β-Lactamase inhibitors
Properties, microbiology & enzymology

DAVID M LIVERMORE

Professor of Medical Microbiology, UEA

Lead on Antibiotic Resistance, Public Health England

β-Lactamase classes

A	*Serine at active site---* *Diverse… TEM, SHV, CTX-M, KPC etc*
B	*Zinc at active site, VIM, NDM etc*
C	*Serine at active site--- AmpC cephalosporinases*
D	*Serine at active site– OXA types--- diverse*

Successive β-lactamase challenges

University of East Anglia

From	Enzyme(s)	Class	Compromised
1940s	Staph penicillinase	A	Penicillin
1960s	TEM-1 penicillinase in G -ves	A	Ampicillin
1960s	Inherent R, *Klebsiella, Enterobacter*,	A,C	Amp/ 1-gen cephs
1970s	High level AmpC, *Enterobacter* etc.	C	2/3-gen cephs
1980s	TEM/SHV, ESBLs in G-ves	A	2/3 gen cephs
2000s	CTX-M ESBLs	A	2/3 gen cephs
2000s	*Acinetobacter* carbapenemases	D	Carbapenems
2010s	Enterobacterial carbapenemases	A,B,D	Carbapenems/All

Increasingly…..Gram –ves have multiple β-lactamases

Determinants of activity of inhibitor combinations

- Type of β-lactamase
  - Mutations can change affinity for inhibitor or substrate
- Partner β-lactam
- Amount of β-lactamase
- Target organism
- pH

Livermore *JAC* 1993;**31 Suppl A**:9

What inhibits which β-lactamase?

	Clav-ulanate	Tazo-bactam	Avi-bactam	EDTA Maleic acids
ESBL	+++	++	+++	-
KPC	Yes, but also hydrolysed		++	-
AmpC	-	+	+++	-
OXA-1	+	+	?	-
OXA-48	-	-	+	-
OXA-23	-	-	-	-
MBLs	-	-	-	++

Some boronates may inhibit all

Resistance to clavulanate & sulphone inhibitor combinations

- Mutations reduce binding of clavulanate & sulphones
  - TEM-31 (IRT-1) Arg244Ser
  - TEM-30 (IRT-2) Arg244Cys
- Resistance mutants occur, selection in therapy rare

Canton *et al., CMI* 2008;**14 Suppl 1:**53
Livermore *JAC* 1993; **31 Suppl A**:9

MICs (mg/L) for CAZ-AVI-selected *bla*$_{KPC}$ mutants:

Single & multi-step mutants (X+Y)	CAZ-AVI 1 mg/L		CAZ-AVI 4 mg/L		Ceftaroline –AVI 4 mg/L	
	Parent	Mutants	Parent	Mutants	Parent	Mutants
Klebsiella						
NCTC13438 (29+2)	8	64->256	1	4-128	0.5	0.5-8
H…643 (24+6)	8	32->256	1	8-128	1	0.5-4
Enterobacter						
H…226 (28+5)	1	16-256	0.5	4-128	0.5	1-8
H…216 (7+0)	1	16-128	0.25	8-64	0.5	0.5-2
Geom. mean rise, Mutants (101) pooled	30.5-fold		34.3-fold		3.3-fold	

Livermore *et al*. *AAC* 2015; **59:**5324

KPC sequences from 13 CAZ-AVI-selected mutants

	Klebsiella NCTC 13438	*Klebsiella* H…643	*E cloacae* H…226	*E cloacae* H…216
Asp163Gly		1		
Pro174Leu			1	1
Asp179Tyr	2	1		1
180Ser181		1		
181 Ser-Ser 182			1	
183 Arg-Ala-Val-Thr-Thr-Ser-Ser-Pro 184				1
Thr243Pro			1	
265Ala-Arg 266				1
None	1			

