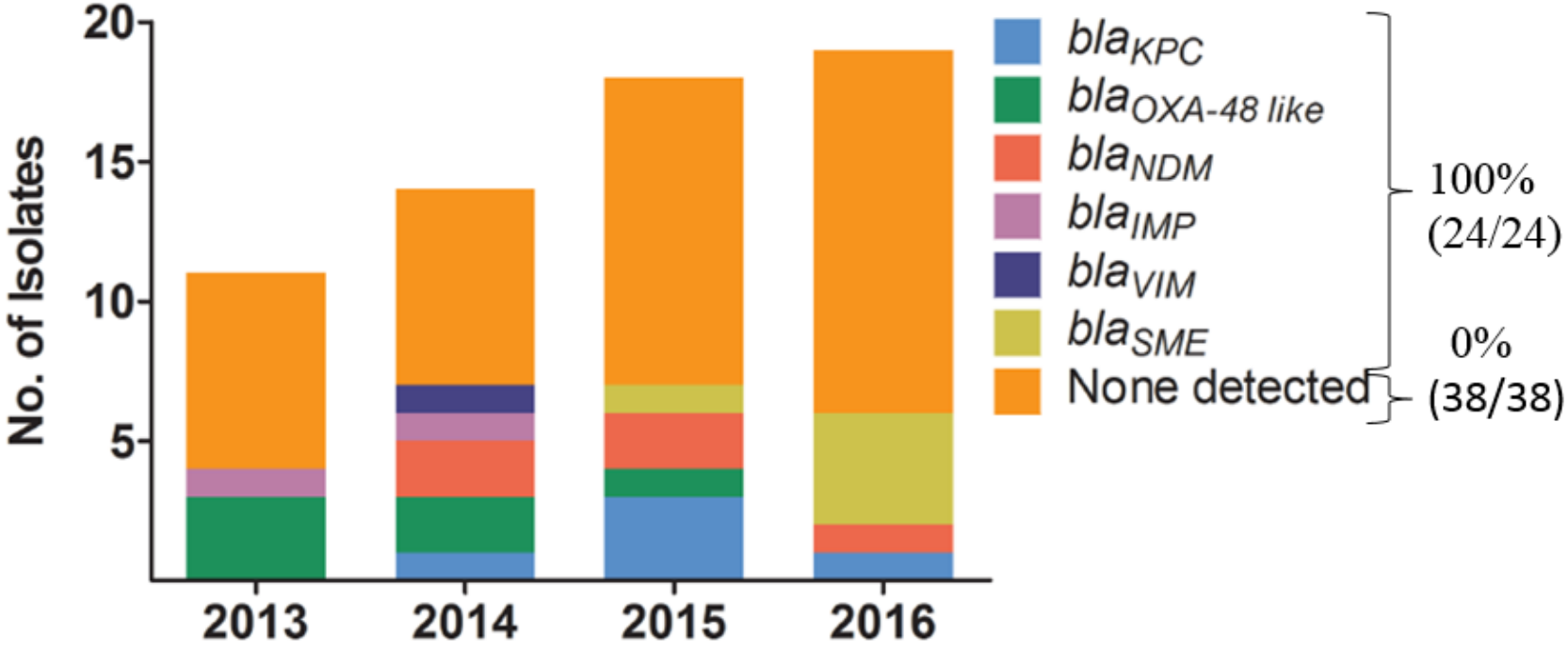
MALDI-TOF spectra of imipenem hydrolysis assays after a 20-min incubation at 37°C

$$\frac{\text{metabolite}}{(\text{metabolite} + \text{imipenem})} \geq 0.82$$

Training set: 77 CPE & 146 non-CPE
Sensitivity: 97.8% (25/25)
Specificity: 97.8% (18/18)

