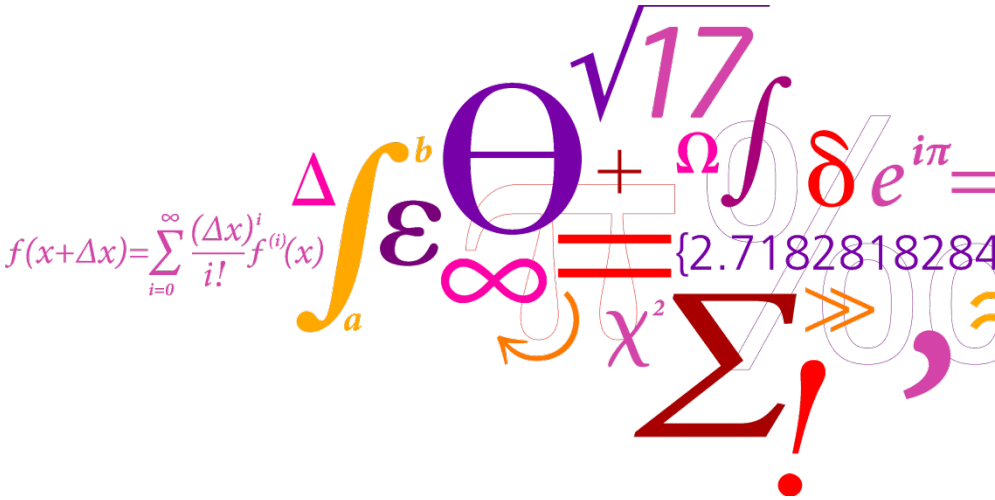
Validation of current interpretative criteria for colistin susceptibility in *Salmonella spp. and for quinupristin/dalfopristin susceptibility in Enterococcus faecium*

Yvonne Agersø



DTU Food
National Food Institute

Background

- The work presented is entitled:

Evaluation of the quinupristin/dalfopristin breakpoints for Enterococcus faecium.

Authored by Hammerum AM, Agersø Y, Garcia-Migura L, Seyfarth AM, Porsbo LJ, Emborg HD, Bogø Jensen L (Int J Antimicrob Agents. 2009)

Background

•The streptogramin family of antibiotics consists of virginiamycin, pristinamycin and Q/D.

•Virginiamycin has been banned as a growthpromoter in Europe but is still used in some other countries.

•Pristinamycin and Q/D are used for the treatment of infections caused by vancomycin-resistant *E. faecium* or meticillin-resistant *Staphylococcus aureus* in humans.

MIC testing may be difficult due to the instability of the compounds. Resulting in increased MICs.

Two breakpoints currently exits CLSI ≥4mg/L and EUCAST >4mg/L as the breakpoint for Q/D.

Activity of drugs

- All streptogramin antibiotics consist of two unrelated compounds, streptogramin A and streptogramin B.

- Individually the A and B components are bacteriostatic, whereas in combination they are bactericidal. Therefore, streptogramin A and streptogramin B are used in combination
- quinupristin (streptogramin B) and dalfopristin (streptogramin A).

- Mechanisms conferring resistance against both components (A and B) are essential for resistance against the combination in *E. faecium.*

Resistance mechanism in *E. faecium*

- Two acetyltransferases encoded by *vat(D) and vat(E) that inactivate* streptogramin A.

- In most cases resistance to the streptogramin B component in *E. faecium* is due to methylation of the 23 S rRNA encoded by *erm(B), which* also encodes resistance to macrolides and lincosamides.

- Two less frequently observed genes encoding resistance to streptogramin B have been detected in *E. faecium* [vgb(B) and msr(A)]

- Presumably, unspecific mutations can create reduced susceptibility.

- Millichap et al. were able to induce reduced susceptibility to Q/D by spreading isolates on plates with Q/D. Stable resistance was obtained for isolates with a minimum inhibitory concentration (MIC) > 8g/mL.

Breakpoint

- CLSI has suggested a breakpoint of ≥4mg/L for Q/D.
- EUCAST has suggested >4mg/L as the breakpoint for Q/D.