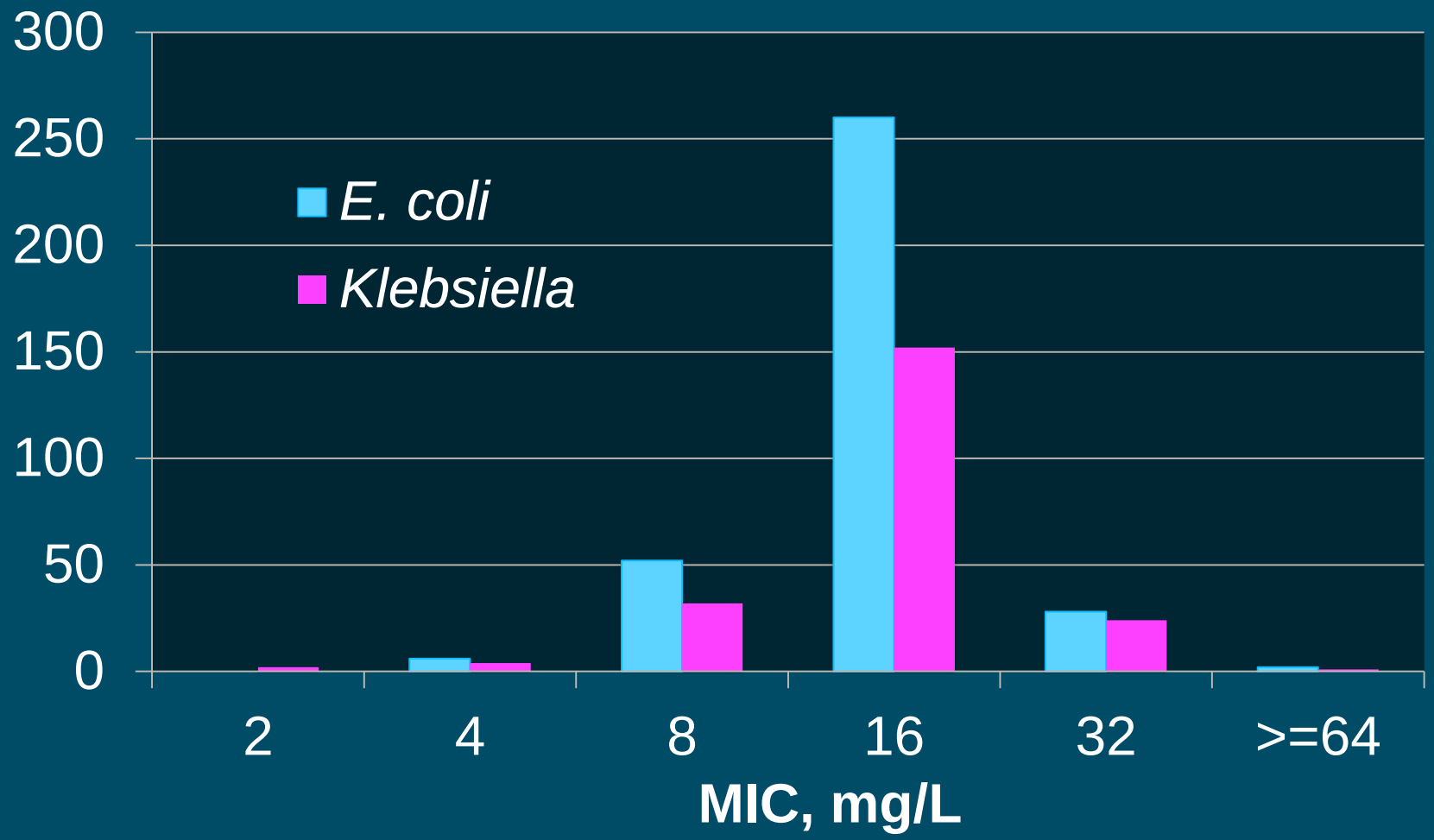
Livermore *et al*. *AAC* 2015; **59:**5324

Why are some β-lactams easier to protect?

- Weaker substrate / lower affinity (=higher K_m)
- High affinity partner β-lactam may protect the enzyme
- Fewer enzymes need to be inhibited if drug is stable to some
  - Many isolates now have multiple β-lactamases
  - Can overcome multiple enzyme if partner is stable to some and inhibitor inactivates others