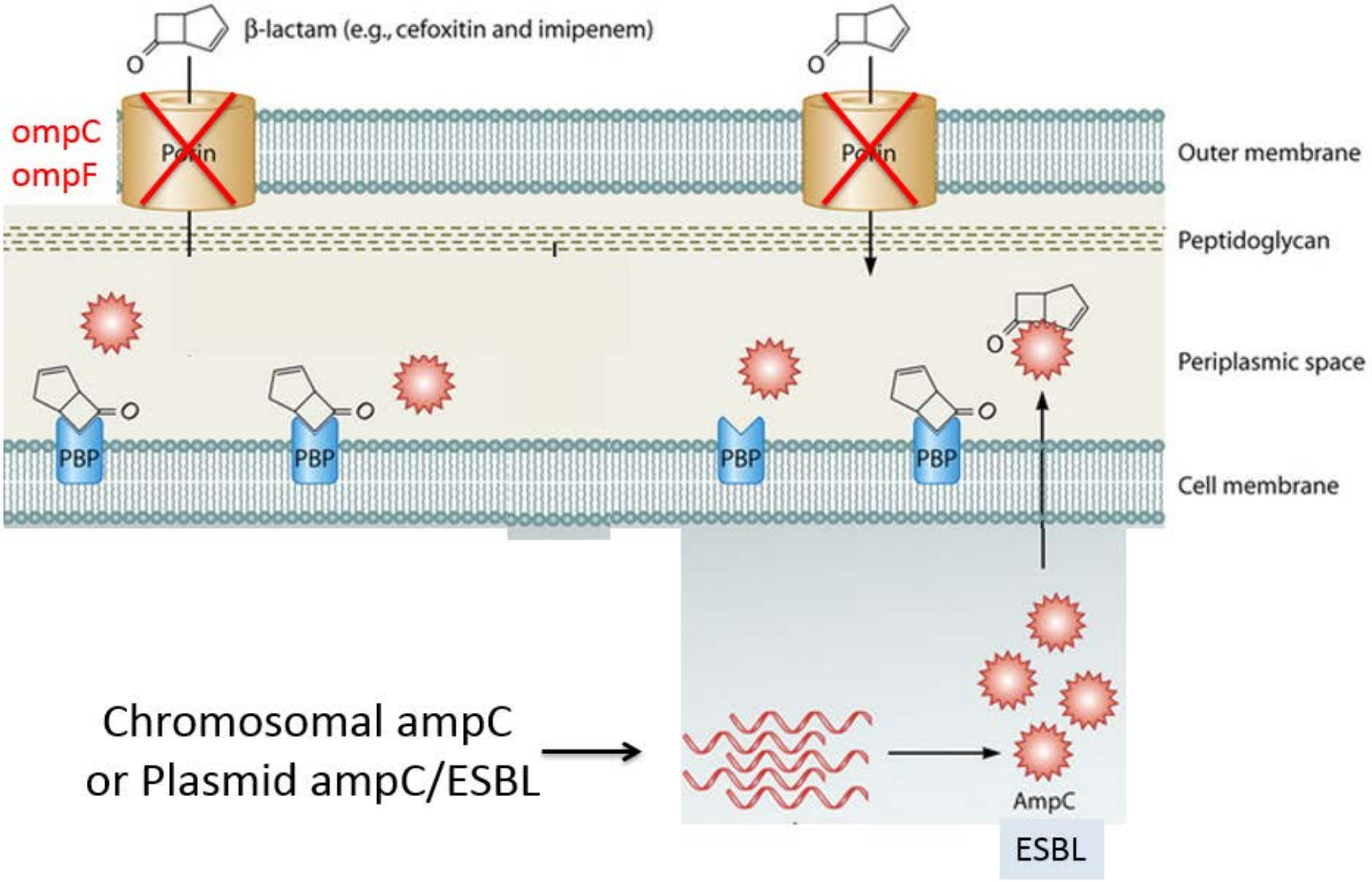
Lasserre et al JCM 2015

Carbapenemase Activity in CRE Isolates at Stanford Health Care

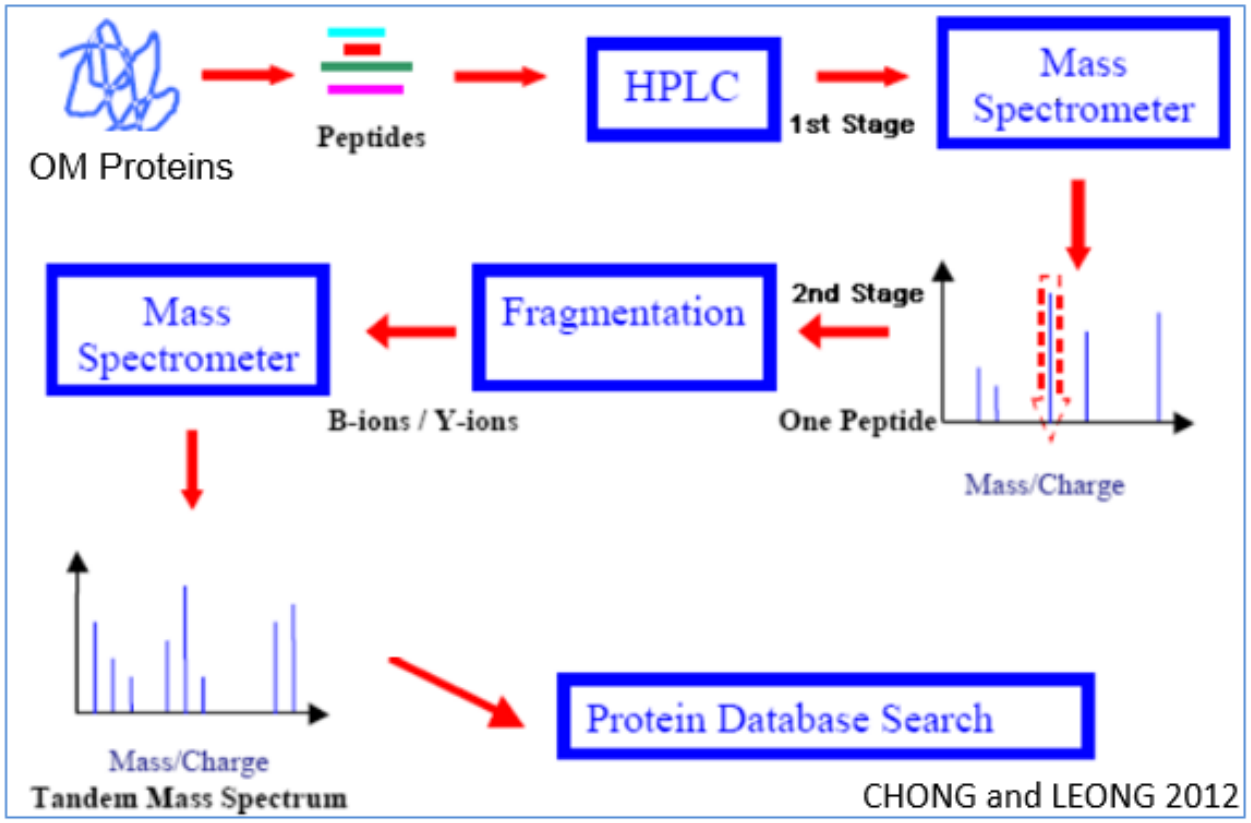


