



External quality assessment (EQA) of antimicrobial susceptibility testing

Lessons from UKNEQAS

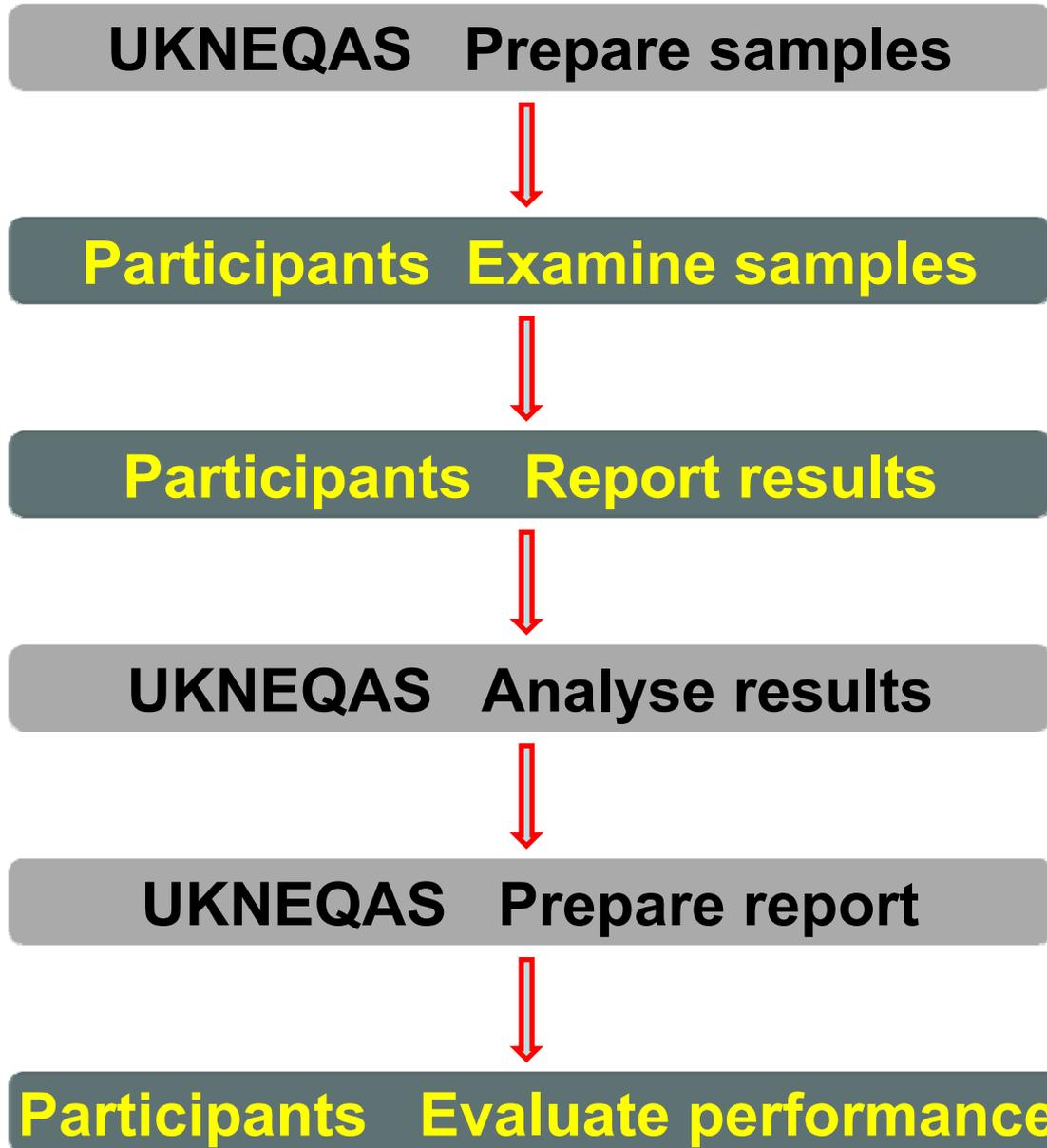
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NordicAST Gothenberg: 31 May 2012

External Quality Assessment (proficiency testing)