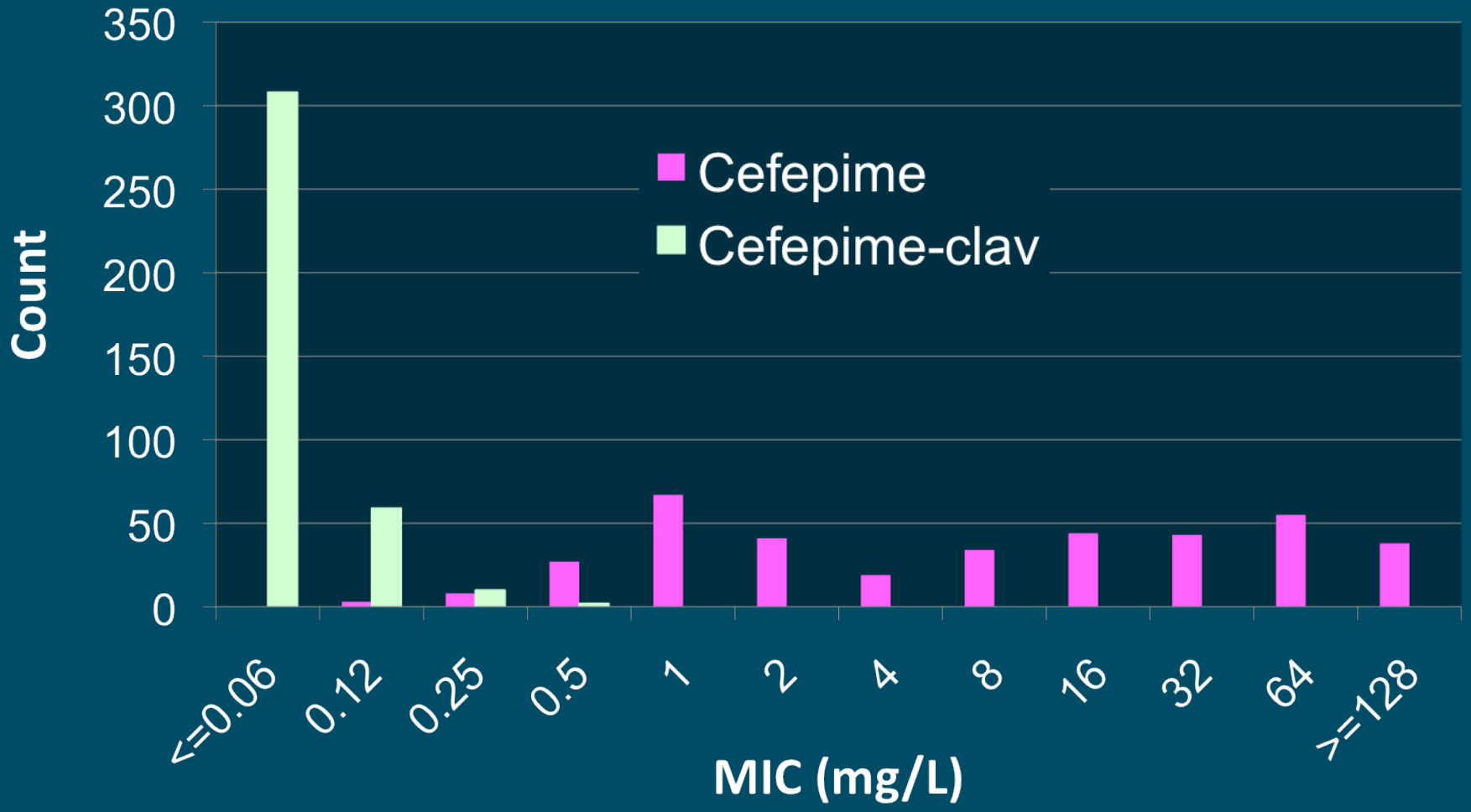
Activity of co-amoxiclav 2:1 *vs.* ESBL +ve *E. coli & Klebsiella*