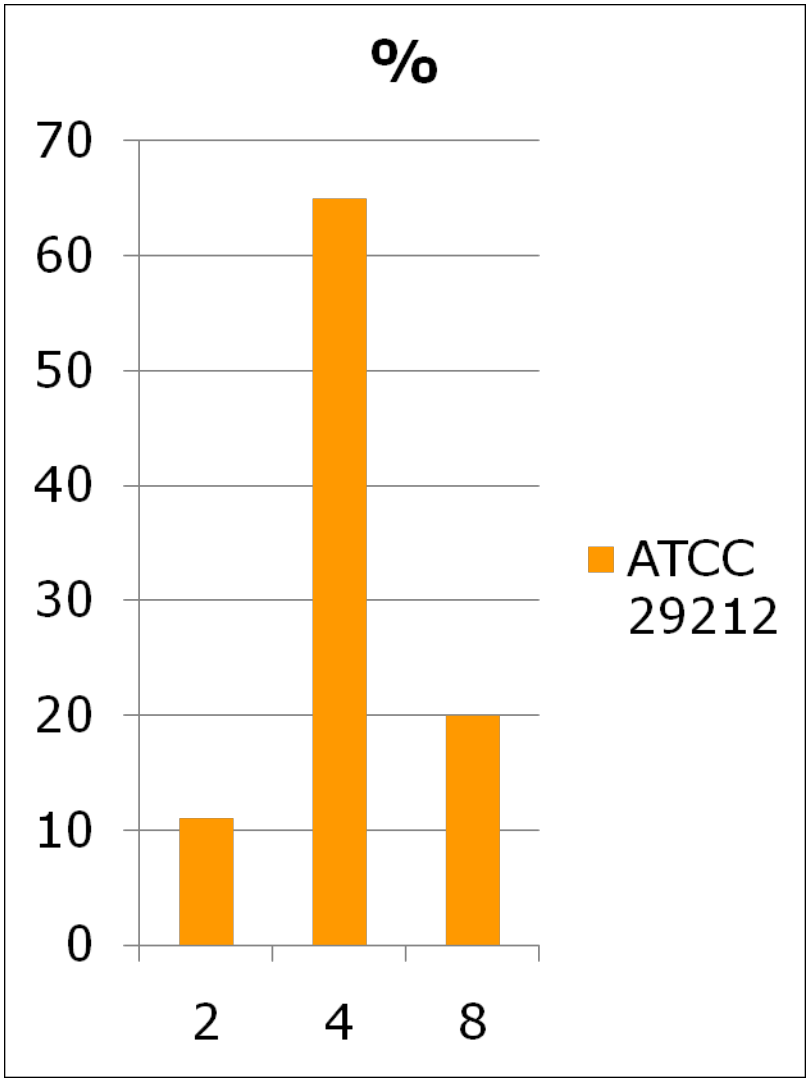
- In DANMAP reports 1998–2006, the MICs for Q/D have been determined using Sensititre (TREK Diagnostic).

Distribution of MICs and occurrence of quinupristin/dalfopristin resistance using two different breakpoints among *Enterococcus faecium from poultry,* pigs and healthy humans from DANMAP reports (1998–2006).

