

**β -Lactam susceptibility tests
in Enterobacteriaceae
Report by mechanism**

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ESBLs matter...

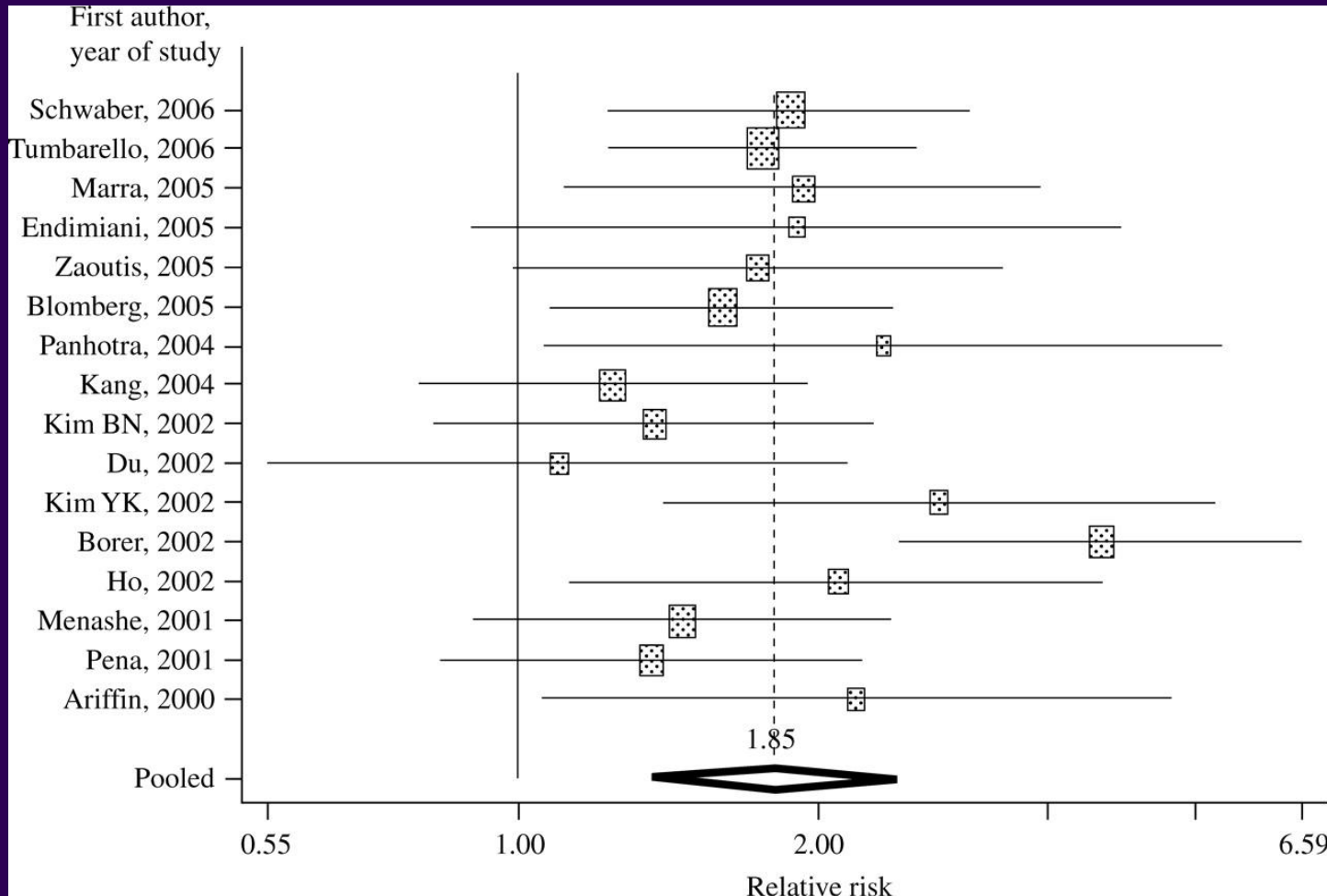
14-mortality ESBL *Klebsiella* bacteraemia



Carbapenem R _x	2/10
No carbapenem	40/61
<i>P</i>	.0121

Paterson *et al.* *CID* 2004, 39, 31

Mortality in ESBL vs. non-ESBL Enterobacteriaceae bacteraemia



ESBLs matter but..... maybe if the MIC is low enough, they don't



	R-	TEM-1	TEM-12	TEM-10	CTX-M-15	CTX-14
Ceftazidime	0.12	0.12	8	128	32	2
Cefotaxime	0.03	0.03	0.12	1-2	256	128
Ceftriaxone	0.03	0.03	0.12	1-2	256	128