University of East Anglia



Livermore *et al. CMI* 2008;**14 Suppl 1:** 189

Cefepime-clavulanate (4 mg/L) *vs.* ESBL *E. coli*



Livermore *et al. CMI* 2008;**14 Suppl 1:** 189

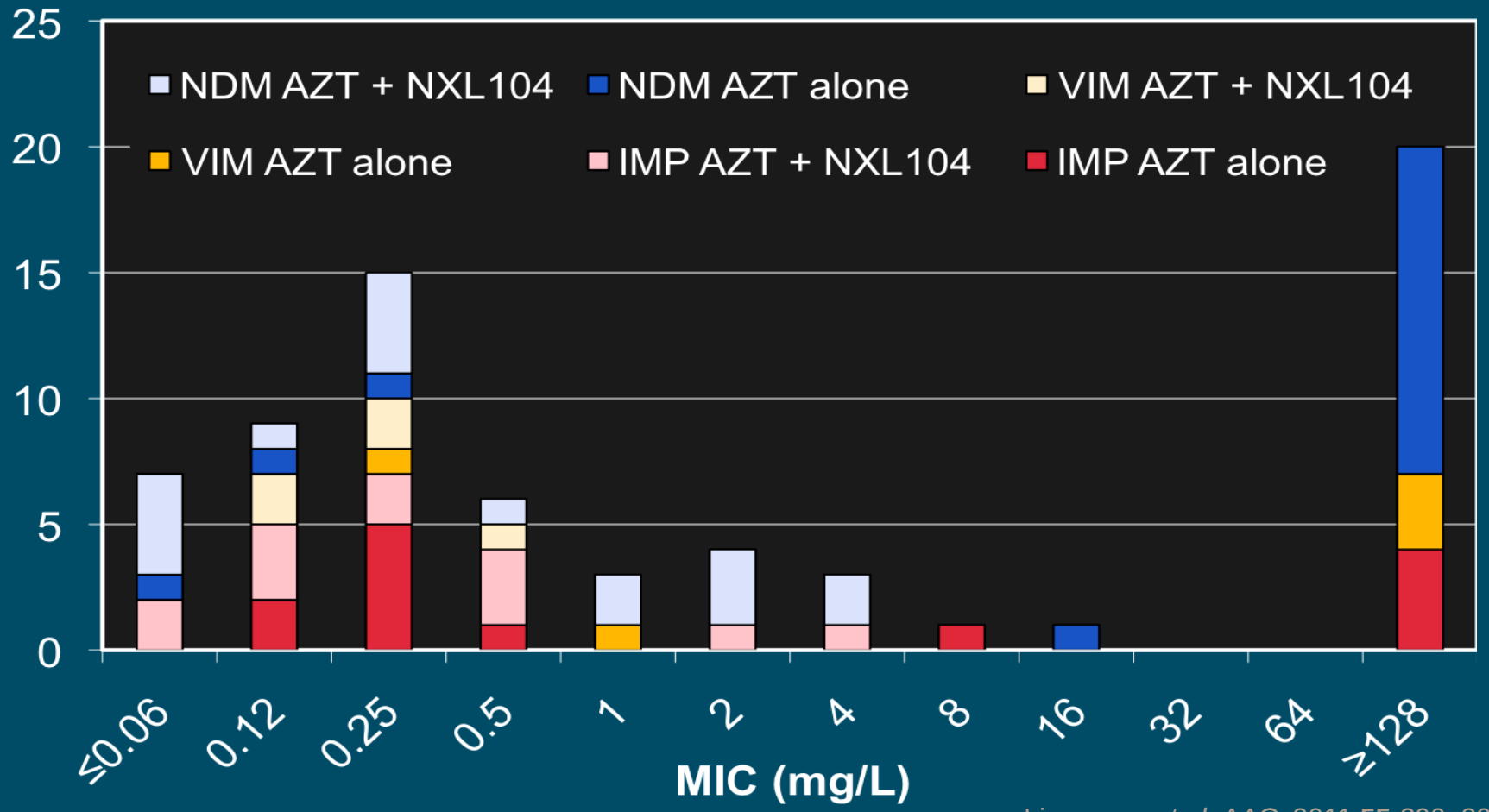
Carbapenems + ME1071 *vs.* 20 NDM Enterobacteriaceae

	k_{cat} (s^{-1})	K_m (μM)	Geom. mean MIC (mg/L)	
			Alone	+128 mg/L ME1071
Imipenem	315	60	42.2	10.6
Meropenem	77	15	84.5	19.0
Doripenem	275	41	68.6	10.9
Biapenem	233	314	7.7	0.78

Livermore *et al JAC* 2013;**68**:153

Aztreonam-avibactam

aztreonam is stable to MBLs anyway….