Animal species	Year	Numbers of isolates tested	% Resistant with use of two different breakpoints		Distribution (%) of MICs for Quinupristin/dalfopristin								
			4	8	0.25	0.5	1	2	4	8	16	32	64
Poultry	1998	151	74.0	59.7	0.7	3.3	5.2	16.9	14.3	21.4	32.5	5.8	
	1999	189	40.7	22.7		14.8	12.7	31.8	18.0	14.8	7.4	0.5	
	2000	189	37.1	27.6		13.2	6.9	42.9	9.5	26.5	1.1		
	2001	131	30.6	7.7		19.8	22.9	26.7	22.9	6.9	0.8		
	2002	102	28.5	21.6		20.6	10.8	40.2	6.9	18.6	2.0		1.0
	2003	123	25.2	6.5		22.0	15.4	37.4	18.7	6.5			
	2004	135	23.7	14.8		13.3	23.0	40.0	8.9	13.3	1.5		
	2005	131	13.0	2.3		46.6	11.5	29.0	10.7	2.3			
	2006	72	5.6	1.4		29.2	20.8	44.4	4.2	1.4			
Pigs	1998	156	46.4	11.1	3.9	9.2	7.8	32.7	35.3	6.5	3.9	0.7	
	1999	202	19.4	4.0		24.8	6.4	49.5	15.4	3.0	1.0		
	2000	182	23.6	15.4		15.9	15.9	44.5	8.2	1.1	7.7	6.6	
	2001	175	8.6	2.3		21.7	9.7	60.0	6.3	2.3			
	2002	194	12.9	0.5		23.7	7.7	55.7	12.4	0.5			
	2003	175	8.6	0.6		19.4	8.6	63.4	8.0		0.6		
	2004	148	12.9	0.7		19.6	6.8	60.8	12.2	0.7			
	2005	105	16.2	1.0		17.1	8.6	58.1	15.2	1.0			
	2006	145	20.0	1.4		13.8	5.5	60.7	18.6	1.4			
Healthy humans	2002	40	2.5	0		30.0	10.0	57.5	2.5				
	2003	55	7.3	0		23.6	14.6	54.5	7.3				
	2004	54	35.2	0		18.5	16.7	29.6	35.2				
	2005	50	54.0	0		26.0	8.0	12.0	54.0				
	2006	24	37.5	0		25.0	16.7	20.8	37.5				

Reproducibility of MIC results

- Testing of *E. faecium* ATCC 29212
- 65 times weekly (>1 yr)
- MIC range 0.5-8 mg/L
- <u>MIC did not seem to be influenced by the age of the panels.</u>



Testing of genes in isolates with ≥4mg/L

	Broilers	17 (MIC=4)	4 (MIC=8)
Broilers	vat(D)	1/17 (6%)	1/4 (25%)
	vat(E)	3/17 (18%)	3/4 (75%)
Pigs	vat(D)	0/45 (0%)	0/3 (0%)
	vat(E)	0/45 (0%)	0/3 (0%)
Healthy humans	vat(D)	0/27 (0%)	
	vat(E)	0/27 (0%)	
Total	positives	3/89 (3%)	4/7 (57%)

Isolates with no genes found are either not 'truly' resistant or other unknowns mechanisms are present

Conclusion of the breakpoint for Q/D

- Variations in MICs owing to the experimental setup can lead to misinterpretation of isolates as resistant because of the overlapping population structure of sensitive and resistant populations.

- We suggest a MIC value >4mg/L as the resistance breakpoint for Q/D, whereas a MIC of 4mg/L should be reported as intermediate-resistant.

- Intermediate-resistant isolates should be tested for the presence of streptogramin resistance genes.

- Our findings support the EUCAST breakpoint of >4g/mL and question the breakpoint recommended by the CLSI (≥4g/mL) for Q/D.

Problems with colistin breakpoint

- Work in preb. Agersø Y, Seifarth AM, Hammerum AM, Møller Nielsen E.
- Colistin resistance in *E. coli* and Salmonella can be used in the microbiology lab. To identify falliure in ID or MIC.

- The later breakpoint by CLSI and EUCAST >2 mg/L.

- Are *E. coli* and *Salmonella* with MIC >2mg/L truly resistant?

Background

- Treatment of *P. aeruginosa* in patients with cystic fibrosis.

Mechanism of action:

➡ Colistin targets the cell envelope

➡ interaction between the cationic polypeptide and negatively charged lipopolysaccharides(LPS)

