Detection of ESBLs

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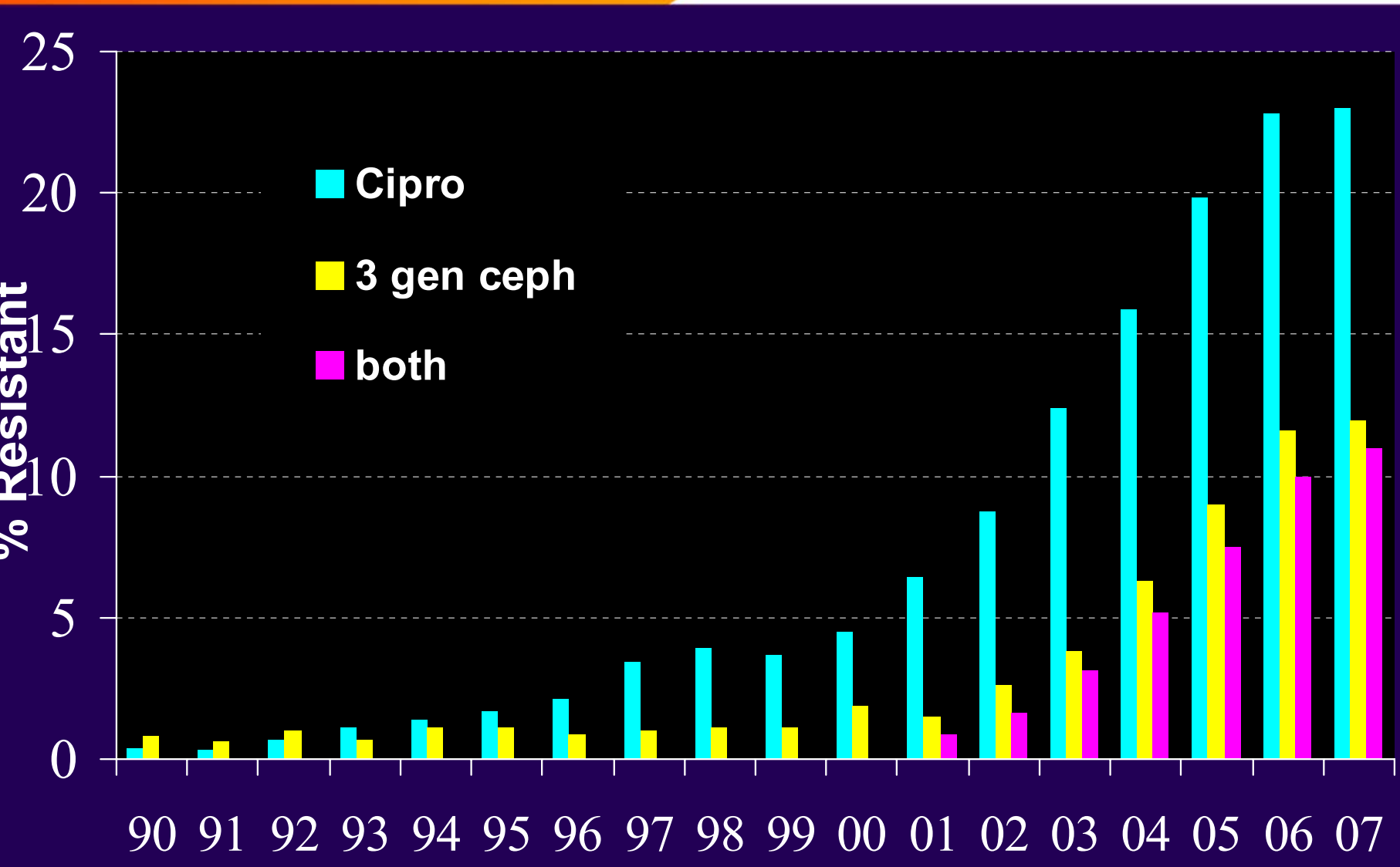
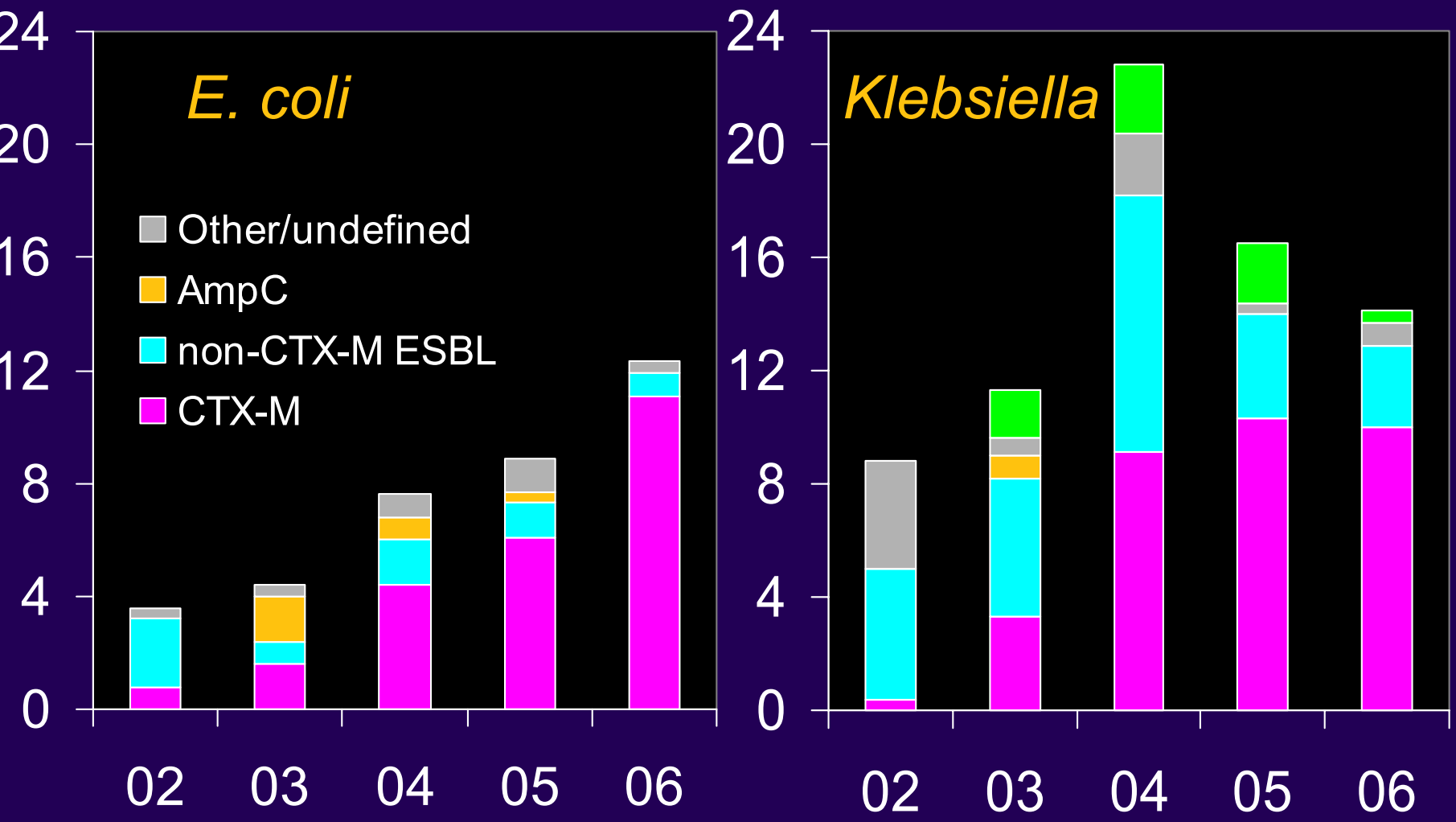
E. coli from blood & CSF