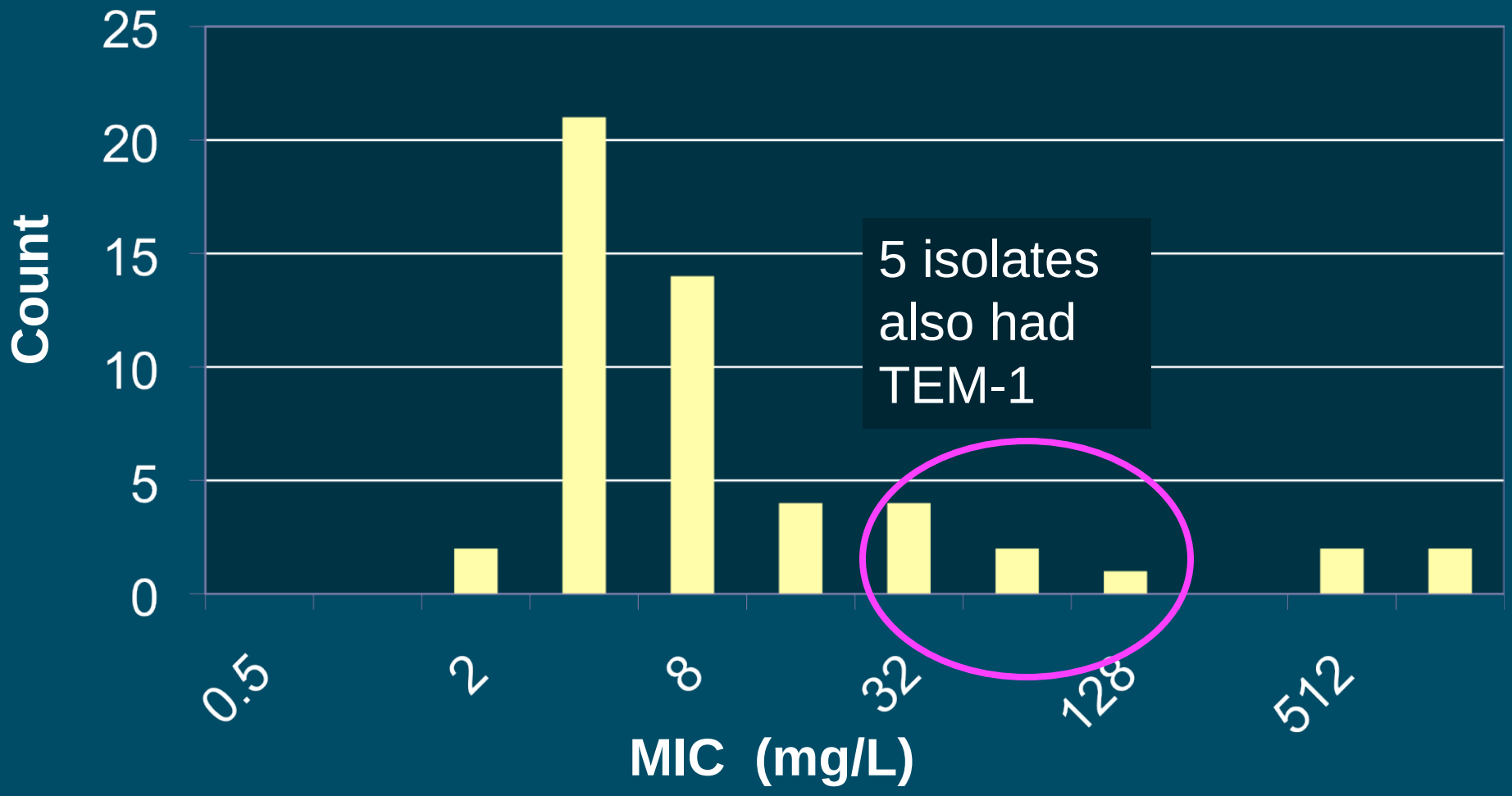
Livermore *et al. AAC* 2011;**55:**390–394

Potentiation in relation to amount of TEM-1 β-lactamase

	Quartile of β-lactamase distribution			
	1	2	3	4
Geom. mean [inhibitor] to bring amoxicillin MIC to ≤8 mg/L				
Tazobactam	1.6	1.7	4.7	14.9
Geom. mean [inhibitor] to bring piperacillin MIC to ≤16 mg/L				
Tazobactam	1.3	1.3	2.7	5.4