**The challenge of laboratory
procedures with specimens of
known but undisclosed content**

The EQA process (UKNEQAS)



Benefits of EQA in antimicrobial susceptibility testing

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Highlights problem areas
- Performance related to guidelines and methods
- (International differences highlighted)
- Gives practical experience of difficult tests (especially if resistance is uncommon)
- Provides background information and discussion of problem tests
- Performance indicator for accreditation

“Limitations” of EQA in antimicrobial susceptibility testing

- Number of specimens distributed is small
- May be considered inappropriate to send some organisms
- Specimens do not reflect routine isolates
- Laboratories may not treat specimens as routine

**Performance may be affected
by breakpoint guidelines used**

S. aureus 7240, ciprofloxacin MIC 0.5 mg/L

Guideline	Breakpoints (mg/L)		Number reporting		
	S \leq	R $>$	S	I	R
CLSI	1	2	334	5	4
BSAC	1	1	167	19	1
SRGA	0.06	2	3	19	0

S. aureus 7876, ciprofloxacin MIC 0.25 mg/L

Guideline	Breakpoints (mg/L)		Number reporting		
	S \leq	R $>$	S	I	R
CLSI	1	2	350	0	0
BSAC	1	1	176	0	1
SRGA	1	1	23	2	1

E. faecalis specimen 0138 (Jan 2011) vancomycin MIC 8-16 mg/L (vanB)

Guideline	Breakpoints (mg/L)		Percent reporting		
	S ≤	R >	S	I	R
EUCAST (n=316)	4	4	5.1	1.9	93.0
CLSI (n=314)	4	16	10.2	35.0	54.8

***P. aeruginosa* specimen 0489 (Aug 2011)
piperacillin-tazobactam MIC 32-64 mg/L**

Guideline	Breakpoints (mg/L)		Percent reporting		
	S ≤	R >	S	I	R
EUCAST (n=340)	16	16	14.1	0.9	85.0
CLSI (n=210)	64	64	55.2	2.4	42.4

***P. aeruginosa* specimen 0489 (Aug 2011)
piperacillin-tazobactam MIC 32-64 mg/L
NordicAST reports**

Country	Number (%) reporting		
	S	I	R
All (n=576)	172 (29.9)	9 (1.5)	395 (68.6)
Denmark (n=13)	3	0	10
Finland (n=19)	1	0	19
Iceland (n=2)	0	0	2
Norway (n=11)	2	0	9
Sweden (n=25)	1	0	24

All reporting S used disk diffusion but three also gave MIC (8, 12, 16 mg/L).

**Guidelines are not always
followed**

S. aureus specimen 0185 (Feb 2011) mupirocin MIC 4-16 mg/L

Guideline	Breakpoints (mg/L)		Percent reporting		
	S \leq	R $>$	S	I	R
EUCAST (n=248)	1	256	13.3	34.3	52.4
CLSI (n=208)	256	256	19.2	11.1	69.7

75% of 93 labs reporting R and using CLSI breakpoints with automated systems reported MICs >8 or ≥ 8 mg/L and therefore cannot distinguish high and low-level resistance

CLSI disk diffusion method requires 200 μ g disk
 21/21 using 200 μ g disk reported S or I
 20/22 using 5 μ g disk reported R

BSAC disk diffusion method requires MIC if resistant with 5 μ g disk
 56/69 using 5 μ g disk reported R, suggesting MIC was not tested

S. aureus specimen 0185 (Feb 2011)

mupirocin MIC 4-16 mg/L

NordicAST reports

Country	Number reporting		
	S	I	R
All (n=480)	78 (16.3)	110 (22.9)	292 (60.8)
Denmark (n=13)	0	2	11
Finland (n=9)	1	5	3
Iceland (n=1)	0	0	1
Norway (n=6)	0	4	2
Sweden (n=9)	2	1	6

Mostly disk diffusion but several also reported MIC, particularly from Norway
 5 or 10µg disk commonly used (will not distinguish I and R)

One reporting S incorrectly interpreted zone diameter (should have been I)

One incorrectly interpreted an MIC (should have been I)