CRE definition based on pre-2015 CDC definition

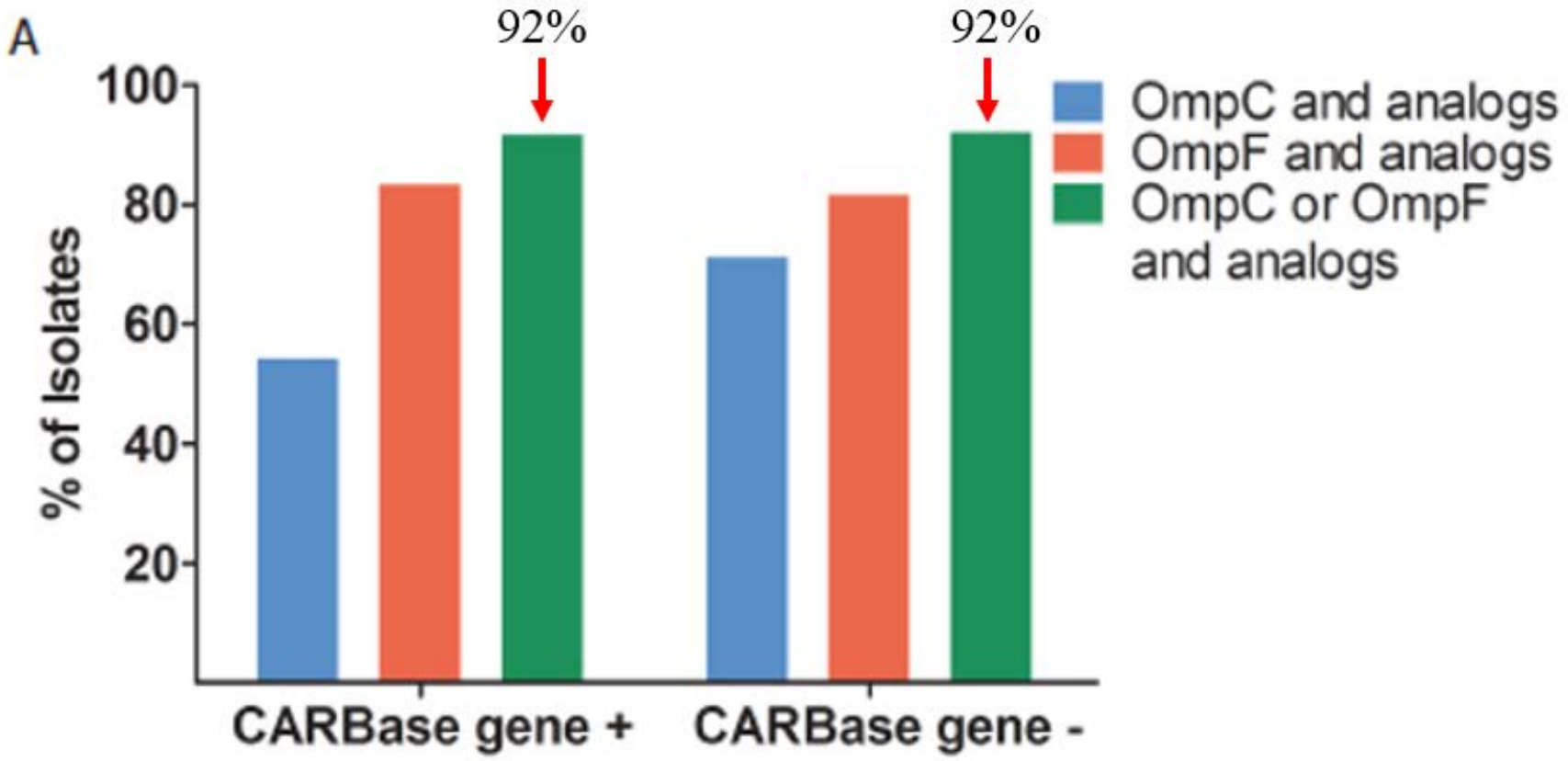
Mechanism of Carbapenem Resistance



Phenotypic Detection of OmpC and OmpF and their Analogs



Porin Levels Down ≥2 Fold in Carbapenemase- Isolates



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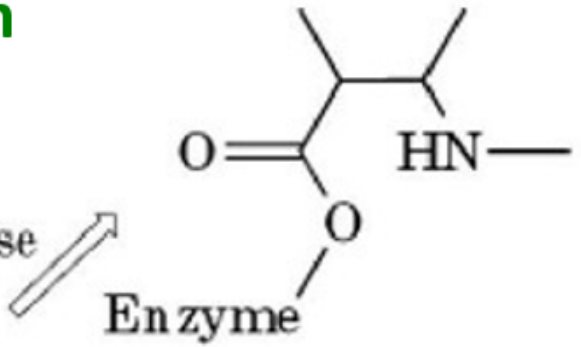
Beta-lactamase Molecular Class Predicts Susceptibility to New Inhibitors