➡ disturbance of the outer membrane

➡ increase in permeability = death

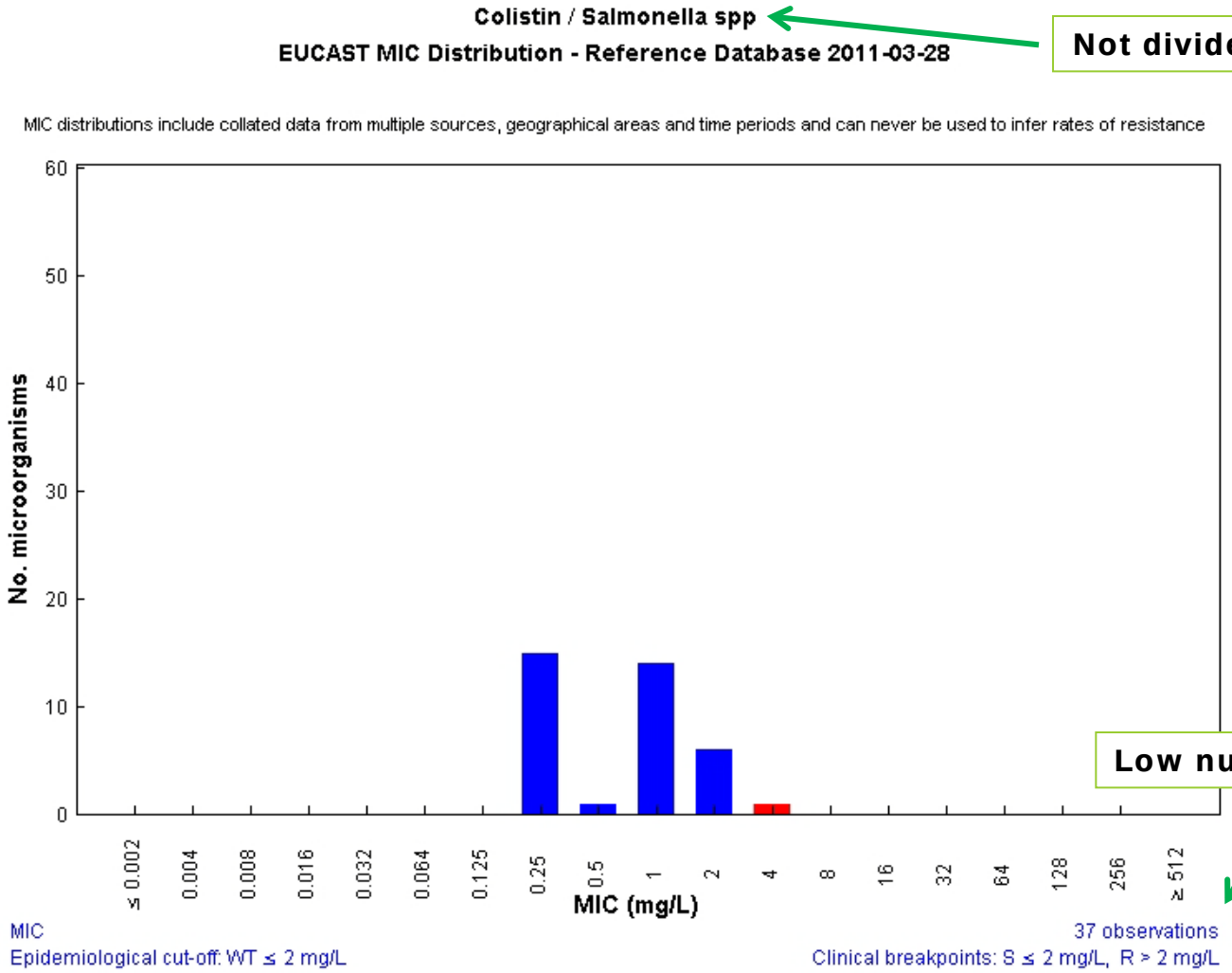
- Eucast breakpoint from >8 µg/ml to >2 µg/ml

Mechanism of resistance

- Mutations conferring colistin resistance on *S. Typhimurium can be introduced in vitro in two* locus named *pmrA og pmrB. Sun et al 2009.*

- *In S. Typhimurium,P. aeruginosa, and A. baumannii, two genes, pmrA and pmrB,* were identified and shown to constitute a two-component regulatory system responsible for polymyxin resistance in all three species.