One reported S with zone 6mm

S. aureus specimen 9889 (Aug 2010) fusidic acid MIC 4-8 mg/L

Guideline	Breakpoints (mg/L)		Percent reporting		
	S \leq	R $>$	S	I	R
EUCAST (n=240)	1	1	0	2.1	97.9
CLSI (n=297)	-	-	1.0	40.1	58.9

***S. aureus* specimen 9889 (Aug 2010)**

fusidic acid MIC 4-8 mg/L

NordicAST reports

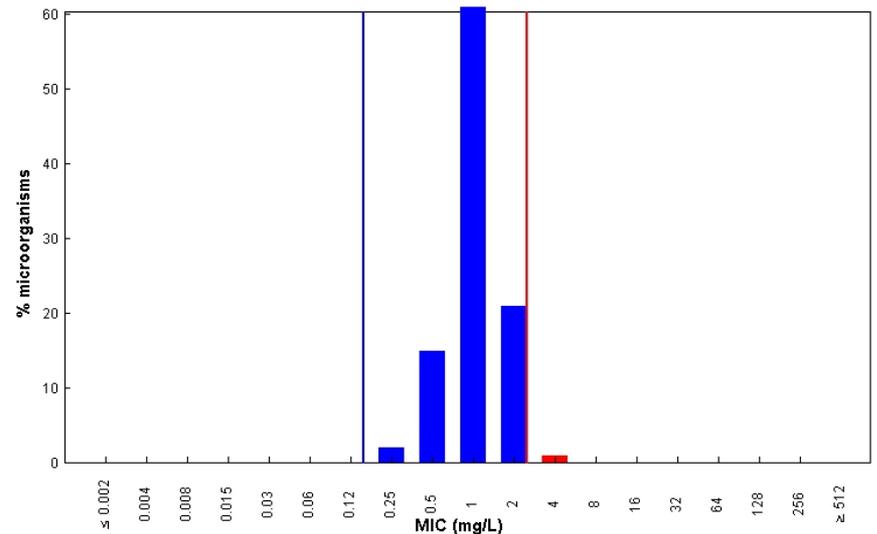
Country	Number reporting		
	S	I	R
All (n=568)	3 (0.5)	136 (24.0)	429 (75.5)
Denmark (n=12)	0	0	12
Finland (n=19)	0	0	19
Iceland (n=0)	0	0	0
Norway (n=8)	0	0	8
Sweden (n=24)	0	0	24

S. pneumoniae specimen 0272 (EARS-Net 2011) ciprofloxacin MIC 0.5-1 mg/L

Guideline	Breakpoints (mg/L)		Percent reporting		
	S ≤	R >	S	I	R
EUCAST (n=202)	0.12	2	30.2	67.8	2.0
CLSI (n=181)	-	-	92.9	14.9	2.2

EUCAST wild type is intermediate – susceptible very rare

CLSI no breakpoints for ciprofloxacin



MIC
Epidemiological cut-off: WT ≤ 2 mg/L

73840 observations (50 data sources)
Clinical breakpoints: S ≤ 0.125 mg/L, R > 2 mg/L

***S. pneumoniae* specimen 0272 (EARS-Net 2011) ciprofloxacin MIC 0.5-1 mg/L NordicAST reports**

Country	Number reporting		
	S	I	R
All (n=406)	225 (55.4)	173 (42.6)	8 (2.0)
Denmark (n=5)	1	4	0
Finland (n=3)	1	2	0
Iceland (n=1)	1	0	0
Norway (n=4)	2	2	0
Sweden (n=10)	5	5	0

**Performance is affected by
method used**

E. faecalis specimen 0138 (Jan 2011) vancomycin MIC 8-16 mg/L (vanB)

EUCAST resistant, CLSI intermediate

Method	Percent reporting		
	S	I	R
Automated (n=333)	3.9	6.2	89.9
MIC (n=71)	2.8	13.9	83.3
Disk diffusion (n=262)	15.5	11.6	72.9

E. faecalis specimen 0138 (Jan 2011)

vancomycin MIC 8-16 mg/L

NordicAST reports

Country	Number reporting		
	S	I	R
All (n=669)	49 (7.3)	123 (18.4)	497 (74.3)
Denmark (n=14)	2	2	10
Finland (n=21)	1	1	19
Iceland (n=2)	0	0	2
Norway (n=9)	1	0	8
Sweden (n=27)	0	0	27