Avibactam

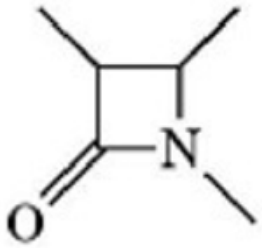
Relebactam **Vaborbactam**

Class A, Class C
Class D β-lactamase

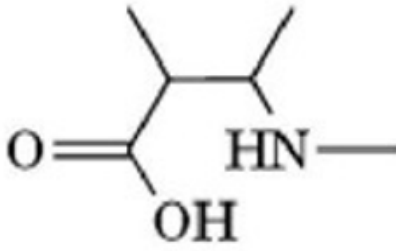
Class A,



H_2O



H_2O

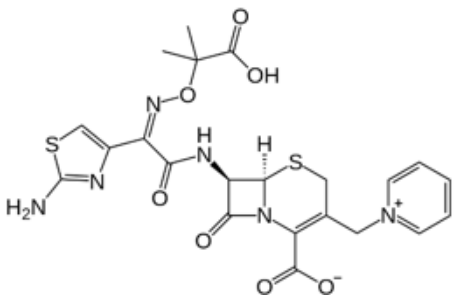


Class B β-lactamase

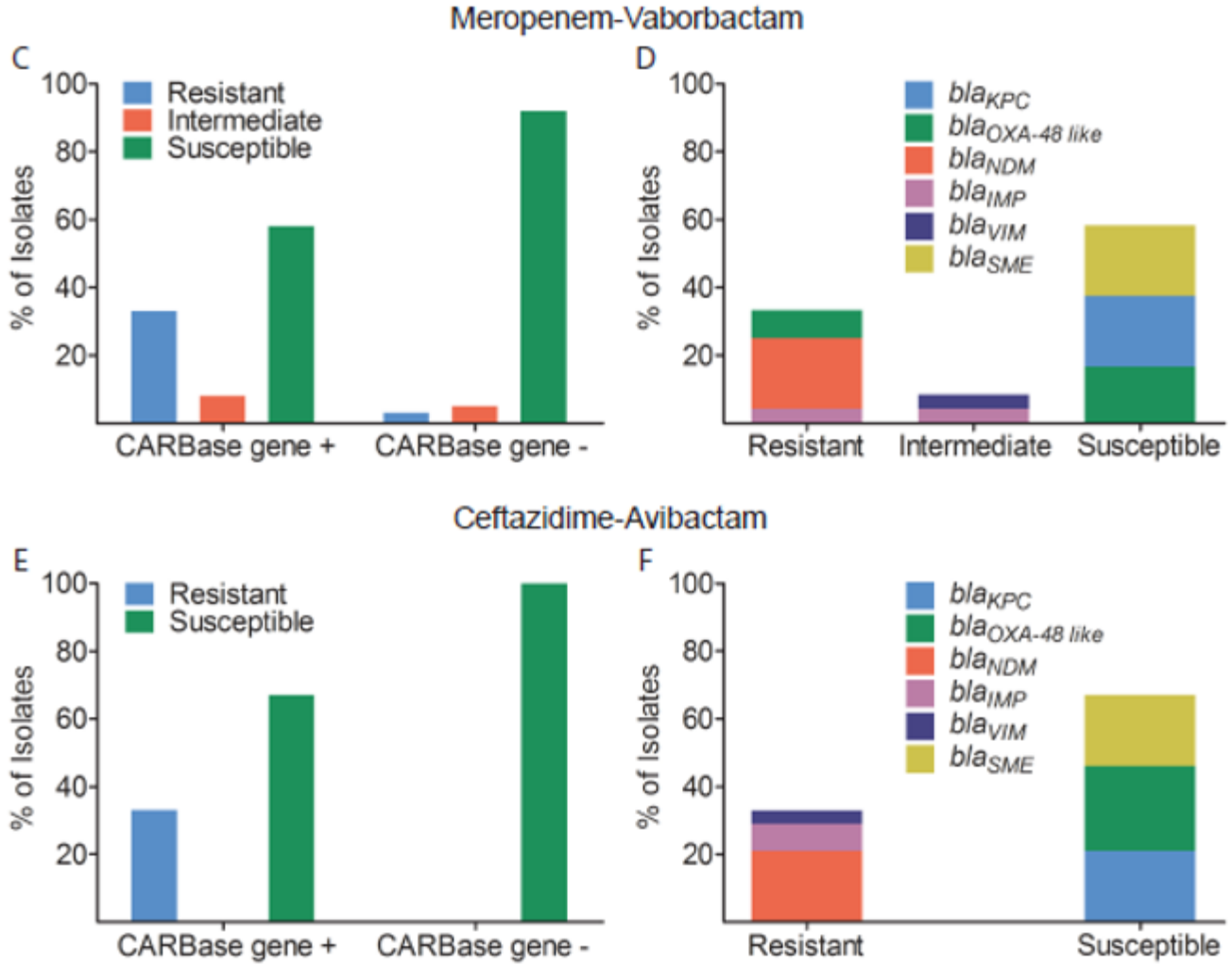
Zn+2

Ceftazidime-Avibactam
Meropenem-Vaborbactam
Imipenem-Relebactam
Ceftolozane-Tazobactam