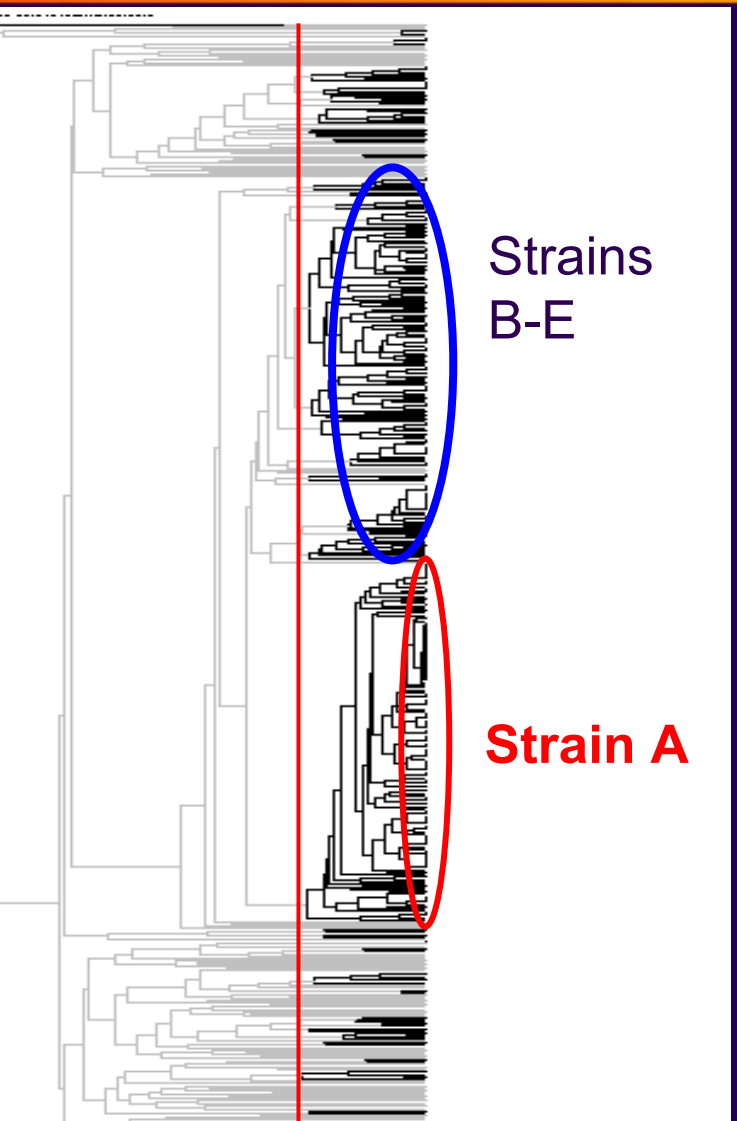


Cephalosporin resistance % Prevalence BSAC surveillance



http://www.bsacsury.org.uk

PFGE: CTX-M +ve E. coli



- 5 major strains, A-E
  - All serotype O25; ST131
- Strain A
  - IS*26* between bla_{CTX-M} & normal promoter; gent S
- Others: diverse/small clusters
- Most (including A-E) have CTX-M-15 & OXA-1 ±TEM-1, large plasmids

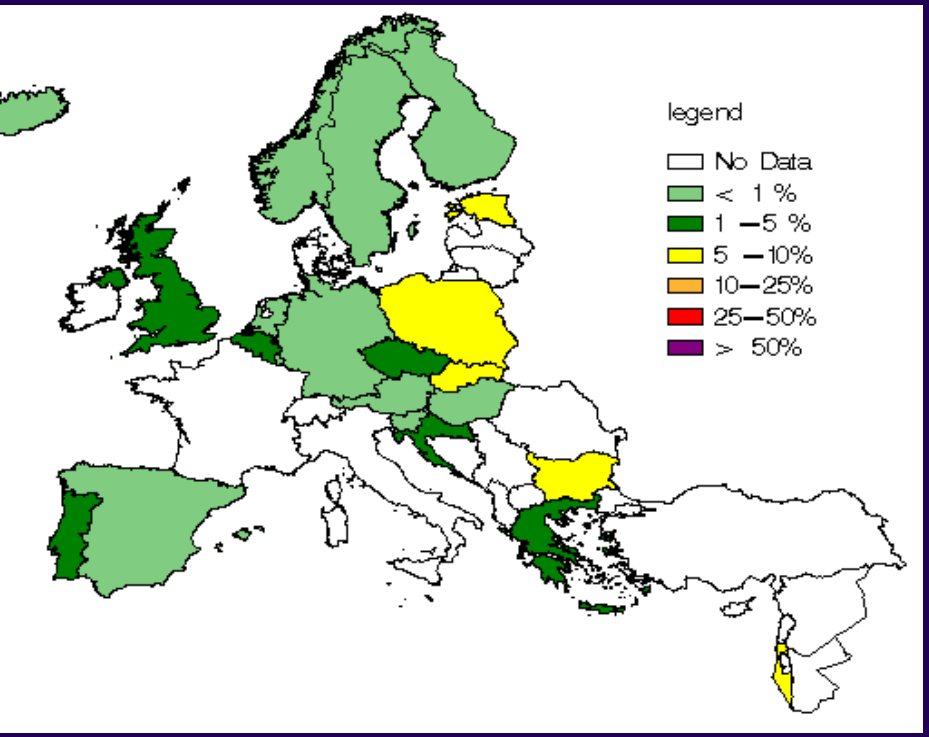
Woodford *et al.* 2004. *JAC* **54**, 73

Geom. mean MICs, (mg/L) CTX-M-15 +ve E. coli

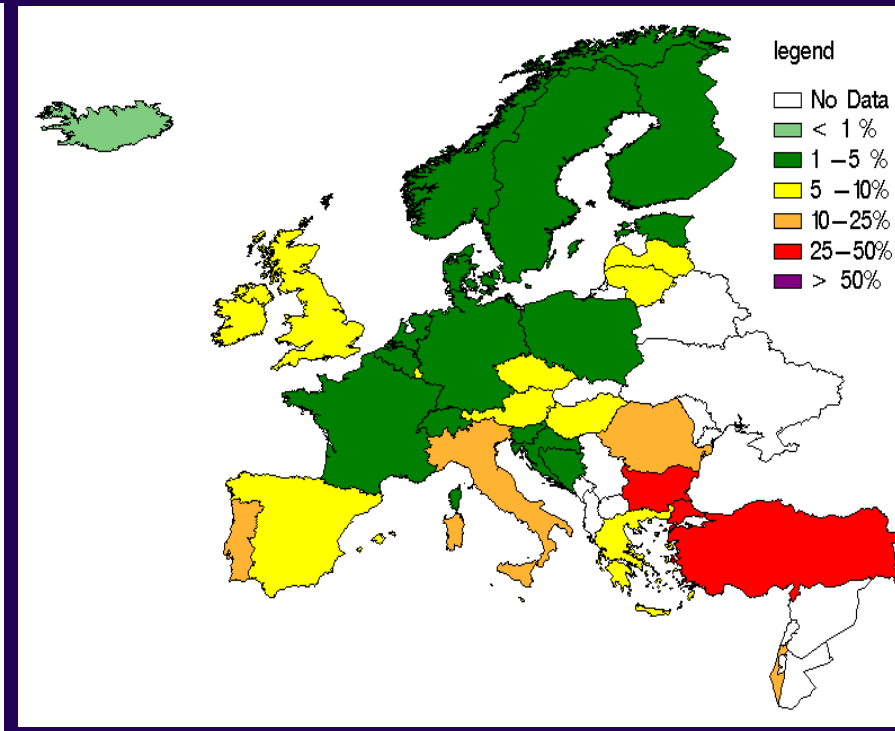
	'Epidemic A'	Other major	Minor
Cefotaxime	37.3	93.2	73.0
Ceftazidime	2.9	23.0	37.9
Pip/taz	20.1	13.2	14.7
Imipenem	**0.2**	**0.2**	**0.3**
Meropenem	**0.05**	**0.1**	**0.06**
Ciprofloxacin	17.5	6.7	6.1
Trimethoprim	256	9.6	45.3
Gentamicin	**1.1**	28.6	12.2
Amikacin	9.0	18.2	9.3
Nitrofurantoin	**8**	**7.3**	**22.6**

