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Phenotypic methods for detection of resistance mechanisms

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PGEC Madrid, 28 September 2010

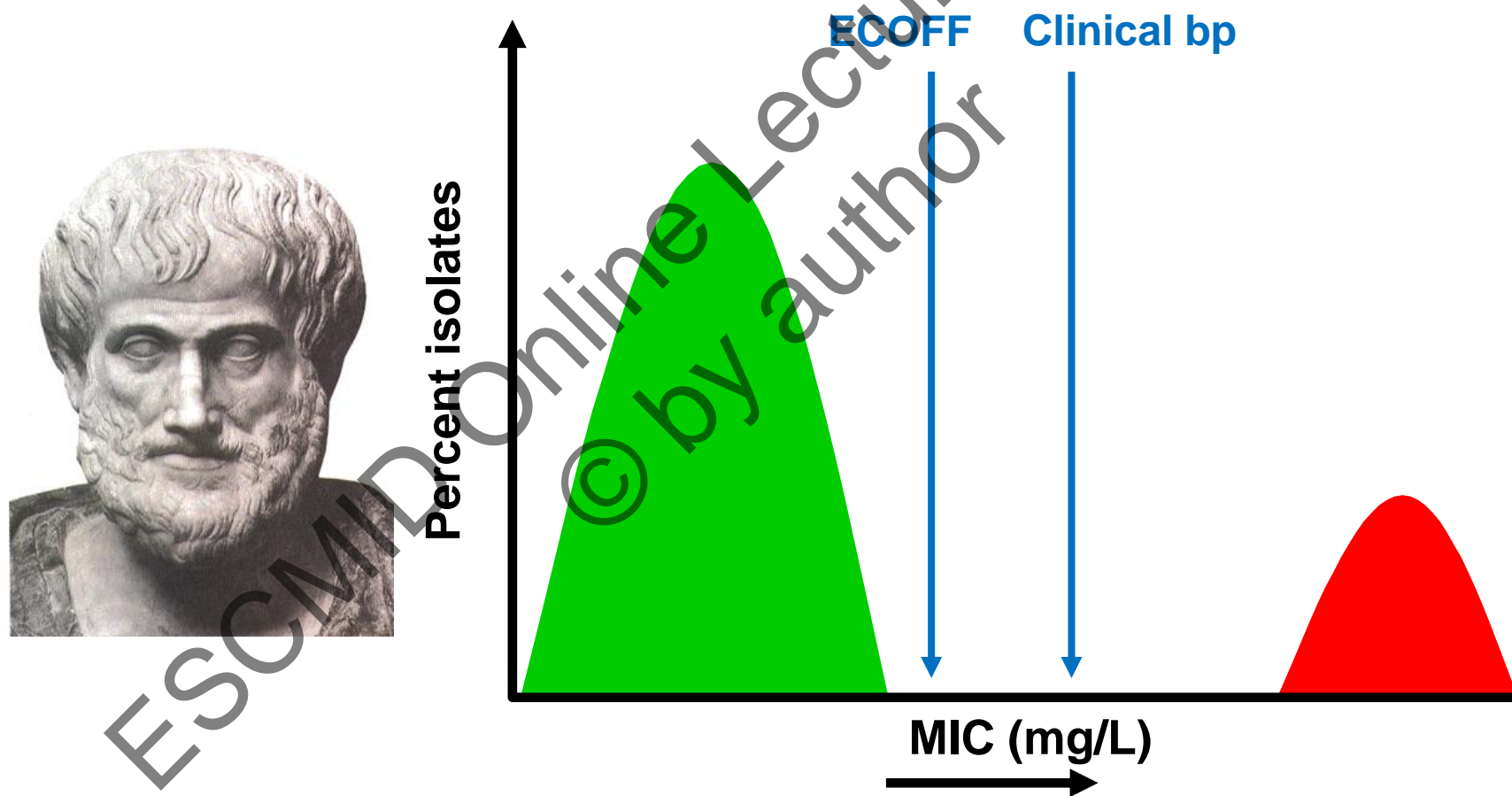
Detection of resistance mechanisms...

- By breakpoints
 - Zone breakpoints vs MIC breakpoints
 - Clinical breakpoints vs epidemiological cutoffs
- By additional tests
 - β -lactamase tests