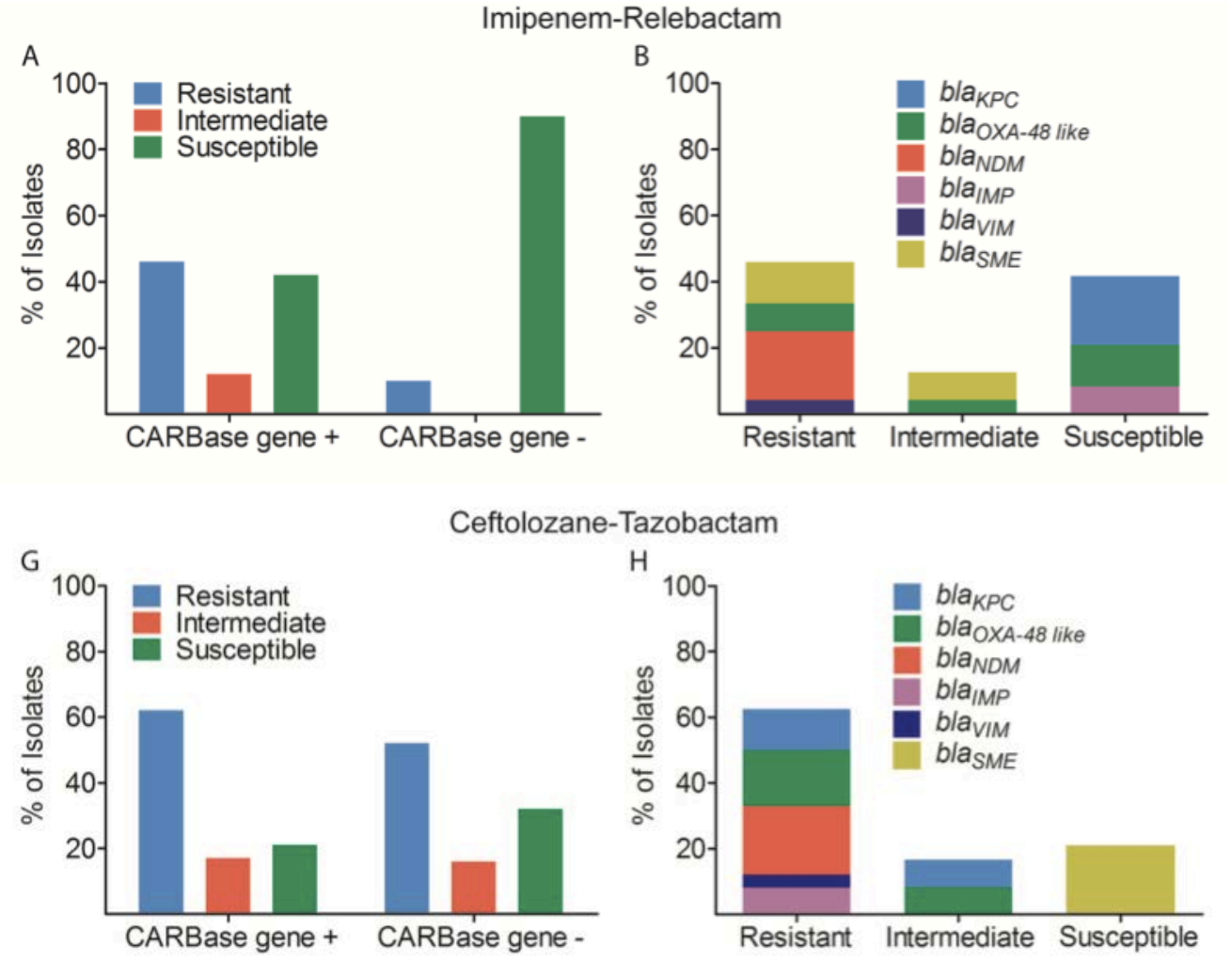
Aztreonam



CRE Susceptibility to Newer β-lactamase Inhibitor Combinations as Predicted

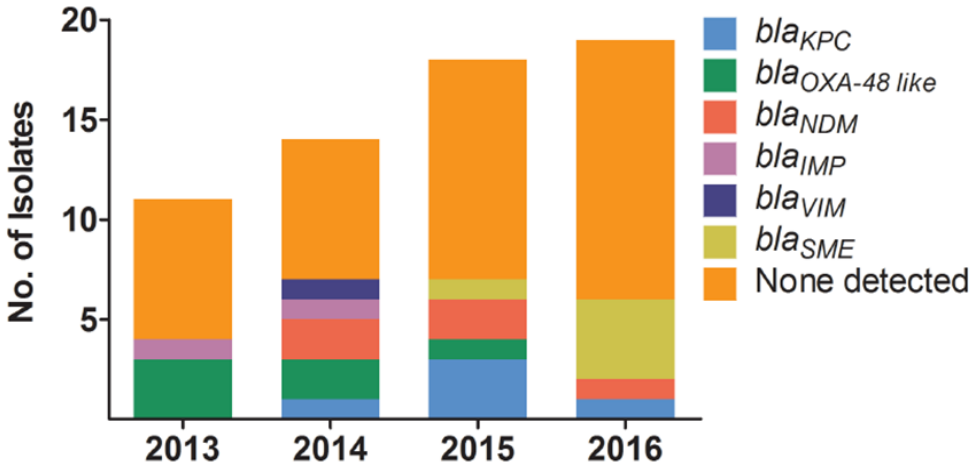


CRE Susceptibility to Newer β-lactamase