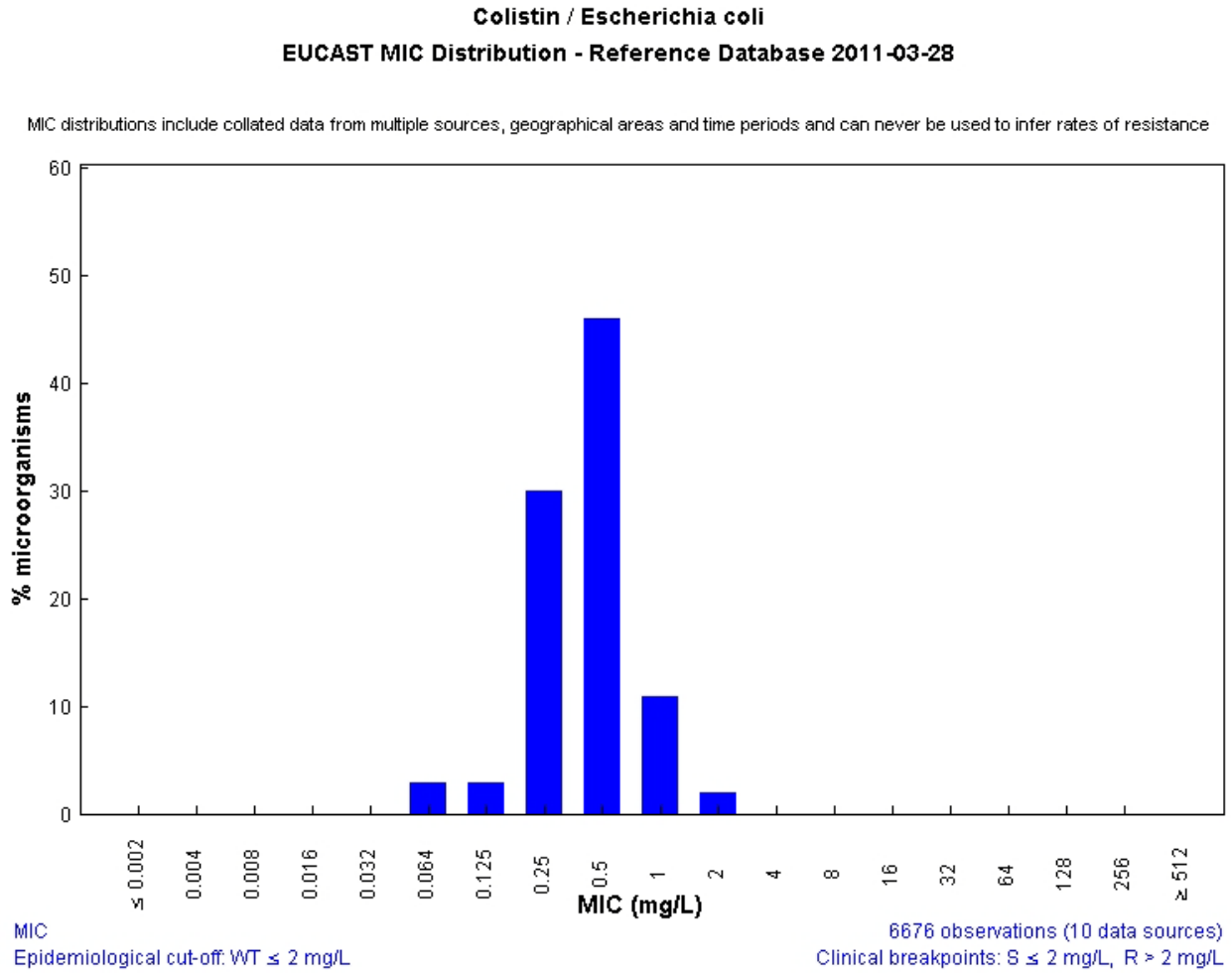
MIC distribution for colistin from Eucast homepage