Woodford *et al.* *JAC* 2004, **54**, 735

EARSS resistance to 3-gen cephs in *E. coli*



2001



2008

http://www.earss.rivm.nl

Why detect ESBLs

Counter argument..... *EUCAST BSAC breakpoints are low and cephalosporins work against the minority of ESBL producers for which the MICs are even lower (≤1 mg/L)....*

So why

- Influences therapy : inhibitor combinations / 4-gen cephs
- Epidemiologically important : ESBLs spreading dramatically; AmpC cephalosporinases aren't

Detecting ESBL producers

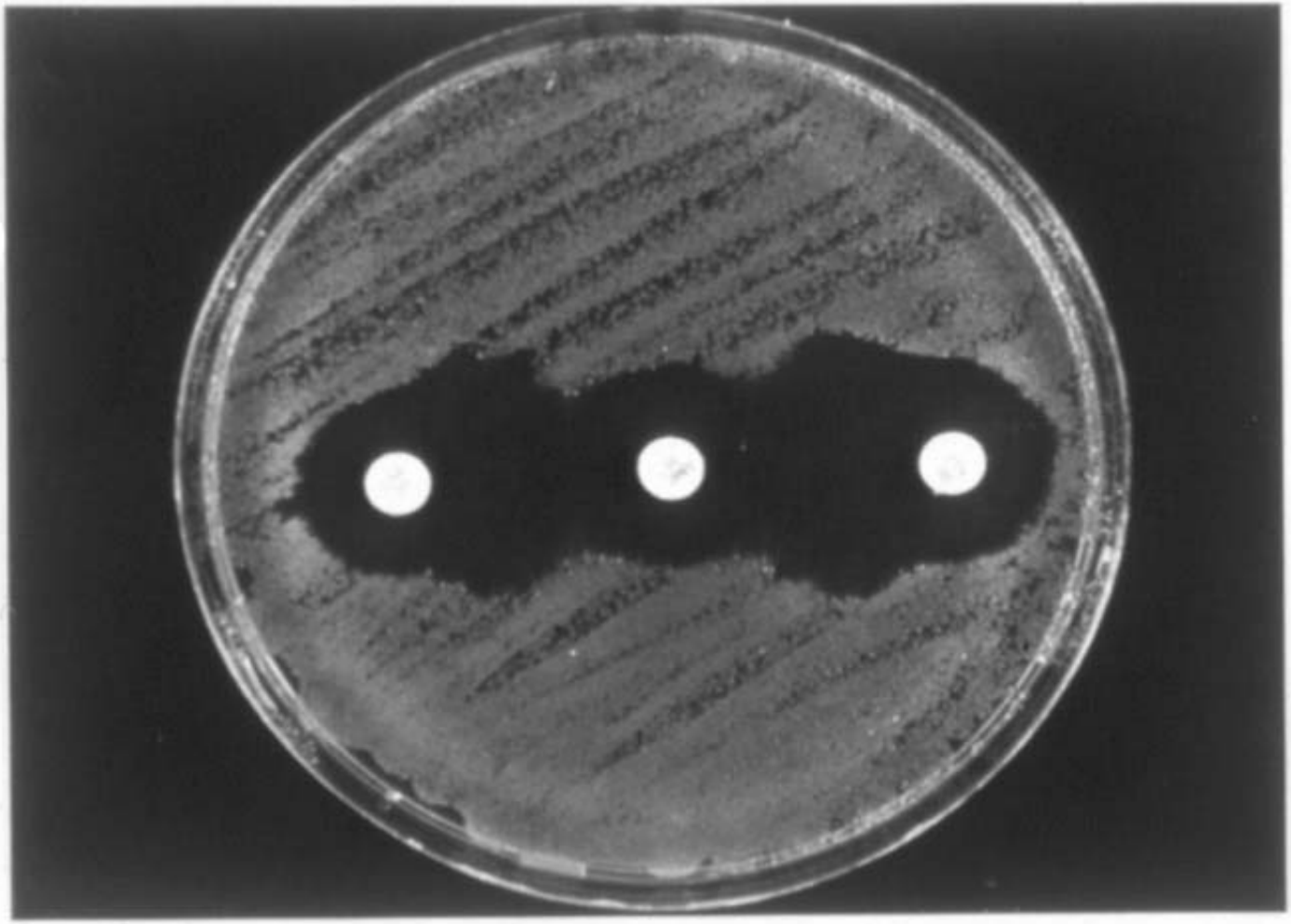
2 steps:

- Screen for resistance with an indicator ceph
- Do confirmatory test on those found resistant

Choice of indicator cephalosporin

	Sensitivity	Specificity
Cefotaxime & ceftazidime	Good	Good
Cefpodoxime	Good	Moderate
Cefuroxime	Poor	Poor
Cephalexin or cephradine	Moderate	Poor
Cefpirome or Cefepime	Poor	Good

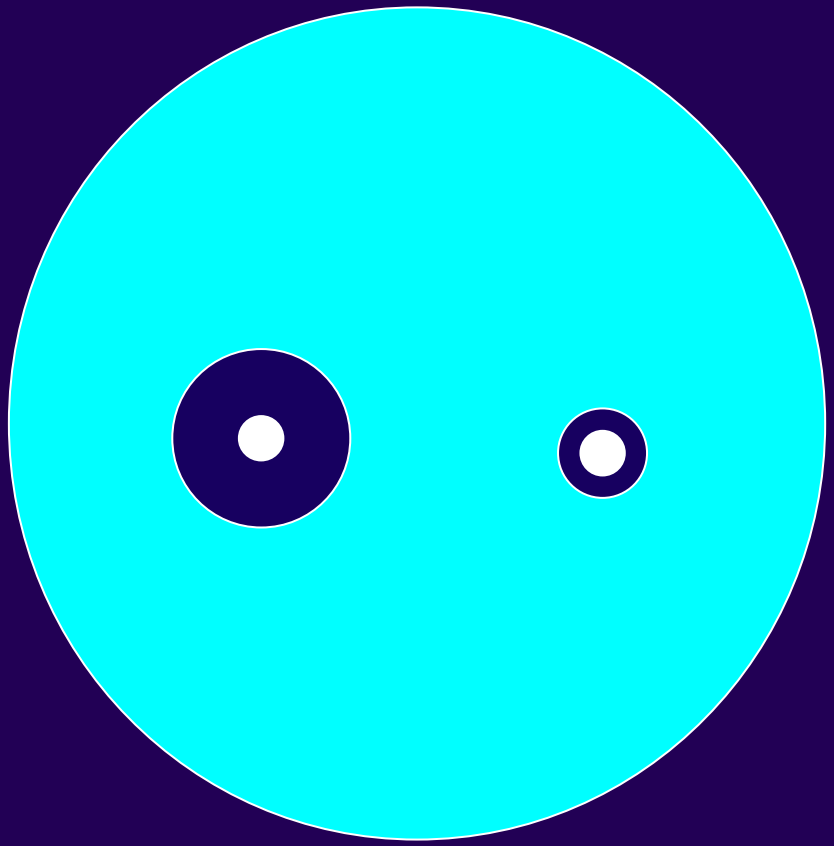
Double disc test for *K. pneumoniae* TEM-3+