2/4 reporting S gave MIC 4 mg/L, stated method disk diffusion

1/4 reporting S gave MIC 8 mg/L, stated method disk diffusion

1/4 reporting S gave MIC 3 mg/L, stated method MIC

**Borderline susceptibility
reduces the reliability of
results**

***E. coli* specimen 0270 (EARS-Net 2011)
piperacillin-tazobactam MIC 8-16 mg/L
(CTX-M-15) S/I EUCAST, S CLSI**

Country	Number (%) reporting		
	S	I	R
All (n=737)	241 (32.7)	175 (23.6)	371 (43.6)
Denmark (n=11)	4	2	5
Finland (n=19)	1	4	14
Iceland (n=1)	0	0	1
Norway (n=14)	6	5	3
Sweden (n=27)	7	8	1

***E. coli* specimen 0270 (EARS-Net 2011)**

piperacillin-tazobactam MIC 8-16 mg/L

- Differences in guidelines used
 - EUCAST reports (n=356) 29.2% S, 27.3% I, 43.5% R
 - CLSI reports (n=341) 38.4% S, 20.8% I, 40.8% R
- Differences between methods
 - Automated 63.1% of 225 reported R
 - MIC 55.3% of 103 reported R
 - Disk diffusion 24.5% of 310 reported R
- Many laboratories edit reports from S to I/R or I to R
 - Automated 9% edited to a more resistant category
 - MIC 24% edited to a more resistant category
 - Disk diffusion 34% edited to a more resistant category

***E. coli* specimen 0270 (EARS-Net 2011)**

piperacillin-tazobactam MIC 8-16 mg/L

Guidelines not being followed - Neither EUCAST or CLSI recommend editing ESBL-producers to resistant.

EUCAST Expert rule 9.1

IF intermediate or resistant to any 3rd generation (cefotaxime, ceftriaxone, ceftazidime) or 4th generation (cefepime) oxyimino-cephalosporin, AND susceptible to amoxicillin-clavulanate, ampicillin-sulbactam or piperacillin-tazobactam THEN report as tested and enclose a warning on uncertain therapeutic outcome for infections other than urinary tract infections (or bacteraemia secondary to UTI).

Uncertainty in reporting penicillin susceptibility in *S. pneumoniae*

- Screen with oxacillin disk
- If resistant, MIC to define susceptibility



S. pneumoniae specimen 0272 (EARS-Net 2011) penicillin MIC 0.5 mg/L

“Intermediate” to penicillin

Susceptible if from pneumonia

Resistant if from meningitis

Reporting	Interpretation		Percent reporting		
	EUCAST	CLSI	S	I	R
Oxacillin (n=523)	R	R	4.6	4.6	90.8
Penicillin					
Test result (n=615)			23.6	69.8	6.6
Pneumonia (n=740)	S	S	76.1	20.8	3.1
Meningitis (n=741)	R	R	5.5	8.1	86.4

***S. pneumoniae* specimen 0272 (EARS-Net 2011) penicillin MIC 0.5 mg/L**

Oxacillin screening test resistant

Countries	Interpretation		Number (%) reporting		
	EUCAST	CLSI	S	I	R
All (n=523)	R	R	(4.6)	(4.6)	(90.8)
Denmark (n=10)			0	0	10
Finland (n=13)			1	0	12
Iceland (n=2)			0	0	2
Norway (n=10)			0	0	10
Sweden (n=13)			0	0	13

S report by EUCAST disk diffusion with zone diameter of 20mm

***S. pneumoniae* specimen 0272 (EARS-Net 2011) penicillin MIC 0.5 mg/L**

Test “Intermediate” to penicillin

Countries	Interpretation		Number (%) reporting		
	EUCAST	CLSI	S	I	R
All (n=615)			(23.6)	(69.8)	(6.6)
Denmark (n=9)			0	0	9
Finland (n=18)			2	16	0
Iceland (n=1)			0	1	0
Norway (n=12)			1	1	10
Sweden (n=17)			0	17	0