EUCAST bpts	Current	Proposed
Ceftazidime	$\leq 1, > 8$	$\leq 1, > 4$
Cefotaxime & Ceftriaxone	$\leq 1, > 2$	

EUCAST proposed advice 'report as found; 'strong arguments to seek ESBLs infection control & epidemiological purposes'

Outcome & MIC bacteraemias with ESBL-Klebsiella



Patient	Source	Ceph	MIC (mg/L)	Outcome
M72	VAP	Ceftazidime	16	Fail
M76	Central line	Ceftriaxone	16	Fail
M58	HAP	Ceftriaxone	12	Fail, died @ 48h
M39	Central line	Ceftriaxone	8	Fail, died @48h
F35	Surg wound	Cefotaxime	4	Fail
M48	Unknown	Cefepime	2	Fail
M49	Peritonitis	Ceftriaxone	1.5	Cure
F73	VAP	Cefepime	1.5	Cure
M25	VAP	Cefepime	0.5	Fail, died septic
F25	Central line	Ceftazidime	0.5	Cure

Outcome & MIC in bacteraemias with CTX-M-3/-14 *E. coli*; ceftazidime 2g q8h



Patient	Source	MIC (mg/L)	Outcome
M62	UTI	8	Cure
F49	Peritonitis	1	Responded, but drainage needed
F36	UTI	2	Cure
M45	Biliary infection	2	Cure
M67	?	2	Cure
F76	HAP	8	Cure
F38	UTI	0.5	Cure

Look directly for ESBL *Klebsiella*– Where it began



		n	Cure	Fail	Relapse
UTI	Ceph	2	2		
	Ceph+ aminoglycoside	2	2		
Non- UTI	Ceph+ aminoglycoside	3		2	1

	MIC mode	Range
Cefotaxime	2	0.5-4
Ceftriaxone	2	0.5-4
Ceftazidime	4	1-16

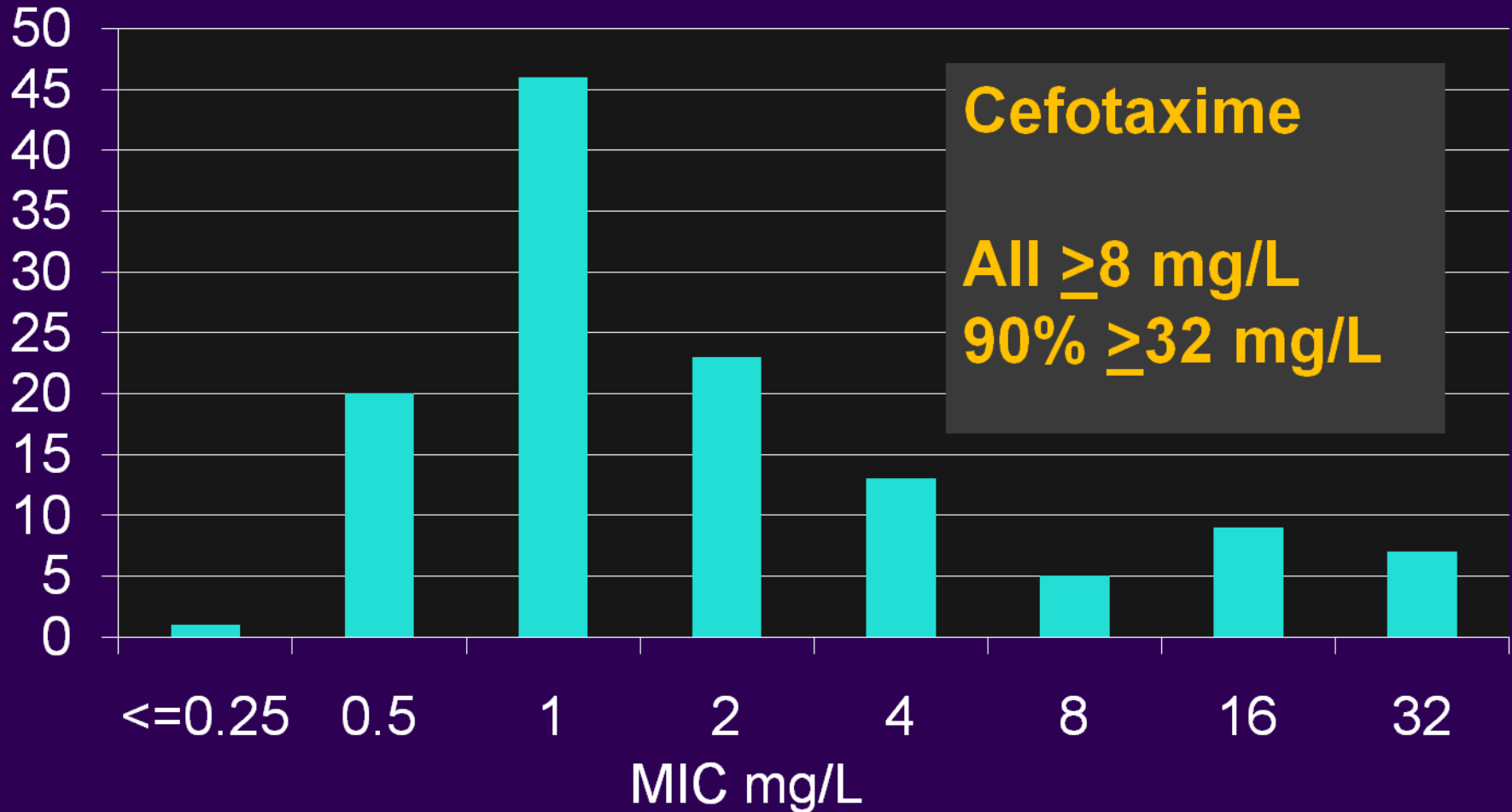
ESBL *E coli* infections treated with ceftazidime: all zones ≥ 18 mm