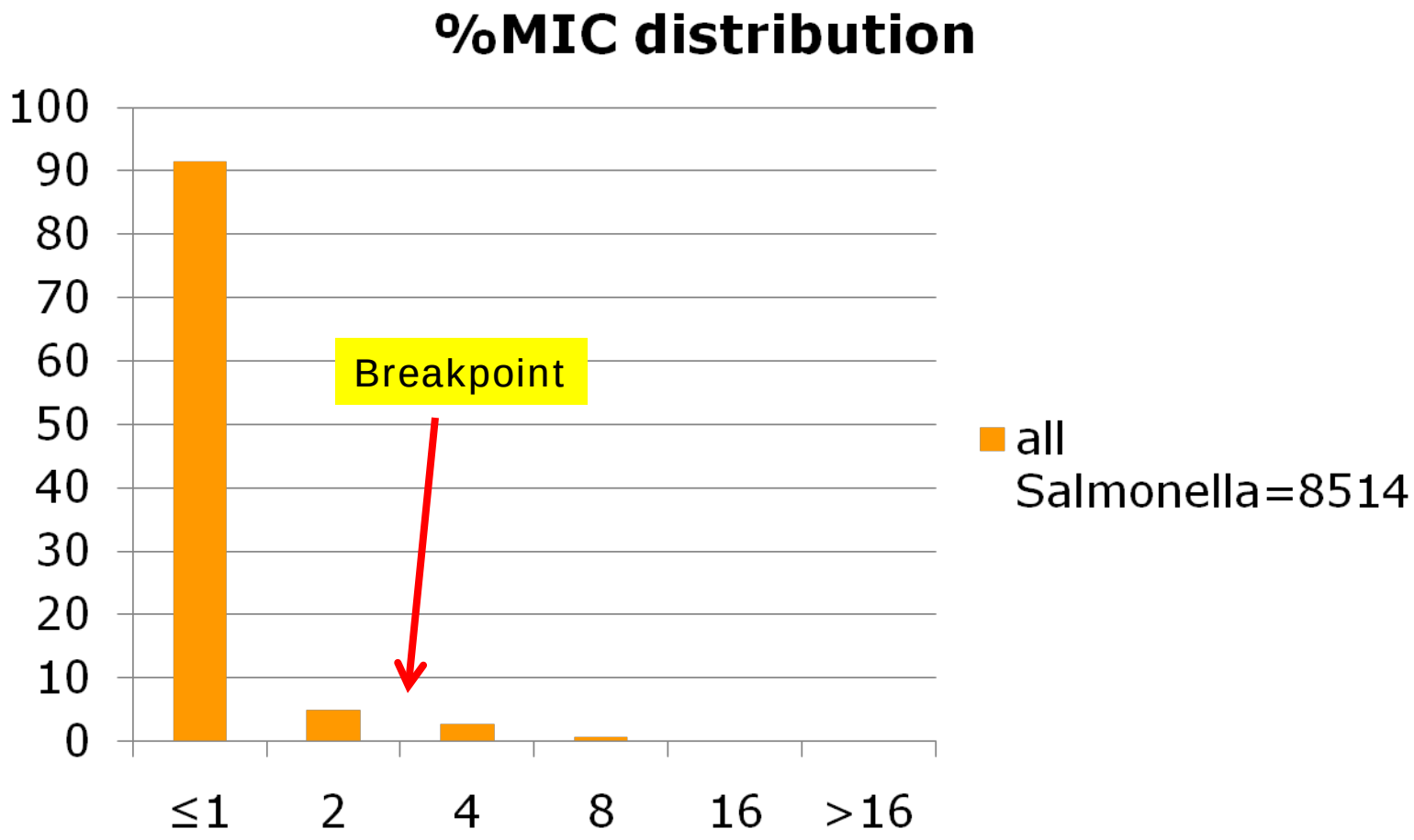
Not divided into serovars

Low number of isolates