Inhibitor Combinations as Predicted

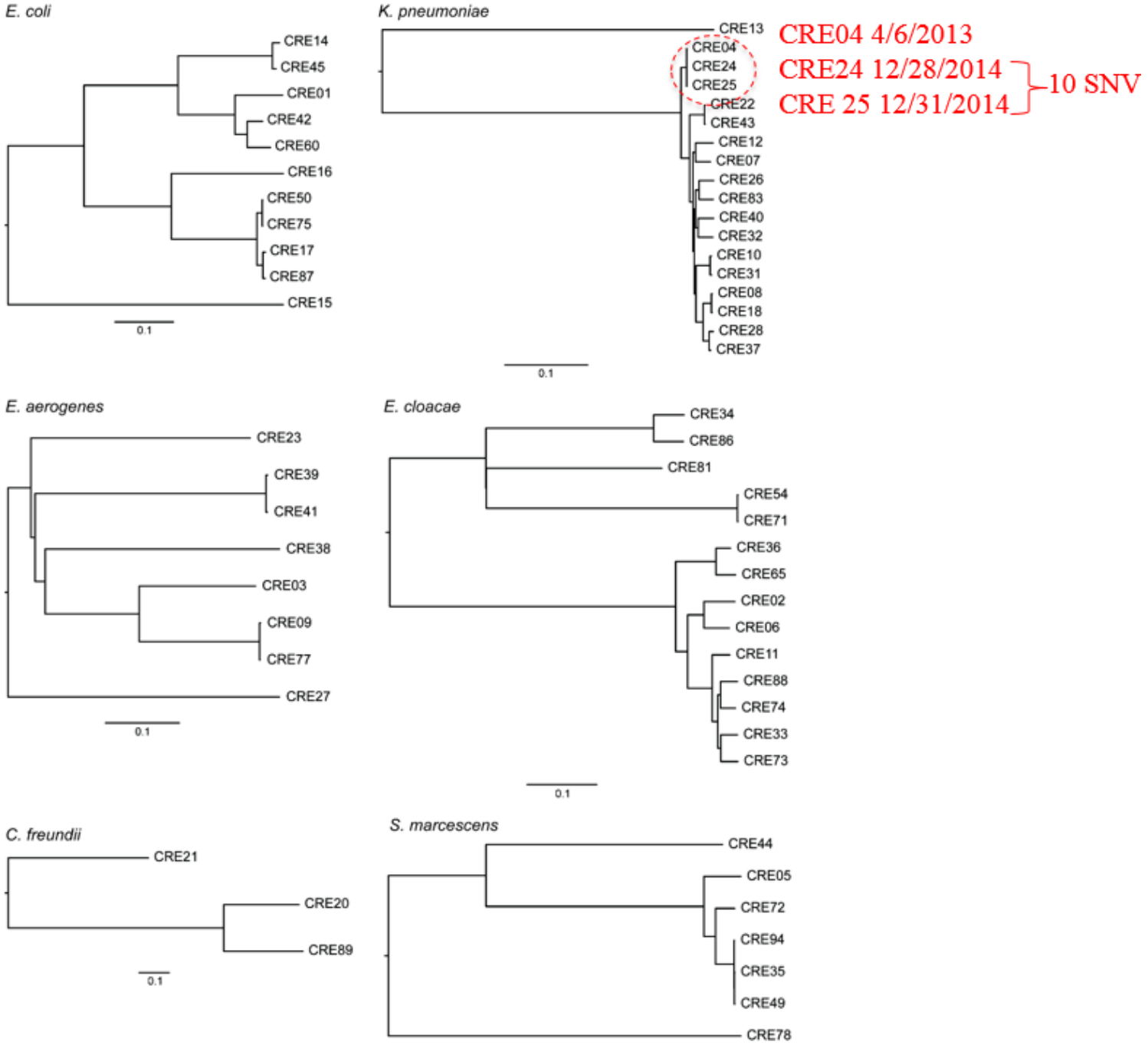


Outline

- Introduction to CRE
- Local Experience
  - Rate
  - Mechanism
  - In vitro susceptibility
  - Transmission
- Future challenges