Patient	Infection	Cure/Fail	Outcome
F70	Peritonitis	Fail	Died, sepsis
F72	UTI	Fail	Died, despite switch to imipenem
F69	UTI	Fail	Resolved on gentamicin
M49	Liver abscess	Fail	Died, persistent infection
F82	UTI	Cured	
M67	1° bacteraemia	Cured	
F83	UTI	Cured	Responded initially to amox-clav

Hong Kong; CTX-M patterns; so probably CTX-M-14

Ceftazidime MICs for Enterobacteria with CTX-M-9/14 ESBLs



First point.....



It's not so clear that cephalosporin MICs of 1-4 mg/L are associated with satisfactory outcomes....

Second point...

The lab doesn't determine measure MICs anyway

E. coli NCTC13352. K-12 derivative with TEM-10, a ceftazidimase



	MIC mg/L
Ceftazidime	>128
Cefotaxime	1-2
Ceftriaxone	1-2
Cefepime	2-4

4 labs each did disc tests 10 times...

NCTC13352: Unedited results for ceftazidime 30 µg discs: 10 tests/lab



	Mean zone (mm)	SD (mm)	S ≥ 30	I 26-29	R ≤ 25
Lab 1	8.1	0.57	0	0	10
Lab 2	6.8	1.75	0	0	10
Lab 3	6.0	0	0	0	10
Lab 4	6.0	0	0	0	10

NCTC13352: Unedited results for cefotaxime 30 µg discs: 10 tests/lab



	Mean zone (mm)	SD (mm)	S ≥ 30	I 24-29	R ≤ 23
Lab 1	28.7	0.82	1	9	0
Lab 2	29.4	0.97	6	4	0
Lab 3	25.9	1.29	0	10	0
Lab 4	31.3	1.06	10	0	0

NCTC13352: Unedited results for cefepime 30 µg discs: 10 tests/lab



	Mean zone (mm)	SD (mm)	S ≥ 32	I 27-31	R ≤ 26
Lab 1	26.4	0.52	0	4	6
Lab 2	28.1	0.74	0	10	0
Lab 3	23.0	1.55	0	0	10
Lab 4	29.1	1.00	0	10	0

Third (& Say it in whispers...)