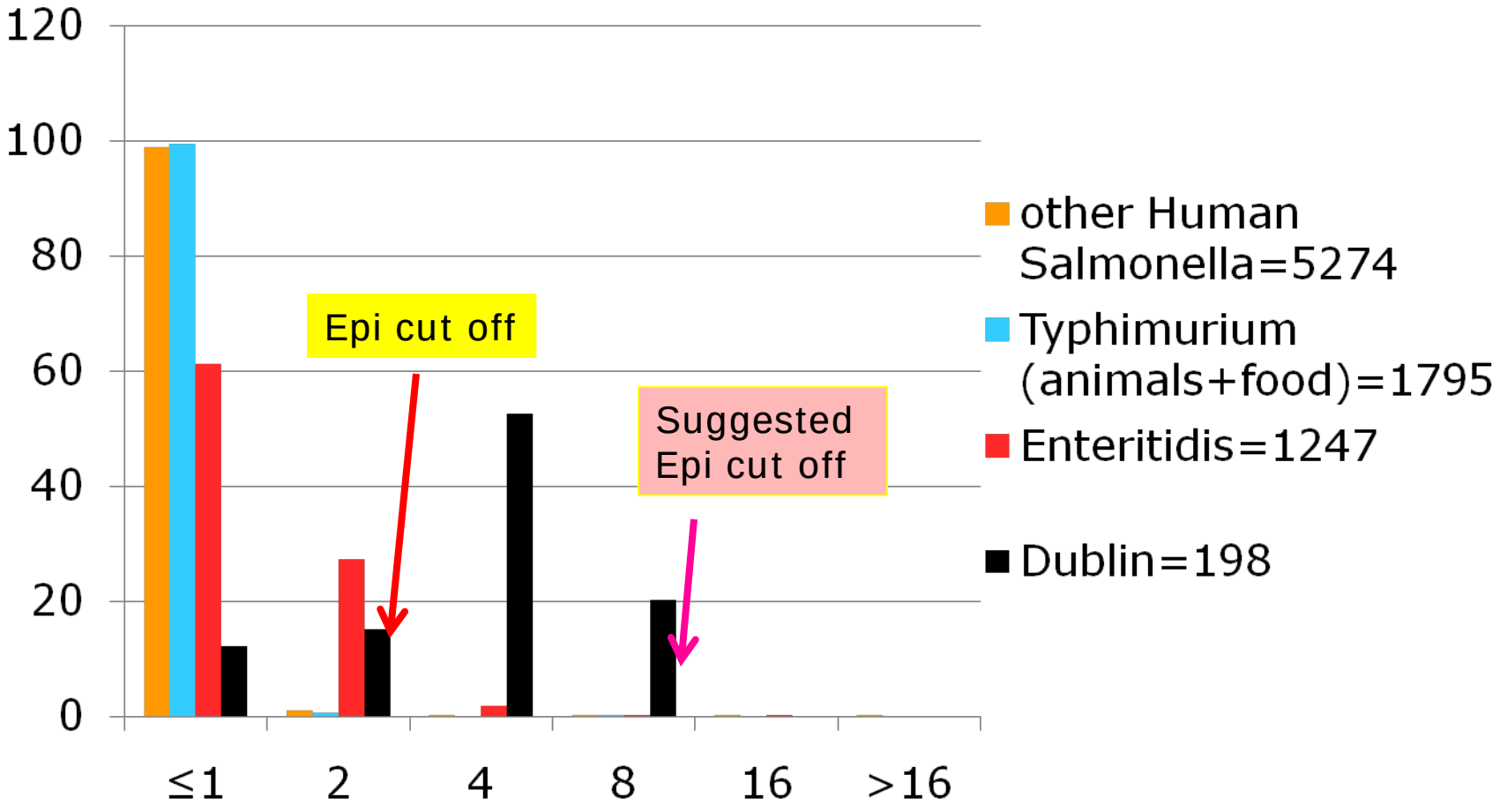
MIC distribution for colistin from Eucast homepage