Performance of breakpoints



Some words about definitions

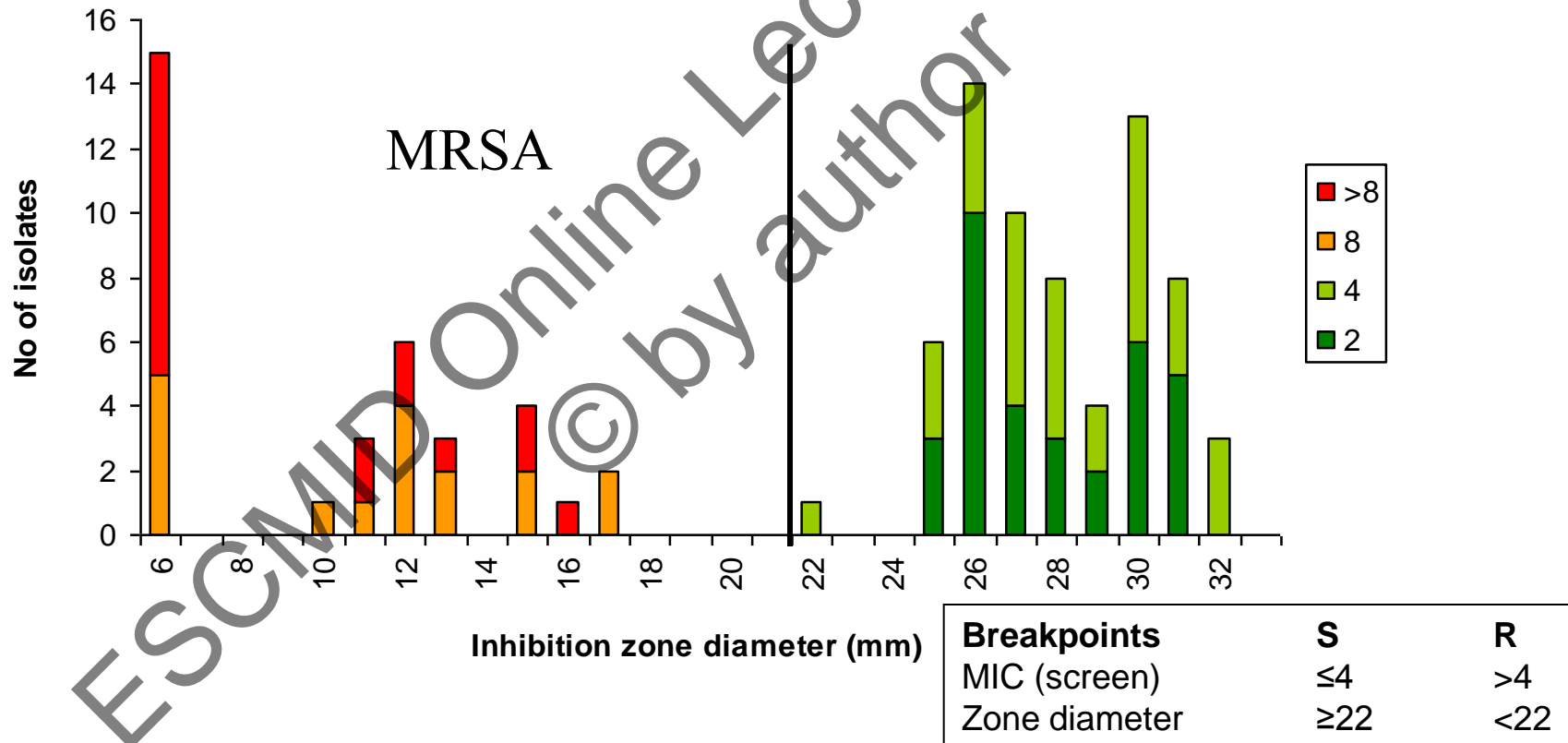


How do the EUCAST breakpoints perform for identifying some important resistance mechanisms?

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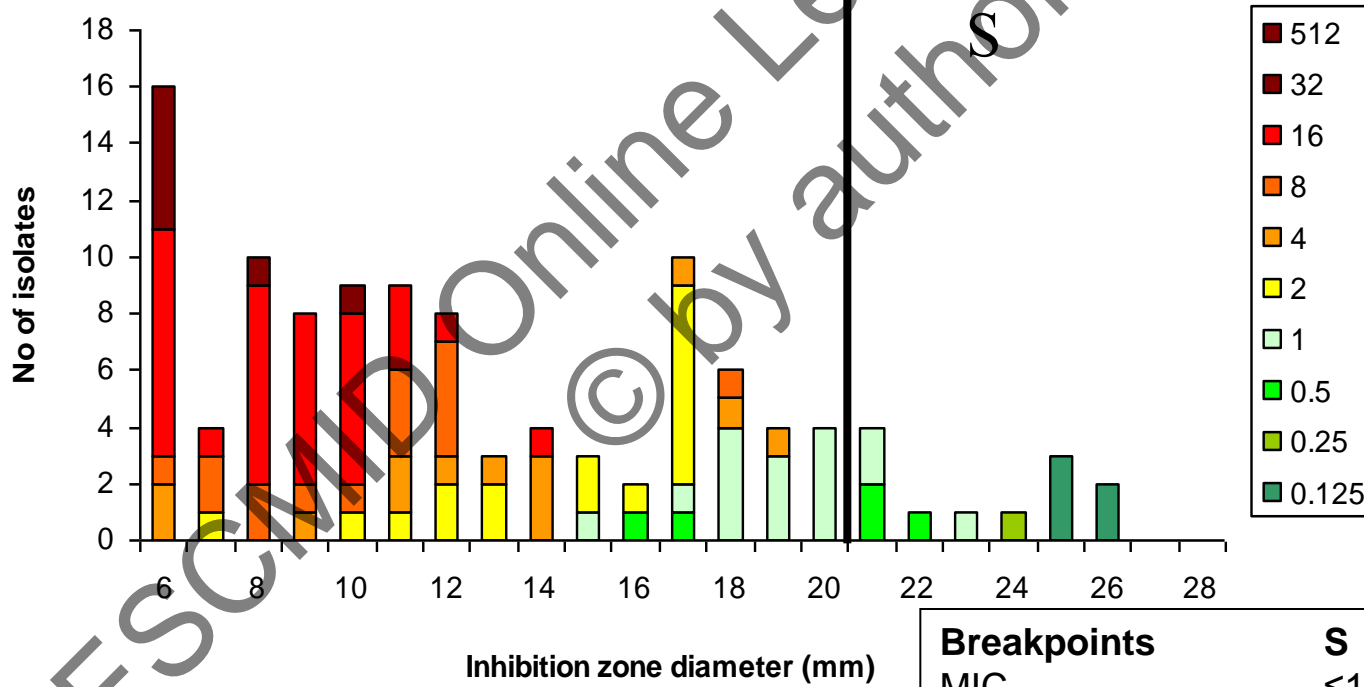
Methicillin-resistant *S. aureus*