Ceftazidime 30 Augmentin 20+10 Cefotaxime 30 µg

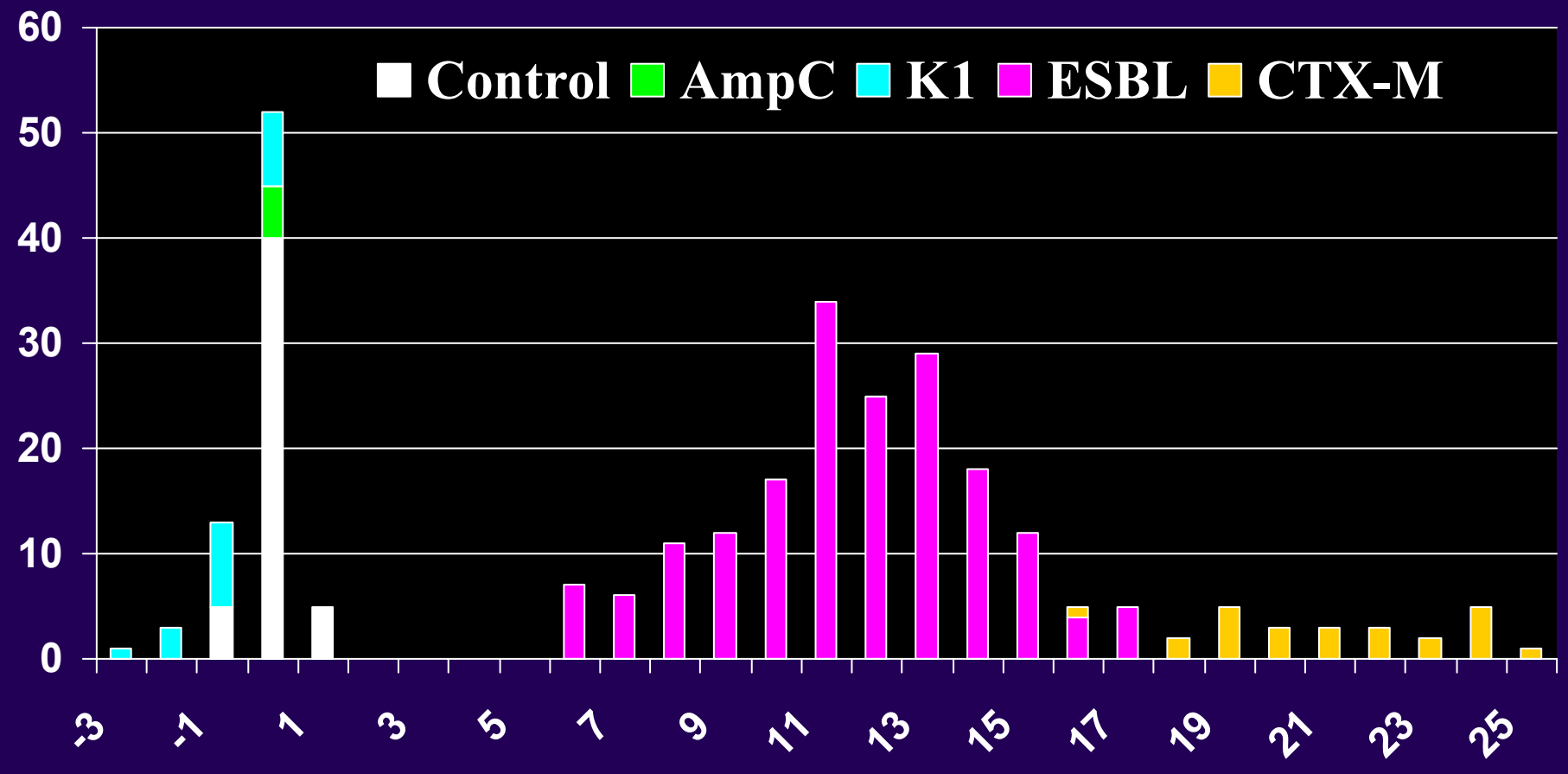
Combination discs

Disc with cephalosporin + clavulanic acid



Disc with cephalosporin alone

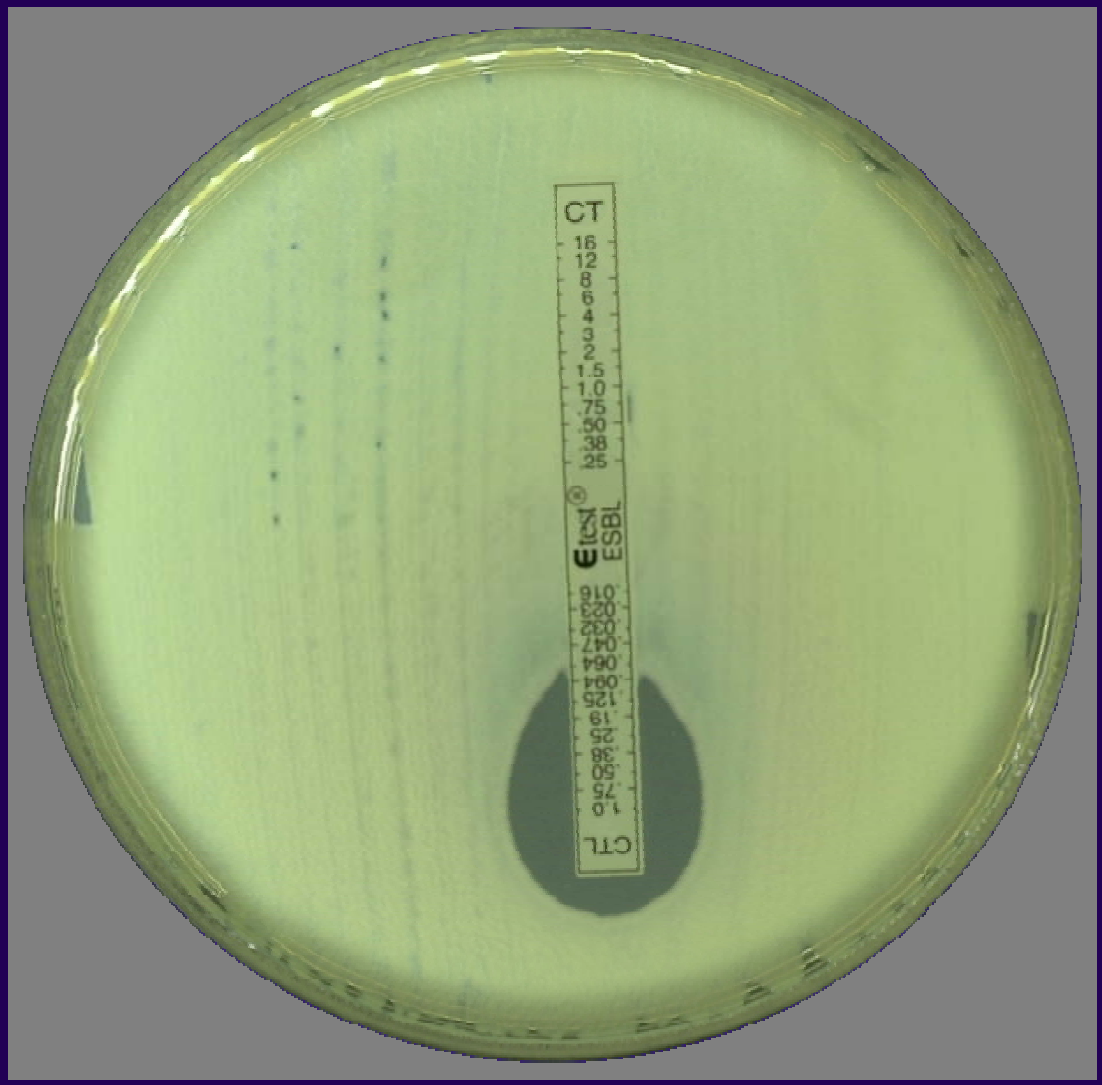
Zone differences (mm), Klebs & E. coli c'pod/clav 10+1 μg - c'pod 10 μg