Phylogenetic Tree for CRE Isolates Based on Whole Genome Sequencing



Summary

- Low CRE rate (0.3%) in our setting
- Comprehensive phenotypic and genotypic characterization identified sporadic occurrence of plasmid-encoded CP-CRE
- Not dominated by blaKPC
- Predictable susceptibility to newer β-lactam-β-lactamase inhibitor combinations based on the mechanism of resistance
- On-demand nucleic acid testing was sufficient for detection of CP-CRE

61 y/o liver and kidney transplant
Respiratory and urine cultures

	4/1/2018 1045	4/18/2018 1330	4/22/2018 1037	4/22/2018 1055	4/28/2018 1314	5/11/2018 2311	5/13/2018 2238	5/13/2018 2250	5/14/2018 0851	5/14/2018 1214	5/19/2018 1825	6/5/2018 1422	6/5/2018 1630	6/8/2018 1800	6/13/2018 1805
CULTURE AND DIRECT...															
BLOOD CULTURE (AER...															
BLOOD CULTURE (2 A...															
LEGIONELLA CULTURE															
RESPIRATORY CULTUR...		C !				!			!				!		
URINE CULTURE										!		!		C !	!

Enterobacter cloacae complex 4/18/18

Susceptibility

	Enterobacter cloacae complex MIC MCG/ML		MIC		NUCLEIC ACID TEST
Amoxicillin/Clavulanic Acid			>16 ug/mL	RESISTANT	
Ampicillin			>16 ug/mL	RESISTANT	
Ampicillin/Sulbactam			>16 ug/mL	RESISTANT	
Aztreonam.			>16 ug/mL	RESISTANT	
Cefazolin			>16 ug/mL	RESISTANT	
Cefepime			>16 ug/mL	RESISTANT	
Cefoxitin			>16 ug/mL	RESISTANT	
Ceftazidime			16 ug/mL	RESISTANT	
Ceftazidime/avibactam	1.0 ug/mL	SUSCEPTIBLE			
Ceftriaxone			>32 ug/mL	RESISTANT	
Cefuroxime (IV)			>16 ug/mL	RESISTANT	
Ciprofloxacin			>2 ug/mL	RESISTANT	
Ertapenem			>4 ug/mL	RESISTANT	
Gentamicin			<=1 ug/mL	SUSCEPTIBLE	
Imipenem			8 ug/mL	RESISTANT	
IMP PCR					NEGATIVE
KPC PCR					POSITIVE
Levofloxacin			4 ug/mL	INTERMEDIATE	
Meropenem			>8 ug/mL	RESISTANT	
Moxifloxacin			>4 ug/mL	RESISTANT	
NDM PCR					NEGATIVE
OXA48-LIKE PCR					NEGATIVE
Piperacillin/Tazobactam			>64 ug/mL	RESISTANT	
Tetracycline			>8 ug/mL	RESISTANT	
Tigecycline			4 ug/mL	INTERMEDIATE	
Trimethoprim/Sulfamethoxazole.			<=0.5 ug/mL	SUSCEPTIBLE	
VIM PCR					NEGATIVE

Enterobacter cloacae complex 6/13/18

Susceptibility

	Enterobacter cloacae complex MIC MCG/ML		MIC	
Amoxicillin/Clavulanic Acid			>=32 ug/mL	RESISTANT
Cefazolin			>=64 ug/mL	RESISTANT [1]
Cefoxitin			>=64 ug/mL	RESISTANT
Ceftazidime			>=64 ug/mL	RESISTANT
Ceftazidime/avibactam	32 ug/mL	RESISTANT		
Ceftriaxone			>=64 ug/mL	RESISTANT
Ciprofloxacin			2 ug/mL	INTERMEDIATE
Doxycycline	8 ug/mL	INTERMEDIATE		
Ertapenem			>=8 ug/mL	RESISTANT
Gentamicin			<=1 ug/mL	SUSCEPTIBLE
Levofloxacin			4 ug/mL	INTERMEDIATE
Meropenem			>=16 ug/mL	RESISTANT
Nitrofurantoin			256 ug/mL	RESISTANT
Piperacillin/Tazobactam			>=128 ug/mL	RESISTANT
Tetracycline			>=16 ug/mL	RESISTANT
Trimethoprim/Sulfamethoxazole.			160 ug/mL	RESISTANT

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