S. aureus with cefoxitin
103 clinical isolates



Extended-spectrum β -lactamases in Enterobacteriaceae - cefotaxime

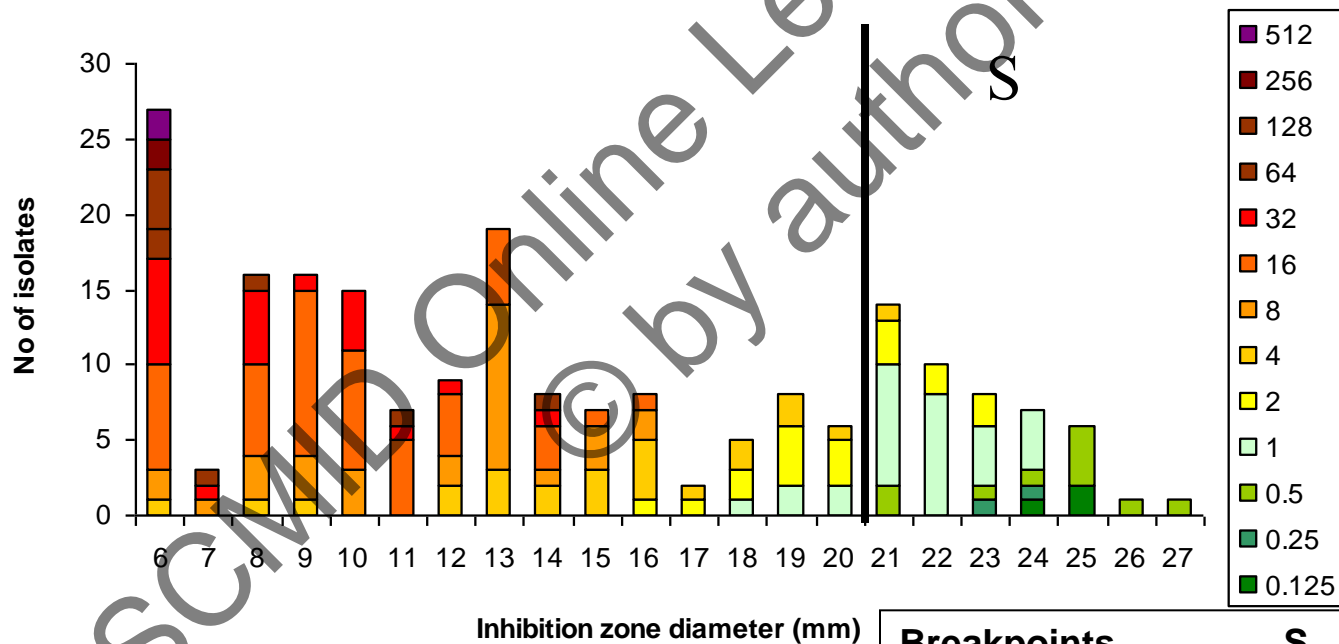
E. coli with cefotaxime 5 ug
112 non-consecutive isolates chose because of resistance



Breakpoints	S	R
MIC	≤ 1	> 2
Zone diameter	≥ 21	< 18

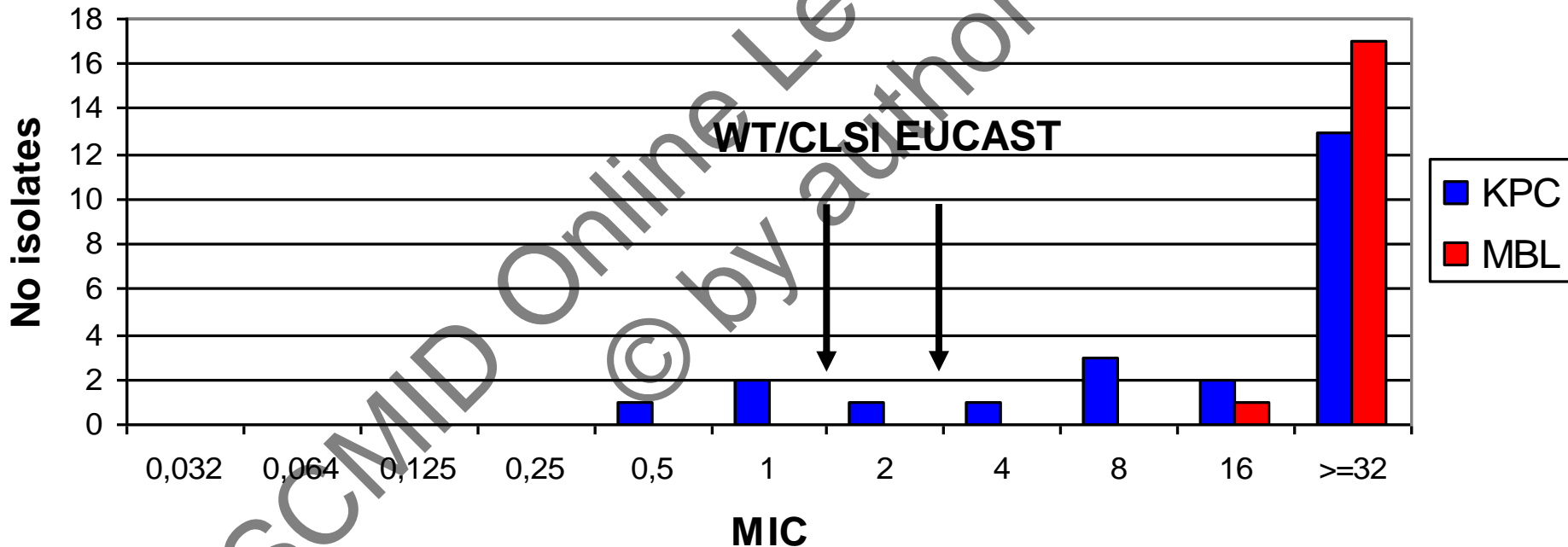
Extended-spectrum β -lactamases in Enterobacteriaceae - ceftazidime