Etest for ESBLs

Health Protection Agency

Cefotaxime



Cefotaxime
+
clavulanate

ESBL tests for AmpC inducible species

- BSAC bacteraemia: *c.* 25% CephR *Enterobacter* have ESBL, not AmpC…..
  - –Strong ceph/clav synergy usually indicates ESBL
  - –But sequencing may find ESBL when synergy –ve
  - –8/23 QnrA +ve Enterobacter had SHV-12, though only AmpC was suspected from antibiogram
- *P. aeruginosa* referred to ARMRL in the UK
  - – 33/44 with +ve ceph/clav synergy test had VEB-1…..

Bottom line- +ve result meaningful; -ve isn't

ESBL confirmatory tests

	Pro	Contra
Double disc	Cheap	Best disc spacing varies with strain
Combination disc	Cheap, sensitive & specific	Batch variation; Controls critical
Etest	Sensitive Internally controlled	More expensive; False +ves with K1 in *K. oxytoca*

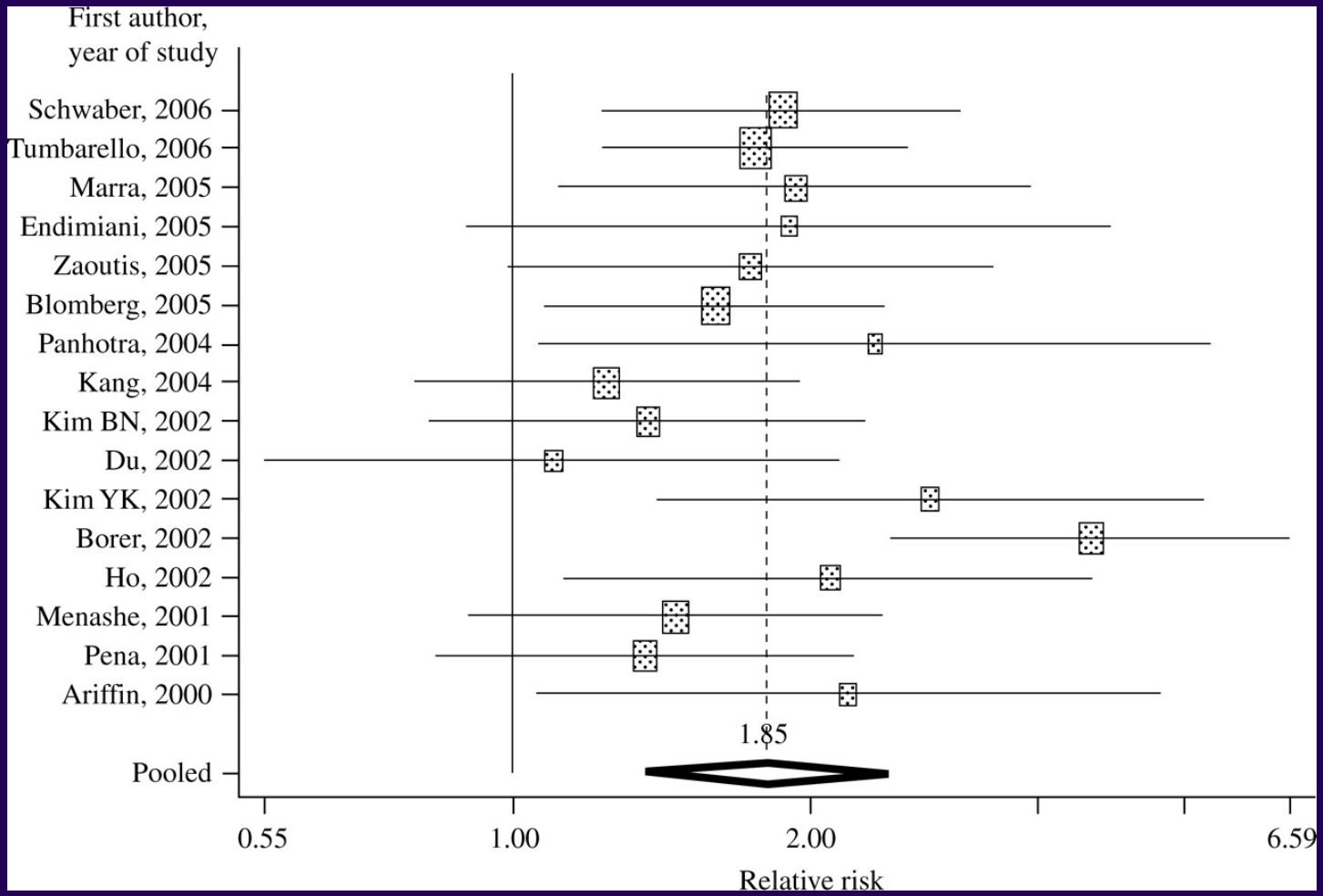
Bacteria not to test for ESBLs

- *Acinetobacter*
  - Often S to clavulanate alone
- *S. maltophilia*
  - +ve result by inhibition of L-2 chromosomal β-lactamase, ubiquitous in the species

What is wrong with standard ESBL detection?