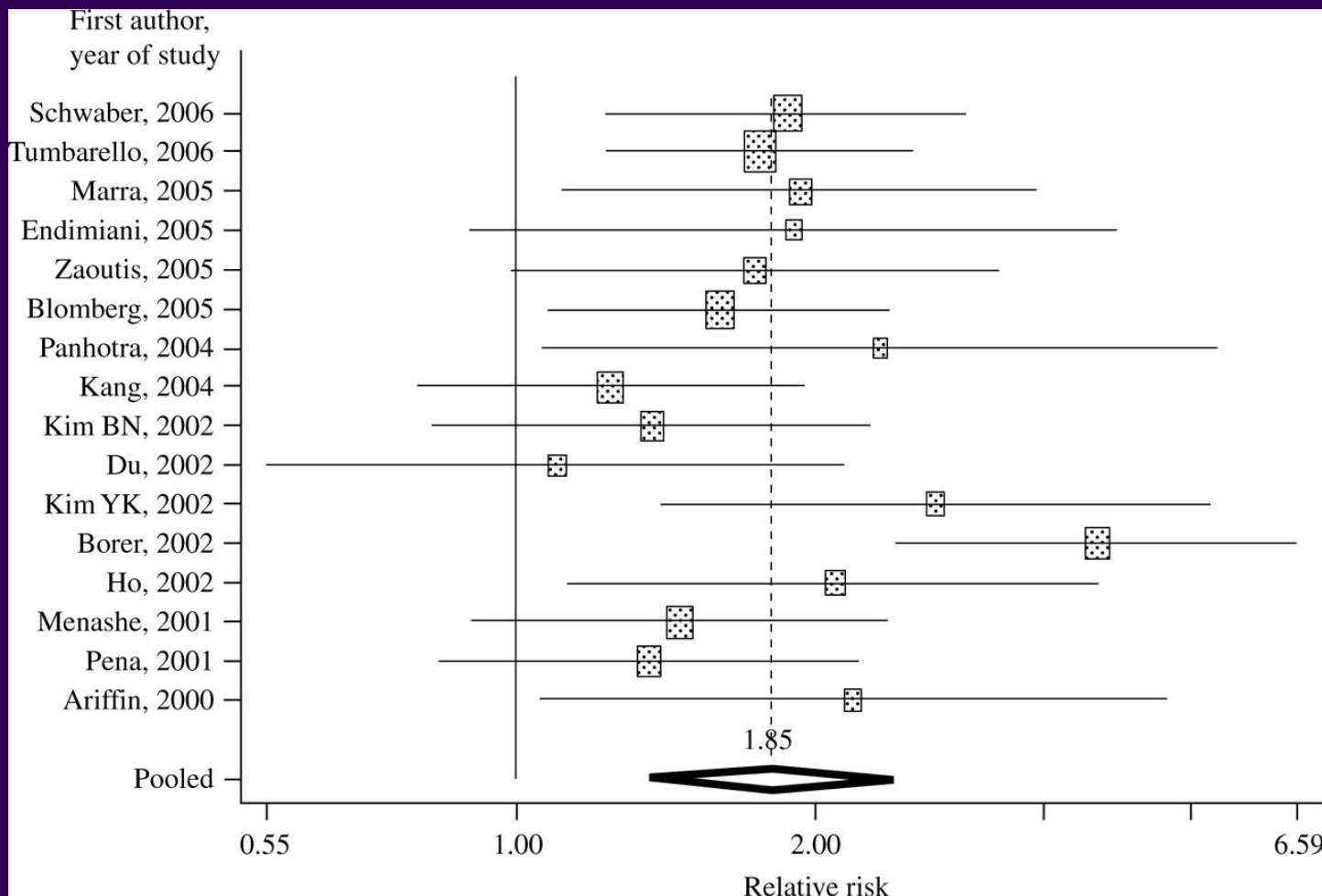


For the seriously-ill patient, fast microbiology is more valuable than precise microbiology...