E. coli with ceftazidime 10 ug
203 non-consecutive isolates chosen because of resistance



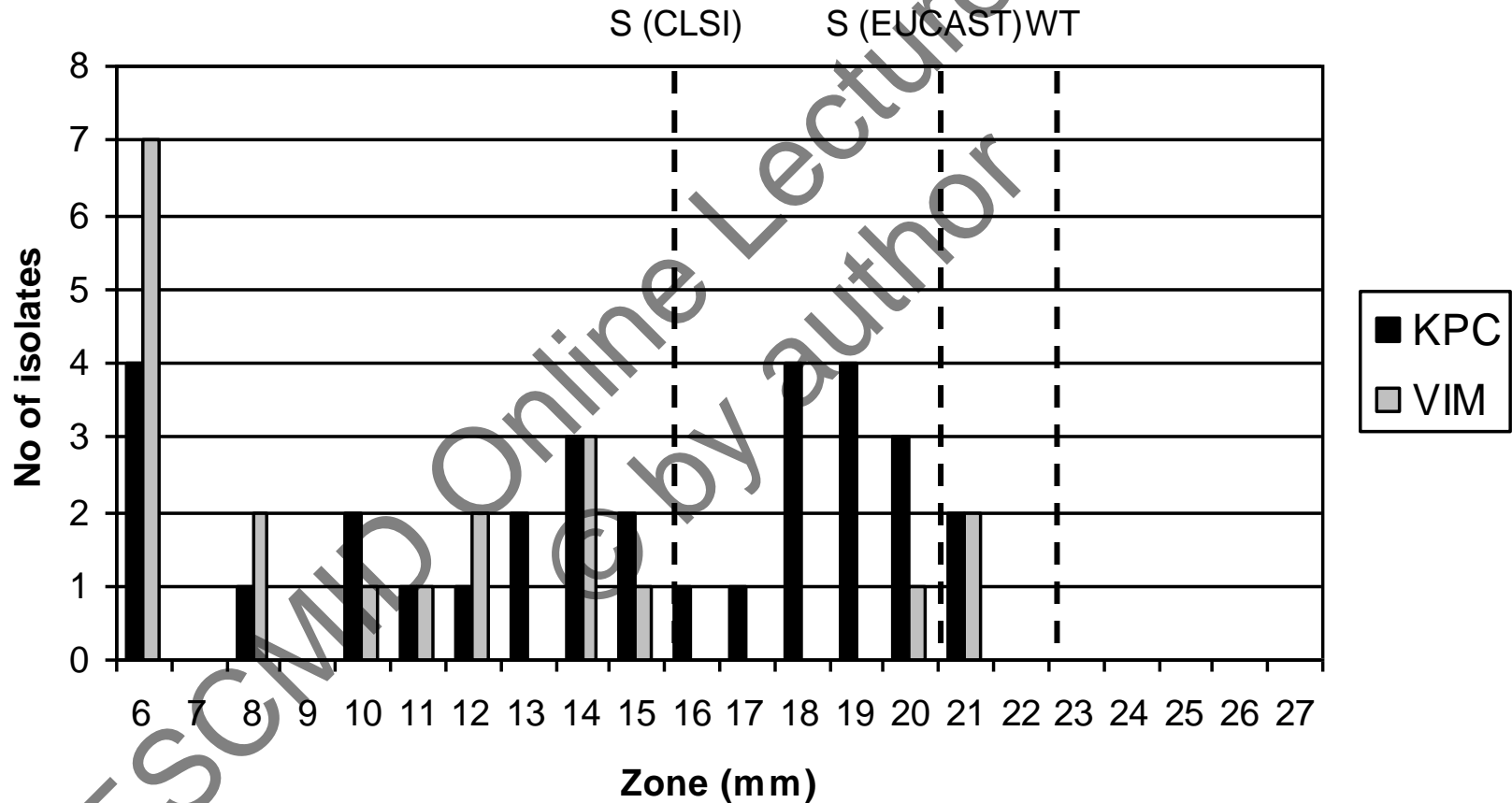
Detection of carbapenemases: *K. pneumoniae* and imipenem