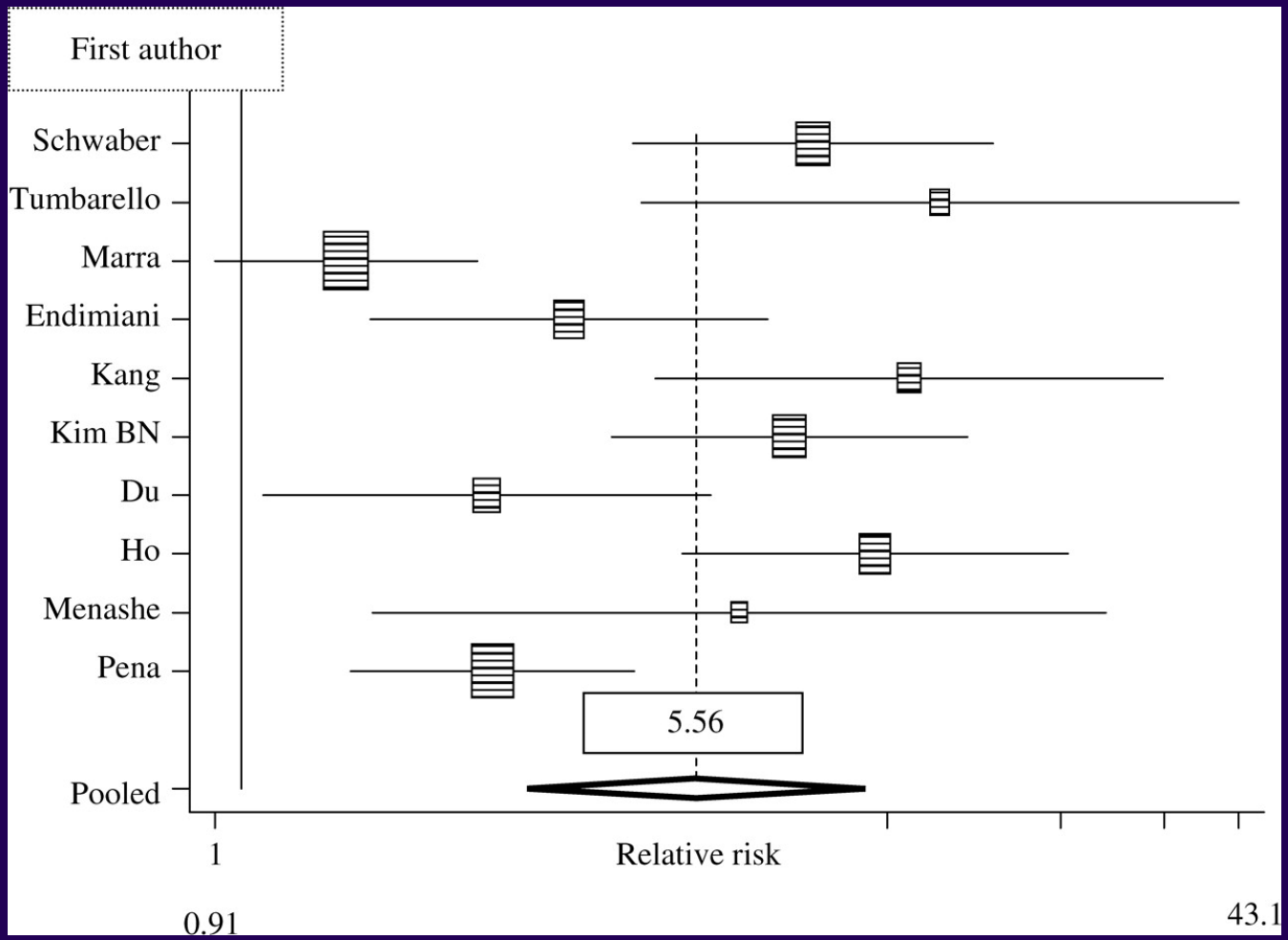
- It's great for epidemiology
  - *But result at 72h*
- Too slow for treatment advice
  - *In step-up: appropriate R_x for patients with ESBL producers delayed*
  - *In step-down, patients kept on broad-spectrum drugs (esp. carbapenems) for too long*
-

Mortality in ESBL *vs.* non-ESBL Enterobacteriaceae bacteraemia



Schwaber & Carmeli *JAC* 2007 **60:**913

Delay in appropriate R_x ESBL *vs.* non-ESBL Enteric bacteraemia



Schwaber & Carmeli *JAC* 2007 **60:**913

ESBL detection, manual & automatic

	Sensitivity	Specificity	PPV	NPV
Mircoscan	83.4	72.9	81.6	75.5
Phoenix	98.8	52.2	75.0	96.6
Vitek 2	85.9	78.0	84.9	79.3
Double disk	94.1	81.4	87.9	90.6
Combi-disk	**92.9**	**96.6**	**97.5**	**90.5**
Etest ESBL	94.1	84.7	89.9	90.9

150 organisms; 3 sites; mixed species ; mixed enzymes; standard cards

Wiengand *et al JCM* 2007: **45,** 1167

ChromID ESBL (bioMerieux)

E. coli **pink** –

K. pneumoniae **green**

P. mirabilis **brown**

- Selective agar with multiple antibiotics including cefpodoxime
- Rapid detection of common ESBL+ enterobacteria within 18-24h of specimen

Chromogenic media to detect ESBL producers- 765 specimens

	True +	False +	False -	Sens-itivity	PPV
ChromID ESBL (bioMerieux)	29	46	4	88%	38%
BLSE (AES)*	28	154	5	85%	15%

ChromID : proprietary, cefpodoxime based

BLSE : 2 compartments : Drigalski agar + cefotaxime, 1.5 mg/L MacConkey + 2 mg/L ceftazidime

Reglier-Poupet *et al. JMM* 2008; **57,** 310

Ceph R but synergy –ve…

Borderline R to cefpodoxime only	???Impermeability / efflux
AmpC- plasmid or chromosomal	S to 4 gen cephs
K1 hyperproducer *K. oxytoca*	R cefuroxime aztreonam, cefpodoxime S ceftazidime, I to cefotaxime May give false +ve ESBL test
Impermeable *E. coli, Kleb*	R cefoxitin & cefuroxime; not 3 / 4-gen cephs
Carbapenemase Metallo or not	Reduced S to imipenem & / or meropenem

Suspect derepressed / plasmid AmpC if:

- Resistant 3-gen cephs, **NOT** cefepime & cefpirome
- Resistant to cefoxitin (but more ESBL producers R, too, nowadays)
- No ceph/clav synergy