One S with borderline zone diameter; one oxacillin R but MIC 0.06 mg/L; one S with MIC 0.25 mg/L

***S. pneumoniae* specimen 0272 (EARS-Net 2011) penicillin MIC 0.5 mg/L**

Pneumonia interpretation susceptible

Countries	Interpretation		Number (%) reporting		
	EUCAST	CLSI	S	I	R
All (n=740)	S	S	(76.1)	(20.8)	(3.1)
Denmark (n=12)			4	0	8
Finland (n=20)			17	3	0
Iceland (n=1)			0	1	0
Norway (n=13)			2	1	10
Sweden (n=17)			1	16	0

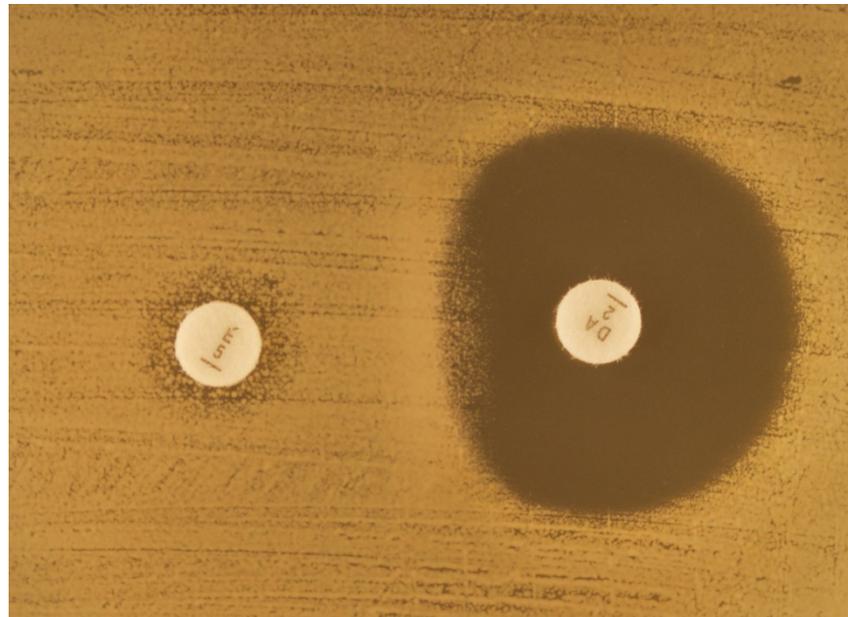
***S. pneumoniae* specimen 0272 (EARS-Net 2011) penicillin MIC 0.5 mg/L**

Meningitis interpretation resistant

Countries	Interpretation		Number (%) reporting		
	EUCAST	CLSI	S	I	R
All (n=741)	R	R	5.5	8.1	86.4
Denmark (n=12)			0	3	9
Finland (n=20)			1	1	18
Iceland (n=1)			0	1	0
Norway (n=13)			0	0	13
Sweden (n=17)			1	1	15

One S with borderline zone diameter; one oxacillin R but MIC 0.06 mg/L

Uncertainty in reporting dissociated (MLSB-inducible) resistance to clindamycin in *S. aureus*



***S. aureus* specimen 0275 (EARS-Net 2011)**

Clindamycin MIC 0.12-0.5 mg/L (S), but
resistance induced by erythromycin

Percent reporting (n=775)		
S	I	R
24.0	1.8	74.2

Reports of susceptible not related to guideline or method

- EUCAST expert rules recommend reporting resistant, or susceptible with warning of possible failure due to selection of resistant mutants. Avoid use in serious infections
- CLSI – report resistant with note that some may respond

***S. aureus* specimen 0275 (EARS-Net 2011)**

Clindamycin MIC 0.12-0.5 mg/L (S), but
resistance induced by erythromycin

Country	Number (%) reporting		
	S	I	R
All (n=775)	186 (24.0)	14 (1.8)	575 (74.2)
Denmark (n=10)	0	0	10
Finland (n=20)	3	0	17
Iceland (n=2)	1	0	1
Norway (n=13)	2	0	11
Sweden (n=17)	0	0	17

**Uncertainty in reporting
cephalosporin susceptibility
for Enterobacteriaceae with
inducible AmpC**

E. cloacae specimen 9581 (Jan 2010)

Inducible AmpC

Agent	Expected test result	% reporting		
		S	I	R
Cefotaxime	S	91.2	0.5	8.3
Ceftazidime	S	90.6	0.4	9.0

Inducible AmpC typical for *Enterobacter* spp. (also *C. freundii*, *Serratia* spp., *M. morgani* and *Providencia* spp.)