Livermore & Seetulsingh, *JAC* 1991;**27**: 761

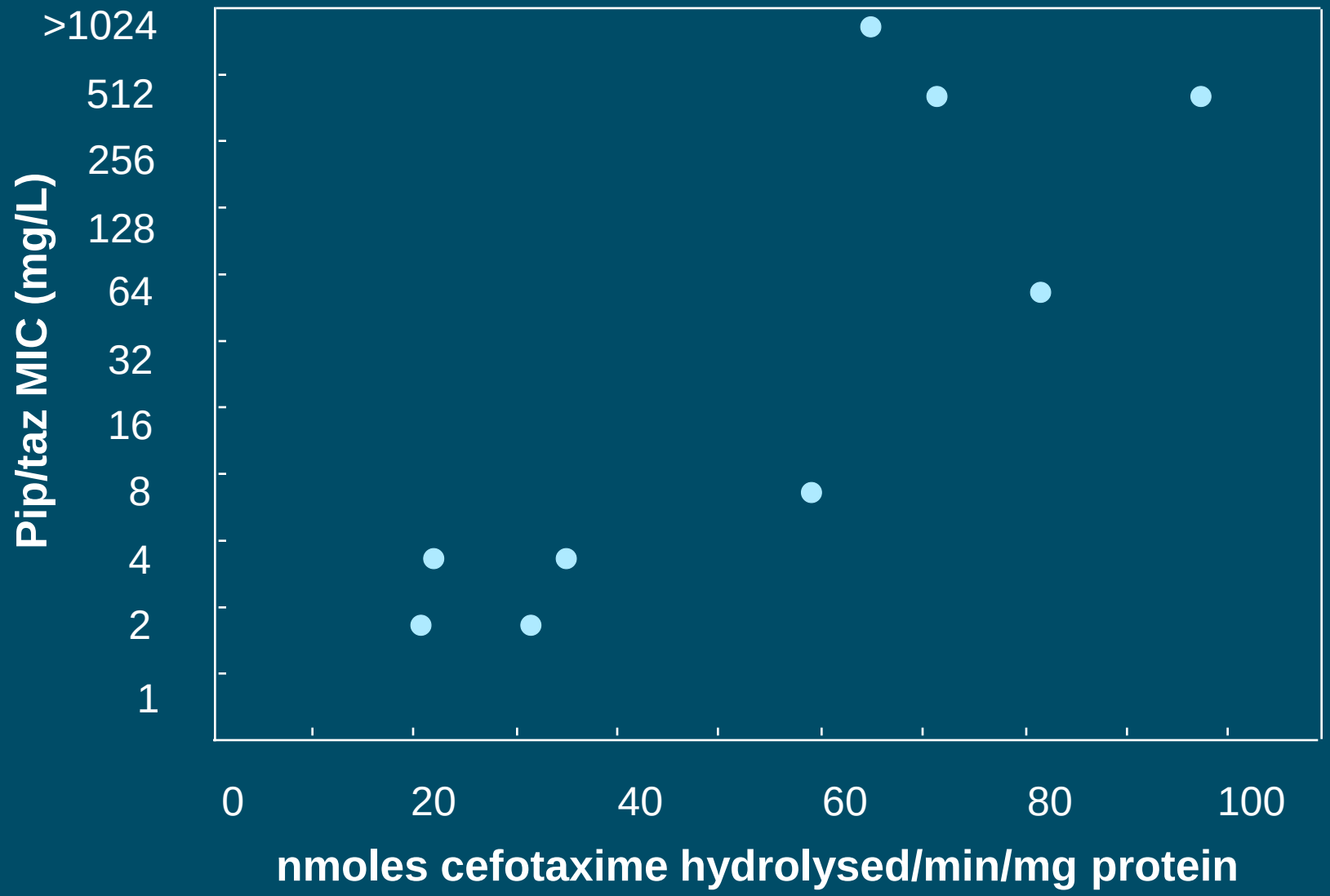
Pip/tazo *vs. K. pneumoniae*
PN1 clone SHV-4