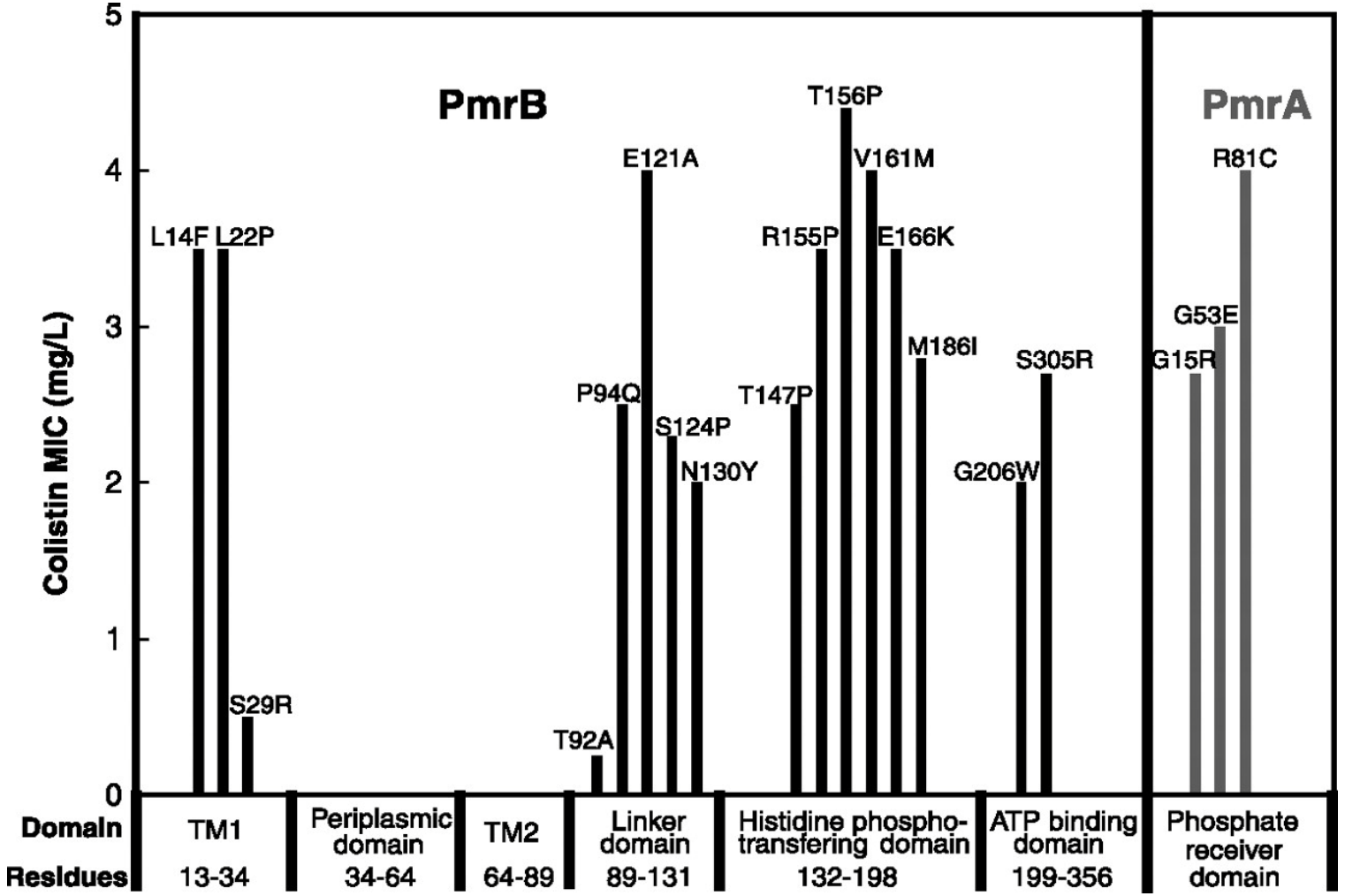


DANMAP isolates 2008-10



% MIC distribution serovar level





Sun et al, 2009

Testing for mutation in *pmgA* and *pmgB*

- 41 clinical isolates out of 1541 from 2009 had a MIC of 4, 8 or >16.
- MIC were repeated and resulted in 26 with MIC of 4,8 or >16. most with a shift from MIC=4 to MIC=2.

Serovar	**≤ 1**	**2**	**4**	**8**	**>16**	**I alt**
Dublin	1	4	12	4	1	22
Enteritidis	1	6	7	1		15
Typhimurium	1			1		2
Concord	1[a]					1
Reading	1[b]					1

[a] *pmgB* mutation
[b] *pmgA* mutation

Results

- All 41 were checked for miss-mutations in *pmgA* and 28 for *pmgB (*Sun et al, 2009).

- *pmgA* was sequenced for all 41 isolates and 1 isolate with a miss-sense mutation in position 266 was found (serovar Reading MIC ≤ 1).

- *pmgB* was sequenced for 28 isolates (bad result for 13).

- 1 isolate with miss-sense mutation in position 1025 (serovar Concord MIC ≤ 1).

- Modified primers showed a miss-sense mutation in all Dublin aa 9 independenly of MIC.

Discussion / conclusion (Colistin)

- Missense mutations in pmgA and pmgB did not correlate with increased MIC value.
- No isolates with increased MIC had mutations in pmgA or pmgB. Except Dublin which had the same mutation in all isolates.
- Increased MIC-værdi for colistin are relatered to serotypes (Dublin and Enteritidis, both O:1, 9,12).
- Merging Salmonella spp into the same MIC distribution has lead to misinterpretation of the epi cut off value.
- Because the breakpoint is in the middel of the MIC distribution for S. Dublin we may recommend the breakpoint is increased from >2 to >8.