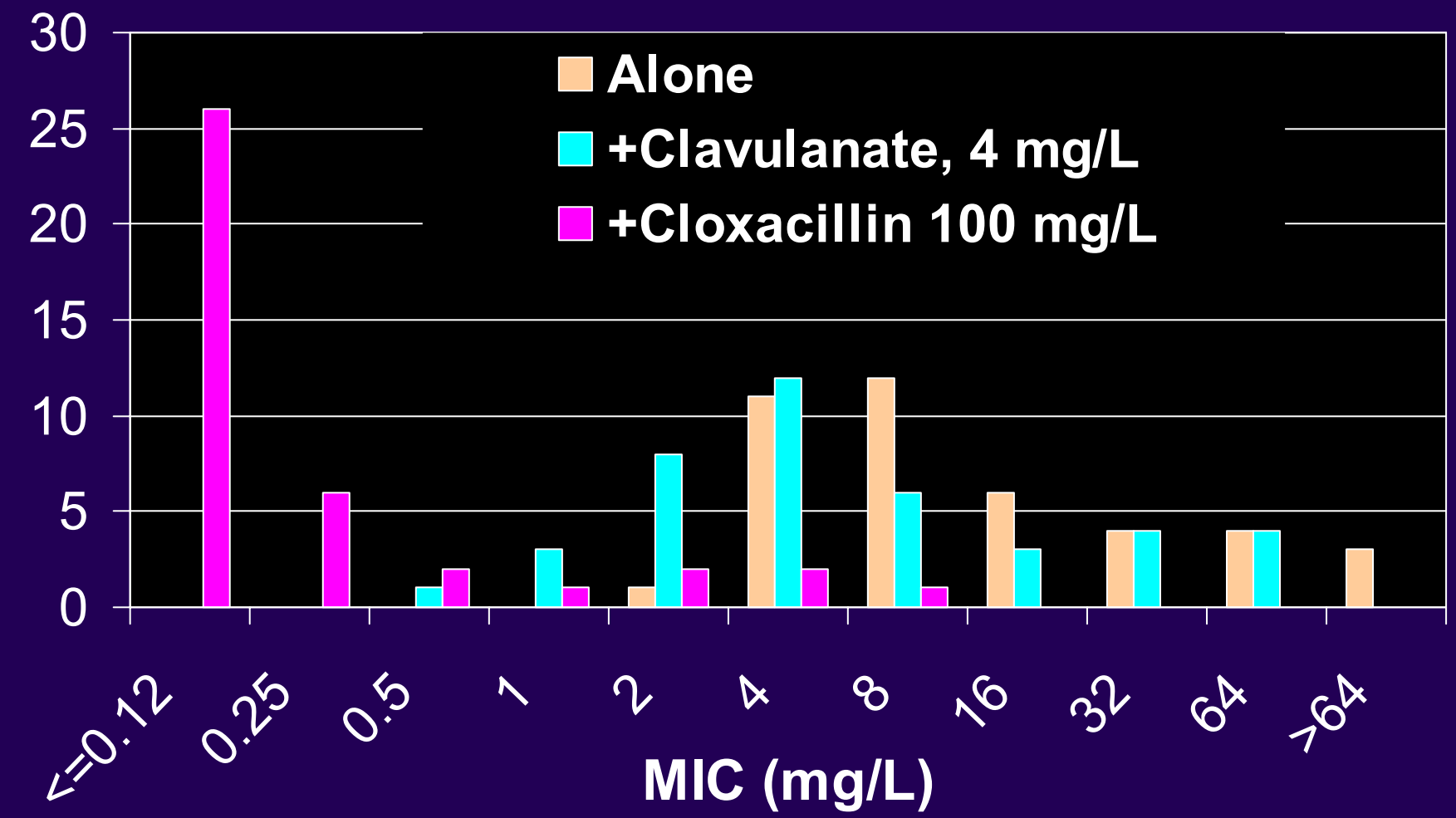
Some wrinkles…

- AmpC-derepressed *M. morganii* are S to pip/tazo
- AmpC derepressed *Serratia* are S to ceftazidime
- Cefoxitin R an unreliable marker for *Providencia, Morganella* & *Serratia* spp.
  - Inducible & derepressed strains may appear I or S
- AmpC derepressed *P. aeruginosa* tend to be S to carbenicillin / efflux mutants are R
- Life complicated if there's an ESBL with the AmpC

Confirmatory tests for AmpC

- *Seek synergy between cefotaxime and either cloxacillin or phenylboronic acid*
  - *Agar dilution*
  - *Disc*
  - *Etest*
- *No agreed interpretive standards*
- *Difficult if ESBL present too*

Cefotaxime combinations *vs.* AmpC *E. coli:* London SE survey



Livermore *et al., CMI* 2008;**14 Suppl 1**:189-93.

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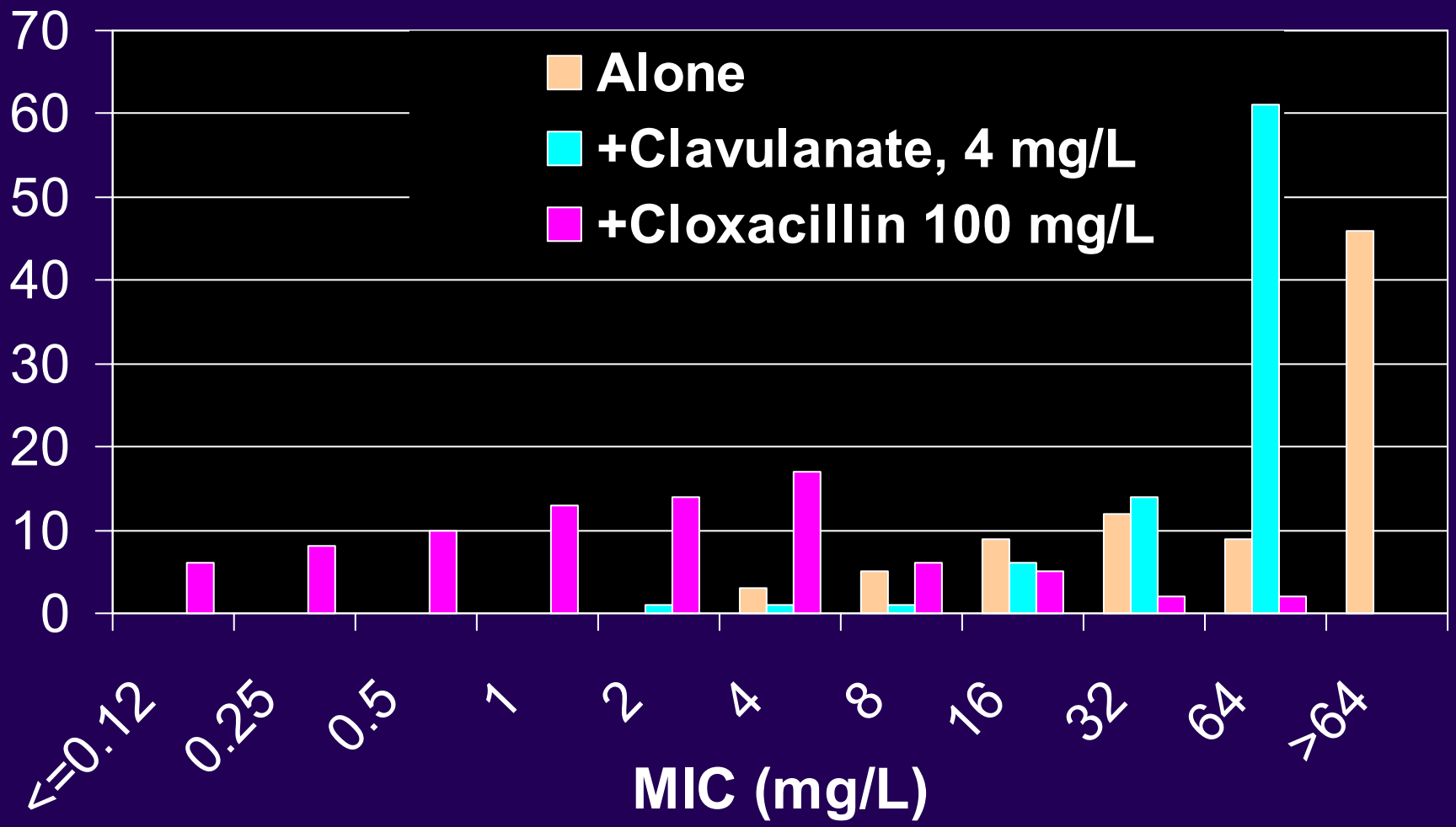


Livermore *et al., CMI* 2008;**14 Suppl 1**:189-93.