May mutate to stably derepressed state during treatment.

Reporting inducible AmpC in Enterobacteriaceae

EUCAST

Expert rule 9.2. If susceptible in vitro, use of cefotaxime, ceftriaxone or ceftazidime in monotherapy (*or in combination with an aminoglycoside*) should be discouraged owing to risk of selecting resistance. Reports should note this or results should be suppressed.

CLSI

Report as susceptible if they appear so in susceptibility tests, but with a warning that resistance may develop during treatment and that repeat isolates should be retested.

E. cloacae specimen 9581, Jan 2010

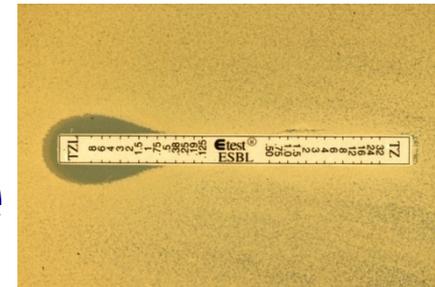
Inducible AmpC

Cefotaxime MIC 0.12-0.5 mg/L (S)

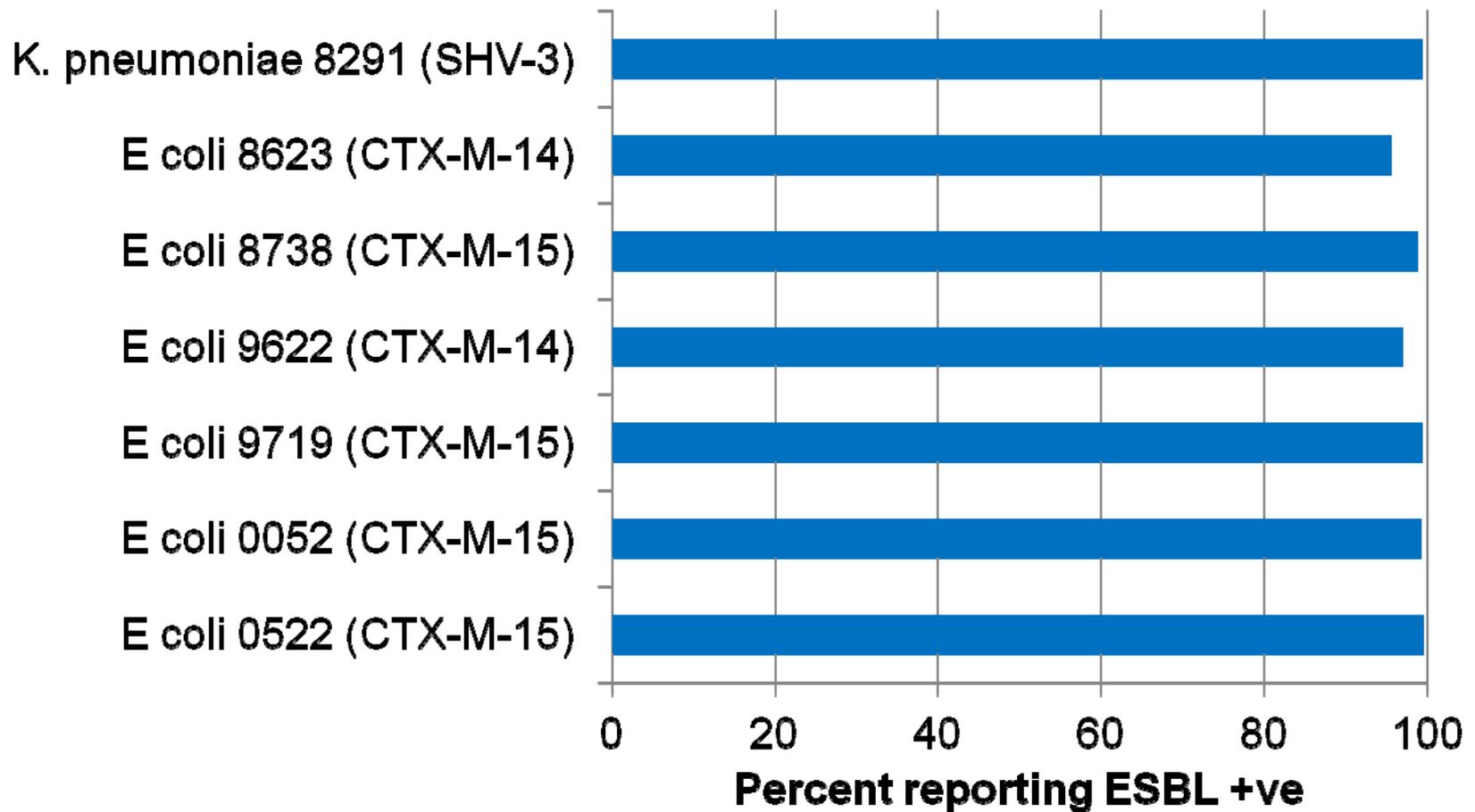
Country	Number (%) reporting		
	S	I	R
All (n=775)	507(91.2)	3(0.5)	46(8.3)
Denmark (n=11)	6	1	4
Finland (n=8)	7	0	1
Iceland (n=1)	0	0	1
Norway (n=7)	7	0	0
Sweden (n=24)	22	0	2