Imipenem MIC: KPC- and MBL-isolates



Vading et al. CMI 2010. In press

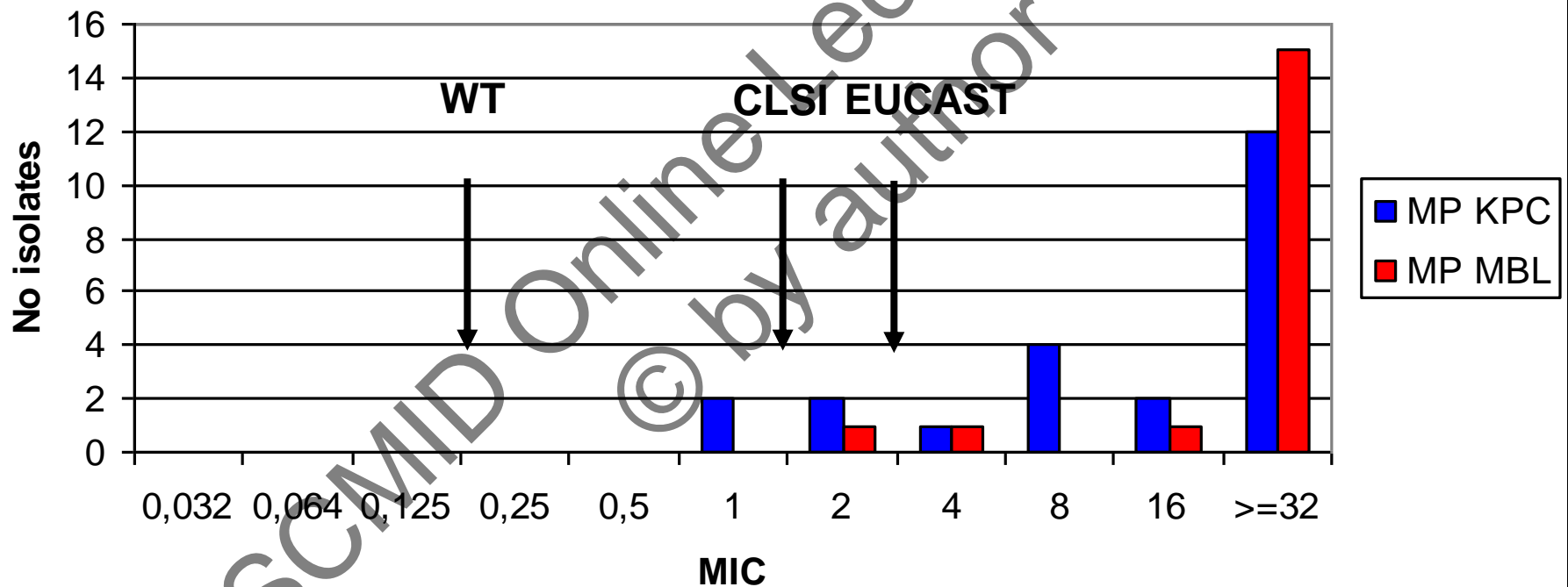
K. pneumoniae and imipenem



Vading et al. CMI 2010. In press

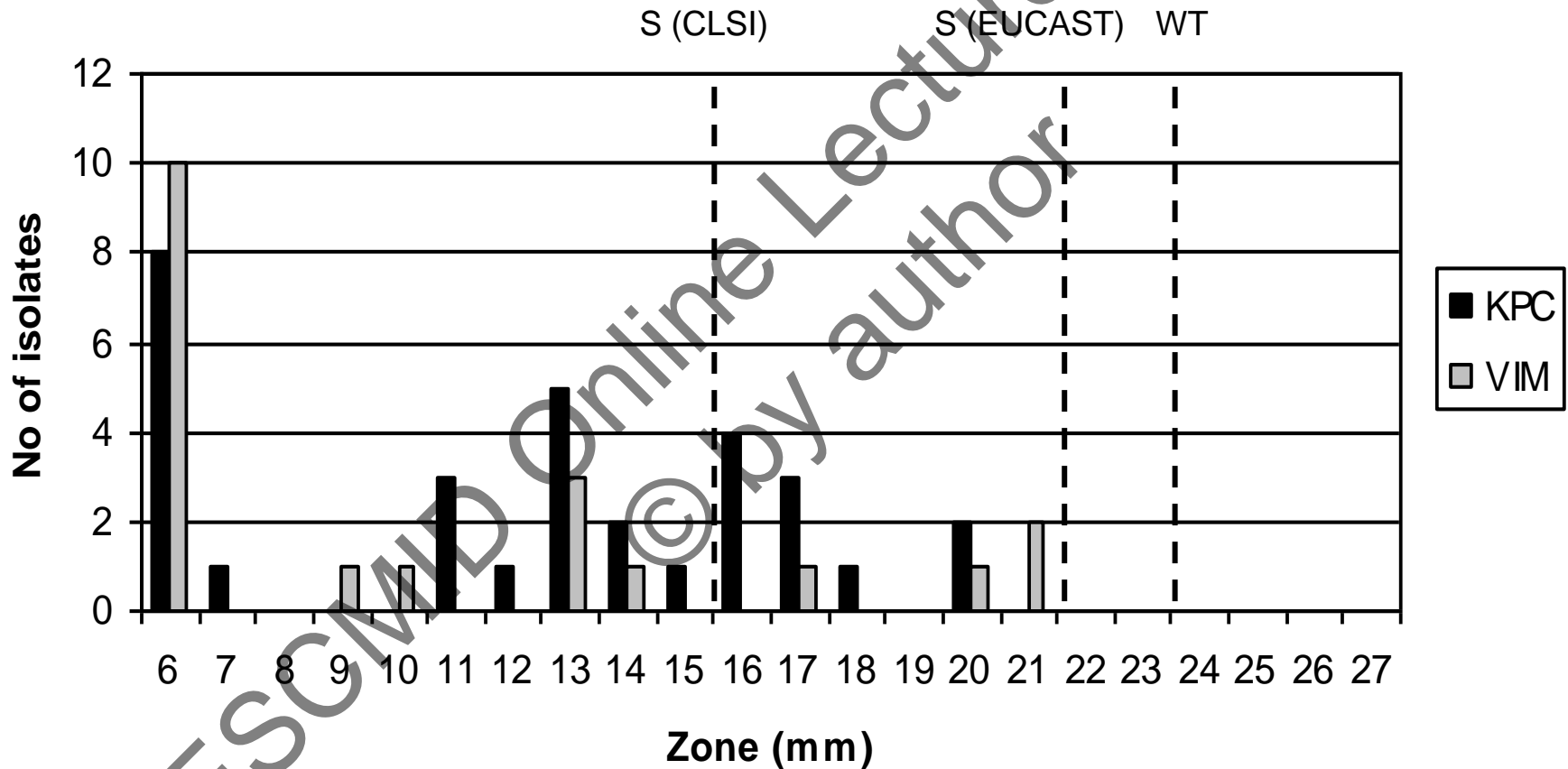
K. pneumoniae and meropenem

Meropenem MIC: KPC- and MBL-isolates



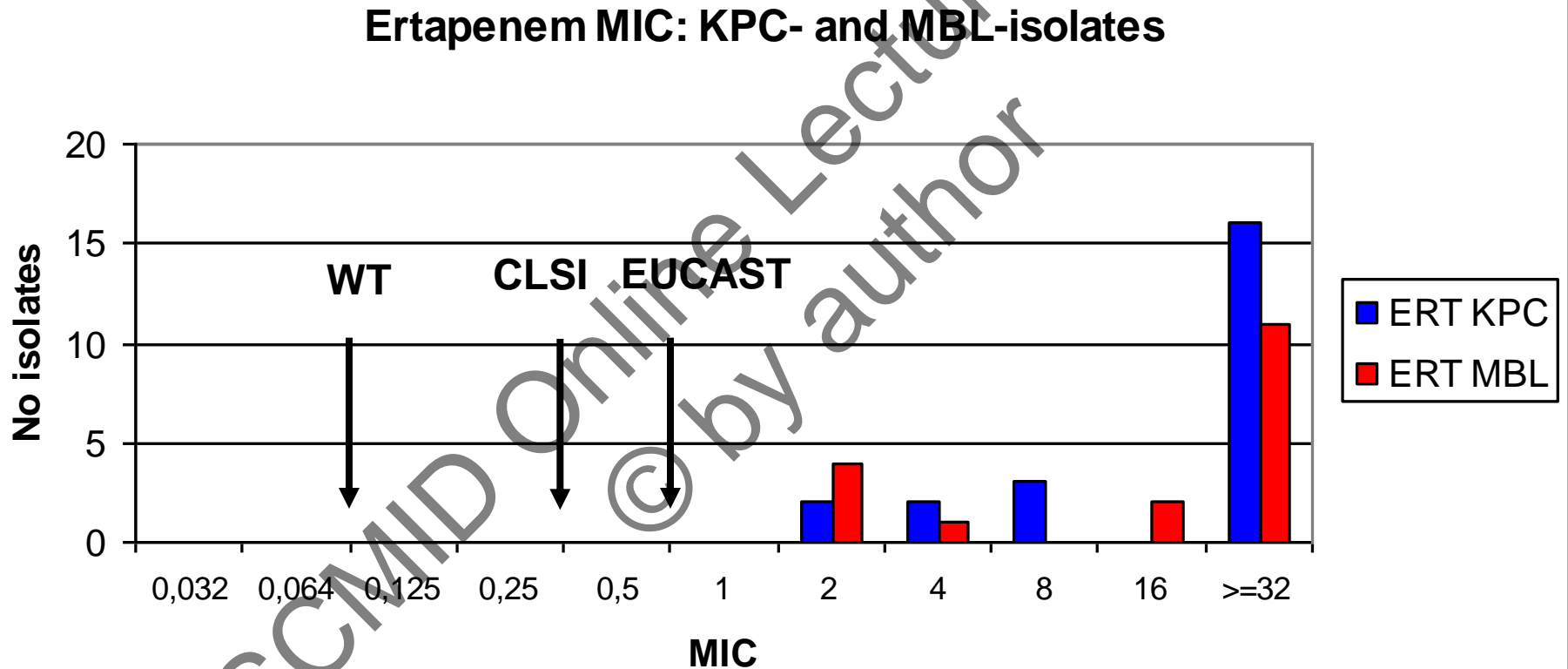
Vading et al. CMI 2010. In press

K. pneumoniae and meropenem



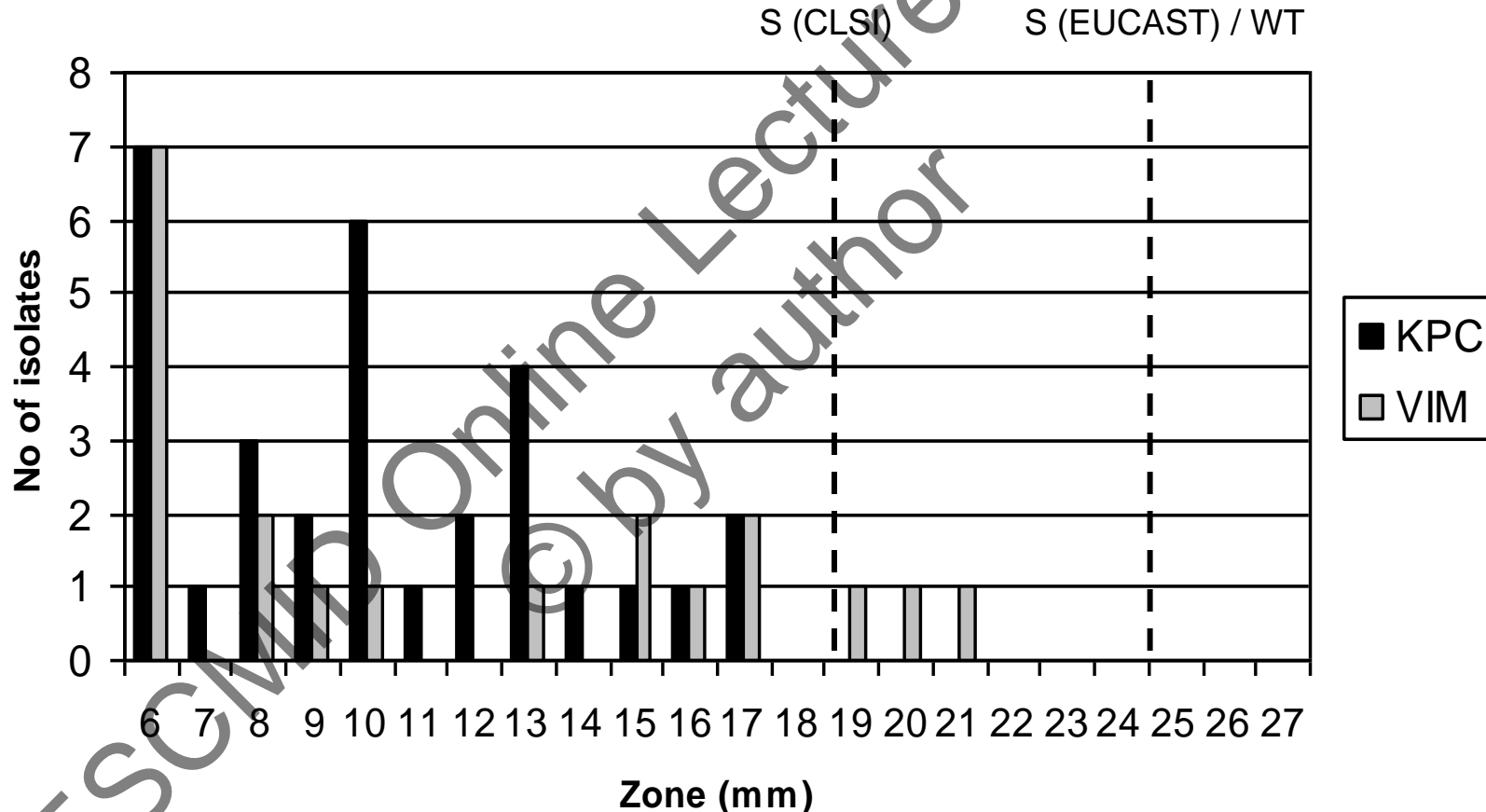
Vading et al. CMI 2010. In press

K. pneumoniae and ertapenem



Vading et al. CMI 2010. In press

K. pneumoniae and ertapenem



Vading et al. CMI 2010. In press

Preliminary conclusions

- MRSA can be identified by the EUCAST disk method
- Clinically relevant ESBL-producing Enterobacteriaceae are identified with the EUCAST disk method
- Clinically relevant carbapenemases in Enterobacteriaceae are identified with the EUCAST disk method
- With MIC-breakpoints ECOFFs are needed for Enterobacteriaceae to identify all carbapenemase-producers

How can you detect important resistance mechanisms with phenotypic methods?

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Case 1: *E. coli* with resistance to cefotaxime and ceftazidime

Antibiotic	Categorization
Cefotaxime	R
Ceftazidime	R
Cefepime	R
Cefoxitin	R
Amoxicillin-clavulanate	R
Piperacillin-tazobactam	R
Imipenem	S
Meropenem	S
ESBL-test	Negative

Which is the mechanism?

- Classical ESBL?
- AmpC – plasmid or chromosomal?
- Combination of ESBL and AmpC?
- NDM-1?
- Impossible to say

Comment - cephalosporinases

- Cefoxitin S = classical ESBL
- Cefepime R = classical ESBL
- Sometimes difficult to detect when both mechanisms are present
 - Cefepime +/- clavulanic acid
 - Addition of AmpC inhibitor in the agar
- Carbapenemases may be a differential diagnosis – should be further investigated if no explanation can be found

Case 2: *E. coli* with resistance to cefotaxime and ceftazidime

Antibiotic	Categorization
Cefotaxime	R
Ceftazidime	R
Cefepime	S
Cefoxitin	R
Amoxicillin-clavulanate	R
Piperacillin-tazobactam	R
Imipenem	S
Meropenem	S
ESBL-test	Negative

How can you verify that it is an AmpC-producer?