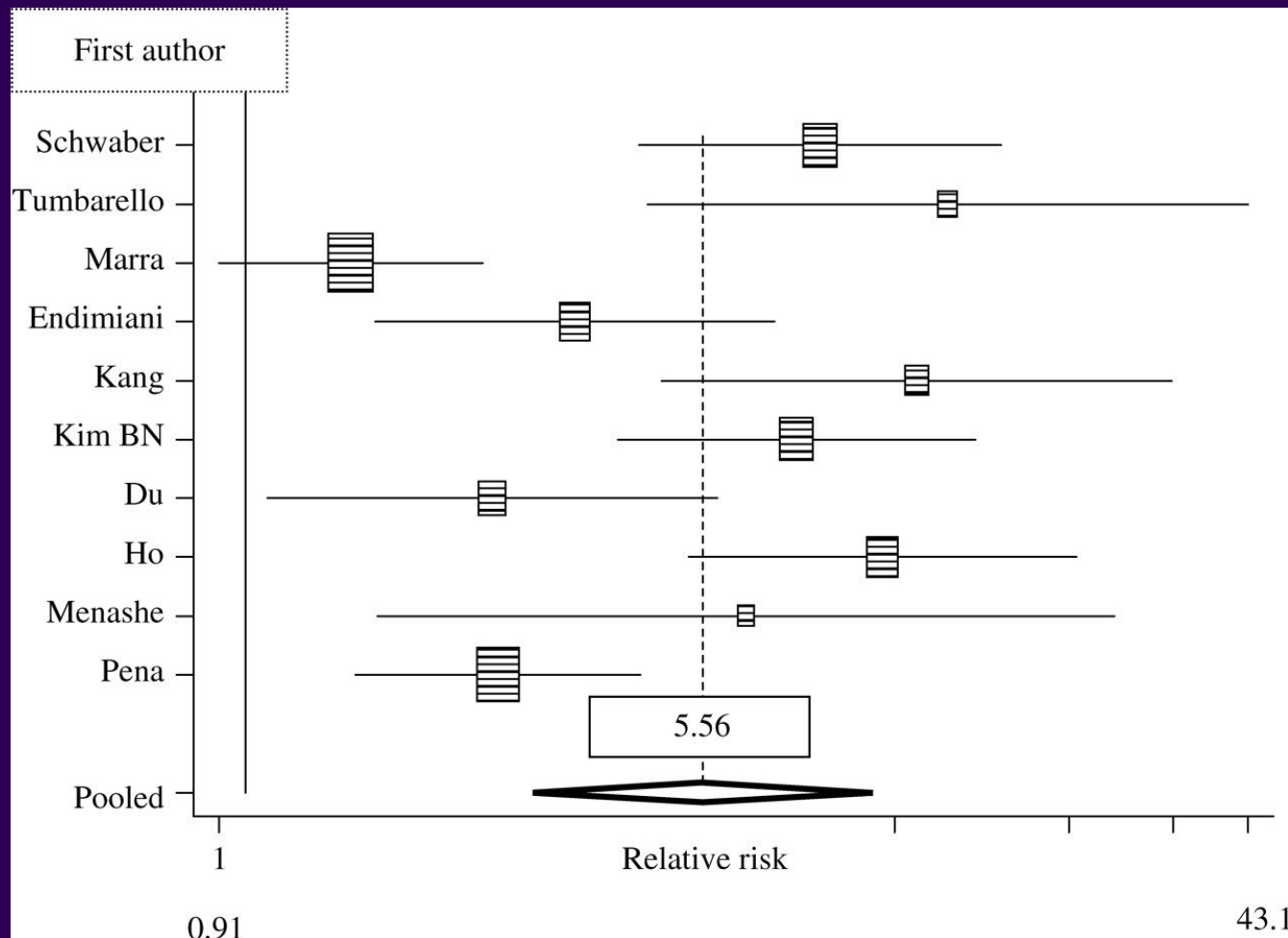
And, mostly, microbiology moves at the pace it did in Fleming's time: 48h from specimen to susceptibility result

Looking for mechanism can be faster....

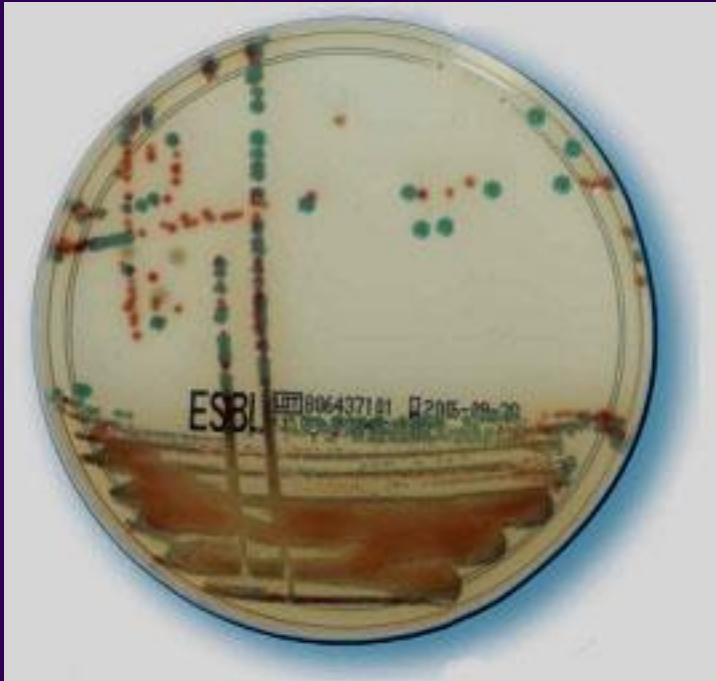
Mortality in ESBL vs. non-ESBL Enterobacteriaceae bacteraemia