All disk diffusion, 5/8 reporting resistant edited S to R, one zone in resistant range, one MIC >64 mg/L, one no data

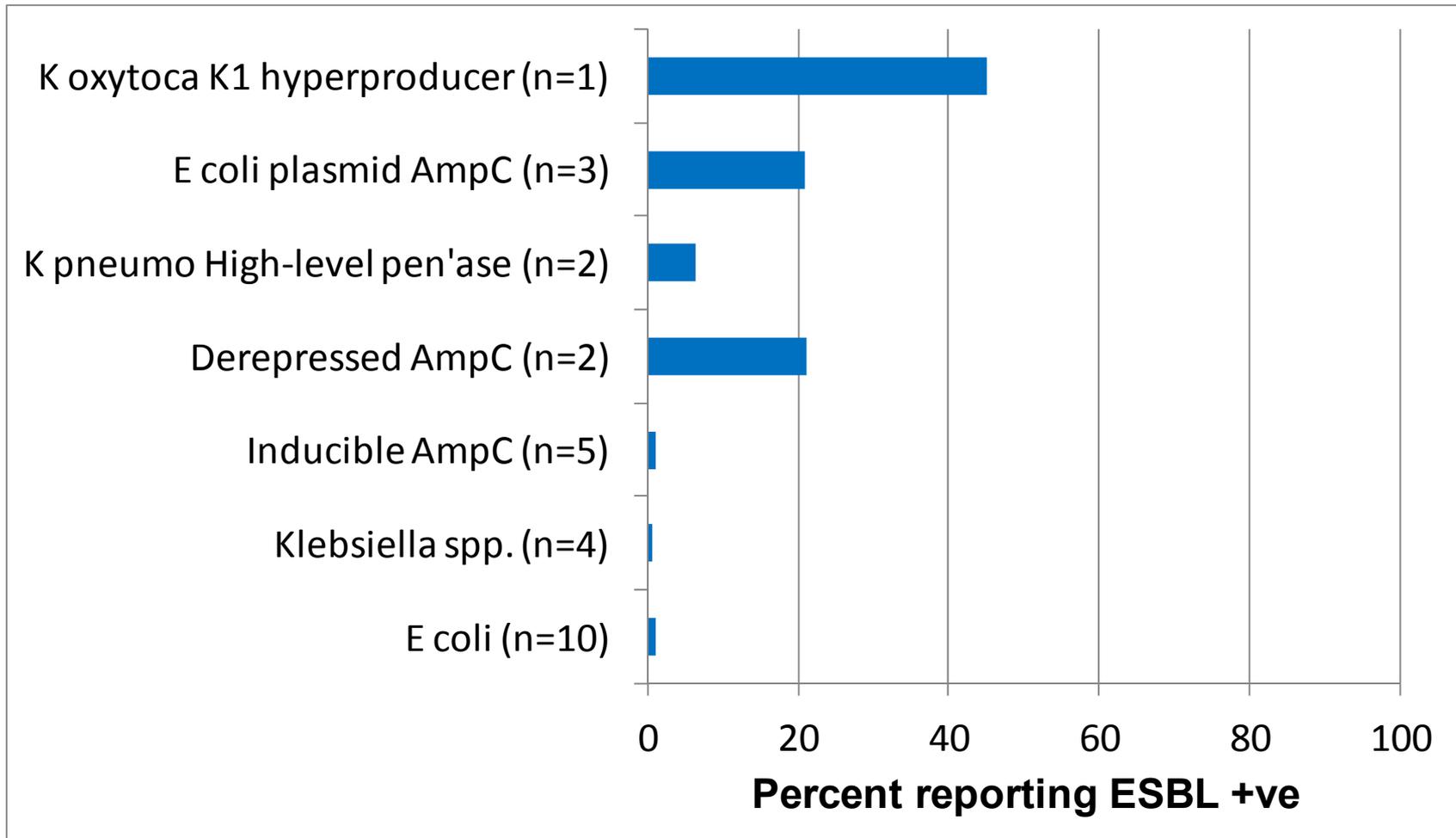
Detection of ESBL-mediated resistance in Enterobacteriaceae