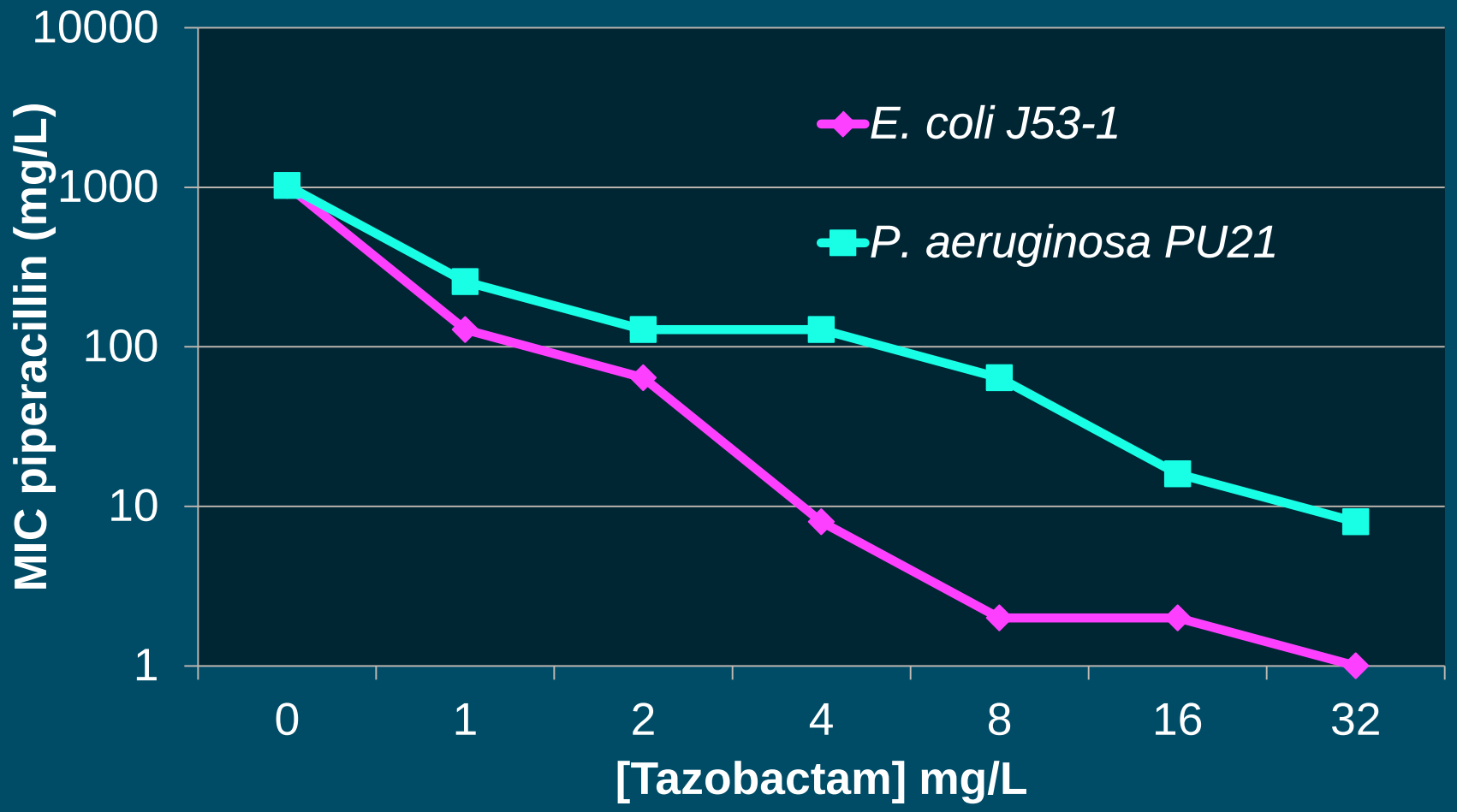
Babini *et al. JAC* 2003;**51**:605

Pip/tazo MICs & SHV-4 activity in K25/PN1 isolates

University of East Anglia



Pip-tazo *vs. E. coli* and *P. aeruginosa* with PR4 plasmid / TEM-2



Livermore *JAC* 1993;**31 Suppl A**:9

pH, TEM-1 & piperacillin-tazobactam….

University of East Anglia

pH	I_{50} (10 min) μM		V_{max}/K_m	MIC (mg/L) *E. coli* K-12		
	Clav	Tazo	Pip	R^- pip	TEM-1 pip-tazo	TEM-1 pip-clav
6.5	0.22	1.1	0.45	1	64	4
7.0	0.29	0.51	0.25	1	4	2
7.5	0.27	0.15	0.25	0.5	2	1
8.0	0.13	0.008	0.17	0.5	1	0.5

Livermore & Corkill *AAC* 1992;**36:**1870

Summary

- **Activity of inhibitor combinations reflects:**
  - Enzyme type….Amount…..Partner ….Organism…..pH
- **Long history of sub-optimal combinations**
  - Who owns what; what's out of patent / known / safe
- **Need to simply dev't of better combinations**
  - If β-lactam A is available with inhibitor I
  - & β-lactam B with inhibitor II
  - Trials should be simplified for A+II