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



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 -	Sensitivity	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

Cica β -Test (Mast)



- Examine hydrolysis of chromogenic oxyimino cephalosporin, 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)



No inhibitor

Mercaptoacetic acid to inhibit MBL

Clavulanate to inhibit ESBL

Boronic acid to inhibit AmpC

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



	Mechanism inferred					
	MBL	ESBL	AmpC	Mixed Other	Pen'ase	No activity
Reference data						
MBL (26)	20	1	0	2	3	0
ESBL (74)	3	63	2	6	0	0
AmpC (25)	2	0	18	3	2	0
<i>K. oxytoca</i> , K1 (10)	0	2	6	2	0	0
OXA carbapenemases (10)	0	0	0	10	0	0
<i>P. aeruginosa</i> 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

Fast molecular detection of resistance



- PCR or gene chip technology on overnight culture... or directly from specimen
- Identify gene and predict resistance
 - But not MIC / direct measure of susceptibility
- Would be available if all ESBLs were *bla*_{CTX-M} variants
 - Has been slow for ESBLs because many are sequence variants of *bla*_{TEM/SHV}
- Feasible for carbapenemase genes

Carbapenem R_x in infections with KPC *Klebsiella*



Patient	Site	MIC imipenem		Days imipenem	Outcome
		Vitek	Etest		
M76	Respiratory	2	0.25	7-mero	Failed
M82	Blood	4	2	14	Cure
M92	Respiratory	4	2	3	Cure
F64	Respiratory	4	2	12	Failed
F69	Respiratory	4	8	6	Failed
F46	Blood	4	8	7	Cure
M77	Respiratory	4	≥32	7	Failed
F61	UTI	2	≥32	7	Cure
M52	UTI	4	16	14	Failed
F60	Blood	≥16	8	10-mero	Failed
M60	Respiratory	≥16	8	7-mero	Cure

Which is more useful?



48 h post-specimen

‘It’s a *Kleb. pneumoniae*. Very resistant. We’ve found an MIC of 4 mg/L for meropenem, though. It might be okay at high dose. Or prolonged infusion. Otherwise there’s colistin.’

‘Yes of course; our lab is fully accredited!’

4 h post-specimen

‘There is something with a KPC gene in this sputum from Mr X with the ventilator pneumonia’

‘It’s likely to be resistant to everything except colistin’

Fourth point: *only by being alert do you spot the unusual*



	1	2	3	4
Cefpodoxime	128	8	128	8
....+clavulanate	1	2	32	4
Ceftazidime	16	1	64	2
....+ clavulanate	0.25	1	32	2
Cefotaxime	128	0.5	64	0.5
....+ clavulanate	0.03	0.5	32	0.5
....+ cloxacillin	64	0.5	2	0.5
Cefepime	32	0.5	2	4
....+ clavulanate	0.03	0.5	1	2
Ertapenem	0.06	0.25	0.5	4
Meropenem	0.03	0.06	0.03	1

Last....ECCMID 2010



Official symposia:

- 2 with ESBL in title
- 1 on carbapenemases
- 1 on β -lactamase inhibition
- 1 on low level resistance
- & this one!

Are you really going to tell me that the mechanism doesn't matter, just the (not very exact) MIC or zone?

Report by mechanism



- Ceph MICs of 1-2 mg/L don't consistently predict response
- Routine tests not exact enough for pD interpretation
- Finding the mechanism can be faster than finding the MIC
 - Early appropriate treatment saves lives
- Thinking mechanisms enables the unusual to be spotted
 - Sometimes something new— OXA-48
- Epidemiologically and clinically important
 - Or ECCMID committee has the programme wrong?