Phenyl boronic acid for detection of plasmid AmpC

	Fold MIC reduction for cefoxitin + 400 mg/L phenyl boronic acid
Kleb MOX-1	128
E. coli LAT-2	64
Kleb DHA-1	128
Kleb DHA-2	64
E. coli ACC-1	4
Kleb ACT-1	64
ALL ESBL +ve	≤2

Coudron, *JCM* 2005 **43** 4163

Boronic acid or cloxacillin to detect AmpC (cefotaxime)

Potentiation sought	4-fold		8-fold	
	Sens-itivity	Spec-ificity	Sens-itivity	Spec-ificity
Cloxacillin 100 mg/L	90	73	85	86
Phenyl boronic acid 100 mg/L	98	82	95	95
BZB, 200 mg/L	95	95	95	95

High sensitivity = few +ves missed....
High specificity = few false +ves

Hope *et al.*, ECCMID 2008

Disc tests for AmpC

60 *E. coli* & *Klebsiella* : cefoxitin MICs reduced >4-fold by 100 mg/L cloxacillin	% with ≥5 mm zone expansion
Cefoxitin + cloxacillin 100 μg	86%
Cefoxitin + BZB 64 μg	89%
Cefpodoxime + BZB 64 μg	97%
Cefpodoxime + clav + BZB 64 μg	100%

BZB: benzo(b)thiophene-2-boronic acid

Brenwald *et al., JAC* 2005, **56,** 600

AmpC detection tests: some traps!

- Cloxacillin tests don't work for *P. aeruginosa*; boronic acid tests do work
- Boronic acid tests give false +ves for strains with KPC carbapenemases
  - Beware if isolate is carbapenem resistant
- Boronic acids difficult to dissolve & handle

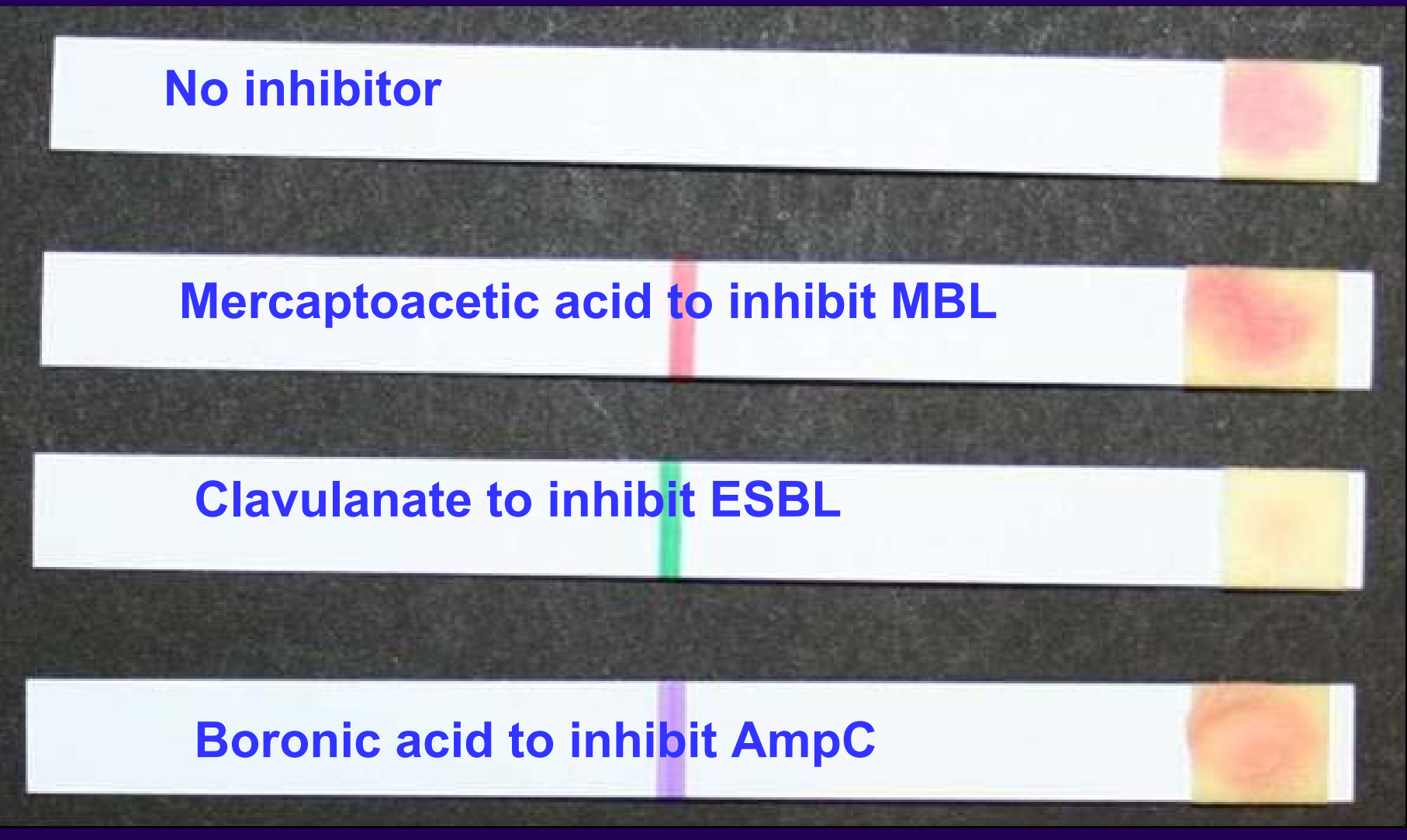
Commercial AmpC tests

- **Rosco -** boronic acid tablets for double disc synergy tests
- **Mast -** cefotaxime + cloxacillin discs
- **AB Biodisk -** cefotaxime / cloxacillin Etests
- **Mast -** Cica β-Test (chromogenic oxyimino-ceph; hydroylsis inhibition by boronic acids)

Cica β-Test (Mast)

- Examine hydrolysis of chromogenic oxyimino ceph, HMRZ-86- yellow to red
- If +ve, test inhibition IN SEQUENCE by:
  - Sodium mercaptoacetic acid – MBL
  - Clavulanic acid – Class A / ESBL
  - Benzo-thiophene-2-boronic acid – AmpC
- Count first positive result

Cica β-Test (Mast)



Cica β-Test (Mast) blind testing of overnight cultures

	Mechanism inferred					
Reference data	**MBL**	**ESBL**	**AmpC**	**Mixed Other**	**Pen' ase**	**No activity**
MBL (26)	**20**	1	0	2	3	0
ESBL (74)	3	**63**	2	6	0	0
AmpC (25)	2	0	**18**	3	2	0
K. oxytoca, K1 (10)	0	2	6	**2**	0	0
OXA carbapenemases (10)	0	0	0	**10**	0	0
P. aeruginosa OXA ESBLs (4)	1	3	0	**0**	0	0
KPC/SME carbapenemase (2)	0	0	2	**0**	0	0
Penicillinase (39)	5	3	1	0	**30**	0

Better but slower to use with antibiogram @ 48h

Livermore et al., JAC in press.

Why not go molecular?

- Good PCRs for ALL *bla*$_{CTX-M}$ & for the 5 sub-groups
- Multiplex PCR for plasmid AmpC

BUT

- Difficult to distinguish genes for TEM/SHV ESBLS from those for classical TEM/SHV...
- Might be achieved with gene chip & multiple probes

Summary

- ESBLs esp. CTX-M types are a rising problem
- Epidemiologically important to distinguish from other modes of ceph resistance
- Classical detection takes 72h
- Automated systems take 36-48h
- Selective media / chromogenic tests take 24h
- Molecular detection possible for CTX-M; tricky for TEM/SHV