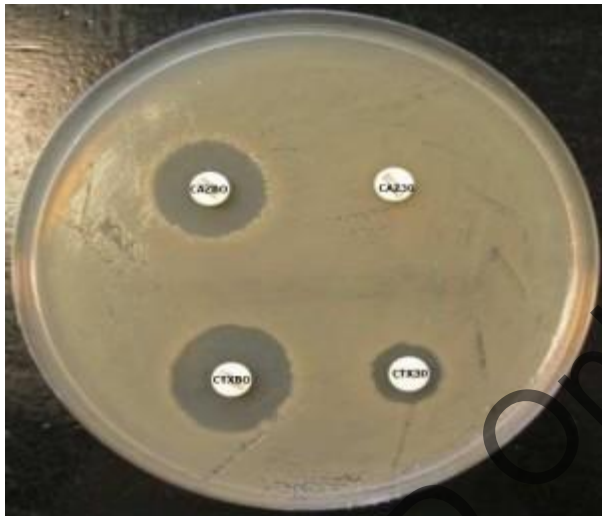


FIG. A

FIG. C

FIG. B

FIG. D

FIG. E

Fig. A: cefotetan +/- cloxacillin
Fig. B: cefoxitin +/- cloxacillin
Fig. C: cefoxitin +/- cloxacillin
Fig. D: cefoxitin +/- boronic acid
Fig. E: EDTA synergy test

Strategy to handle putative AmpC-producers

- FOX-resistance in *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Salmonella* spp. and *Shigella* spp.
- AmpC-testing (commercial or in-house method)
- No phenotypic method to separate plasmid-mediated from chromosomal AmpC in *E. coli* and *Shigella* spp.

Case 3: *K. pneumoniae* with resistance to cefotaxime, ceftazidime and meropenem

Antibiotic	Categorization
Cefotaxime	R
Ceftazidime	R
Cefepime	R
Cefoxitin	R
Amoxicillin-clavulanate	R
Piperacillin-tazobactam	R
Imipenem	S
Meropenem	I
ESBL-test	Positive

Which is the mechanism?

- Classical ESBL plus porin loss?
- AmpC – plasmid or chromosomal plus porin loss?
- Combination of ESBL and AmpC?
- NDM-1?
- KPC?
- Impossible to say

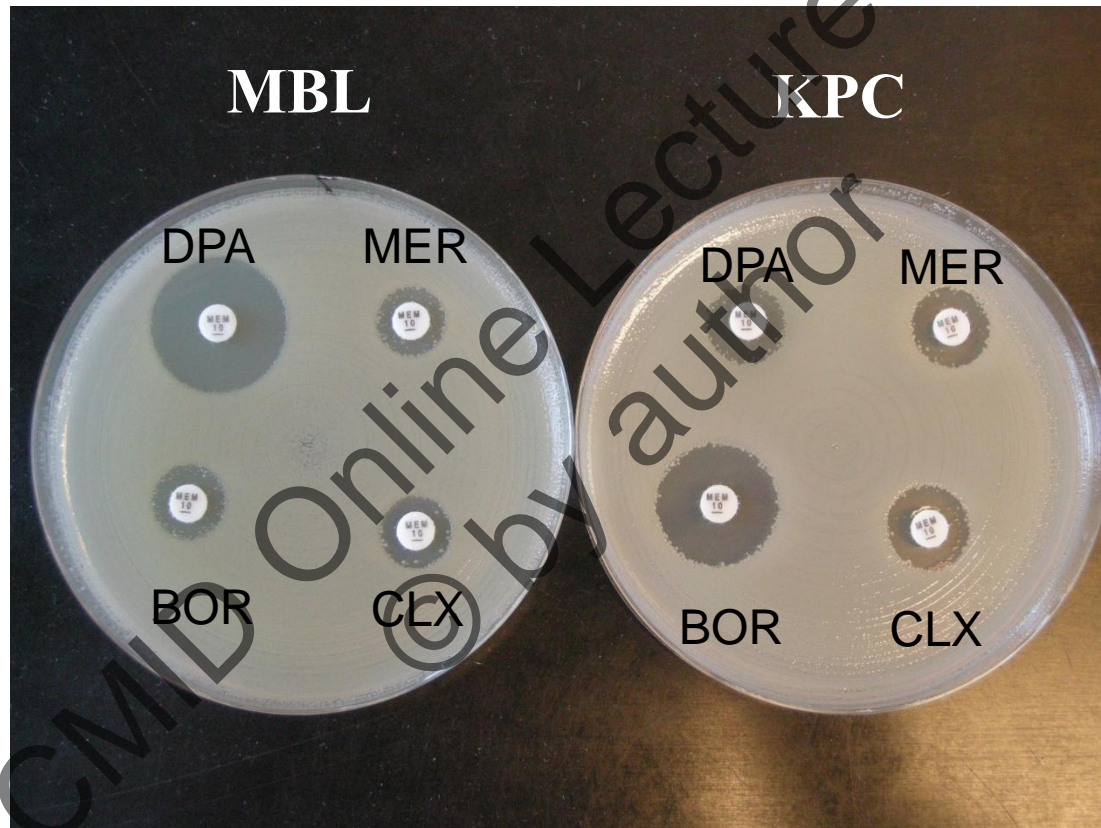
Comment

- When using ESBL combination disks
 - 21/31 KPC-producers were positive
 - 2/20 VIM-producers were positive
 - Vading et al. CMI 2010. In press
- Beware of non-susceptibility to carbapenems, even when the ESBL-test is positive
- How should one proceed to elucidate whether it is ESBL+porin loss or a carbapenemase?

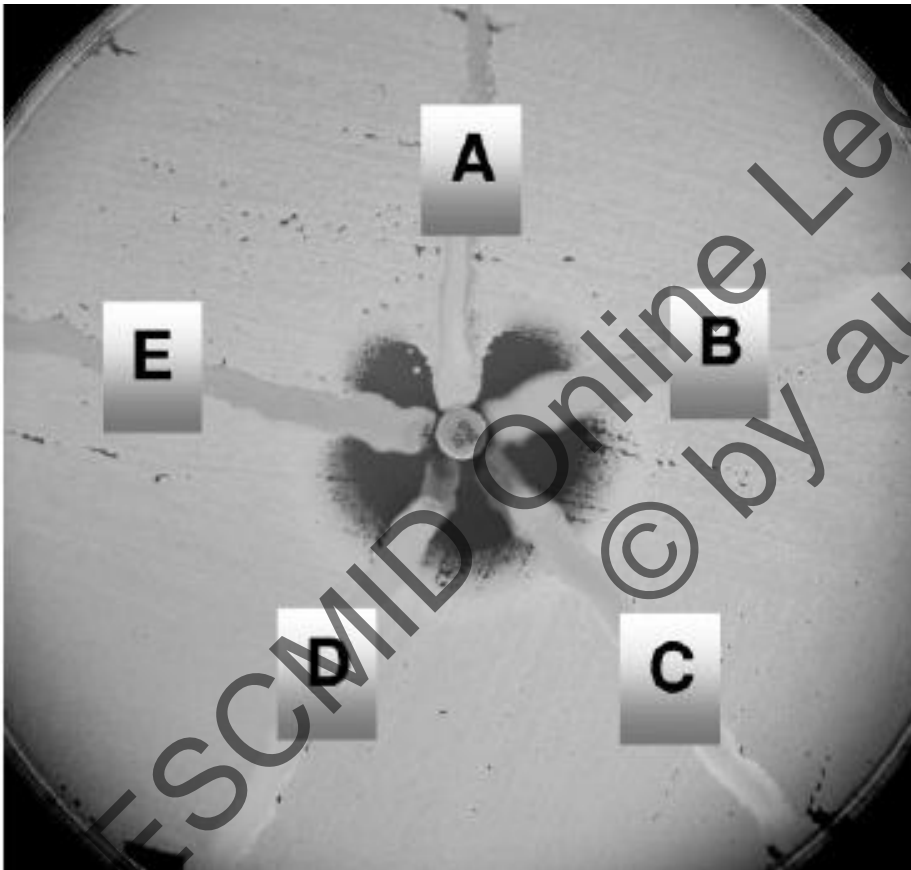
Phenotypic detection of carbapenemases

β-lactamase	Inhibited by		
	EDTA/DPA	Boronic acid	Cloxacillin
MBL	Y	N	N
KPC/class A carbapenemases	N	Y	N
OXA-48	N	N	N
ESBL	N	N	N
AmpC	N	Y	Y

Positive phenotypic tests: KPC and MBL



Modified cloverleaf (Hodge) test



- 1:10 dilution of 0.5 McF suspension of *E. coli* ATCC 25922
- 10 µg imipenem disk in the center
- Each test isolate streaked from the the disk to the edge of the plate
- Isolate A is positive, isolates B-D are negative

Increase in zone diameters

β-lactamase	Increase in zone diameter (range)		
	DPA	Boronic acid	Cloxacillin
MBL (n=25)	5-15	-1-2	0-2
KPC (n=34)	-1-3	4-16	0-2
OXA-48 (n=9)	-4-4	-1-4	0-2
ESBL (n=9)	-3-2	-2-3	-1-0
AmpC (n=9)	-3-1	1-7	0-7

Giske et al. CMI 2010. In press

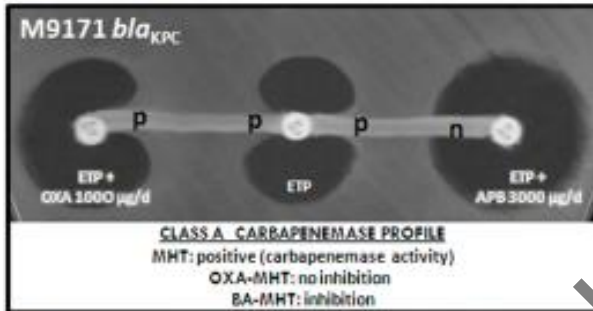
Sensitivities and specificities

Test	β -lactamase	Sensitivity	Specificity
APBA	KPC	100%	98%
APBA+CLX	AmpC	80%	100%
DPA	MBL	100%	100%
EDTA	MBL	100%	88%
Modified cloverleaf (Hodge) test	Carbapene- mases	100%	77%

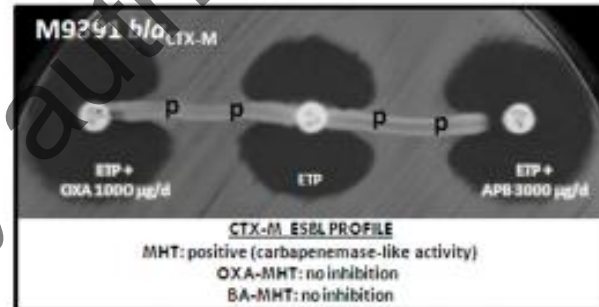
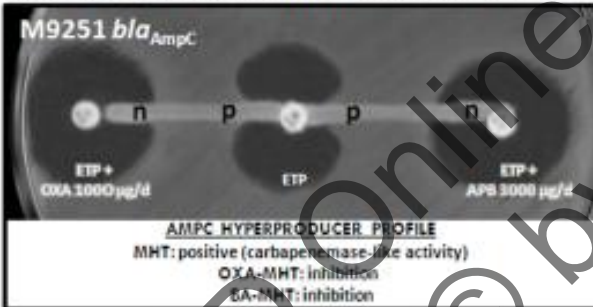
Giske et al. CMI 2010. In press

"Double modified" cloverleaf test

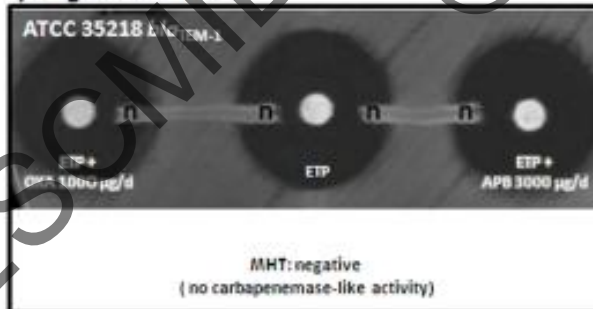
a) Carbapenemases



b) Non carbapenemases (MHT false positives)



c) Negative control



Pasteran et al. JCM 2010
 (Epub doi:10.1128/JCM.01771-09)

Conclusions

- Possible to detect most particularly important mechanisms of resistance by disk diffusion
- Simple phenotypic tests can differentiate between various types of extended-spectrum β -lactamases, AmpC and carbapenemases
- Is it important to detect mechanisms of resistance?
- Depends on whether you are concerned about what is circulating in your hospital....