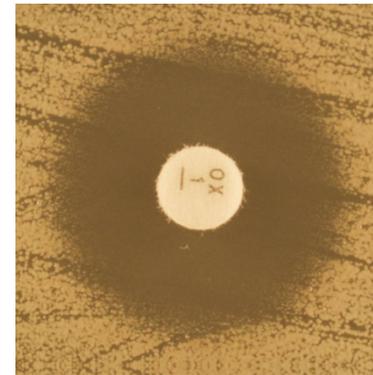
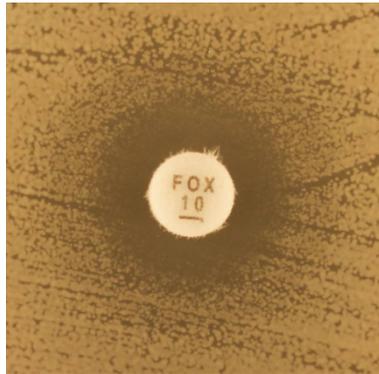
Detection of ESBLs in Enterobacteriaceae



Reports of ESBLs in Enterobacteriaceae without ESBLs



Detection of methicillin resistance in *S. aureus* in tests with cefoxitin and oxacillin



Detection of oxacillin/cefoxitin resistance in *mecA* positive *S. aureus*

Specimen	Oxacillin MIC range (mg/L)	Percent reporting R	
		Oxacillin	Cefoxitin
8701	4-128	95.6	94.1
8858	>128->128	100	99.2
9141	8-32	92.8	94.8
9179	64->128	98.5	96.2
9376	>128->128	99.8	99.8
9487	128->128	99.3	99.6
9992	8-32	92.8	95.0
0185	>128->128	99.1	99.6
0364	64->128	97.5	96.6
0448	64->128	99.2	98.9

Reports of oxacillin/cefoxitin resistance in *mecA* negative *S. aureus*

Specimen	Oxacillin MIC range (mg/L)	Percent reporting I/R	
		Oxacillin	Cefoxitin
9582	0.12-0.12	0.2	0.2
9670	0.12-0.5	1.6	0.6
9757	0.12-0.25	0.9	1.3
9807	0.5-0.5	1.6	1.3
9848	0.5-1	7.6	3.1
9889	0.12-0.12	0.7	0.6
0137	0.12-0.5	0.9	1.0
0280	0.12-0.5	1.0	1.0
0404	0.12-0.25	0.8	0.8
0521	0.25-1	0.8	1.1

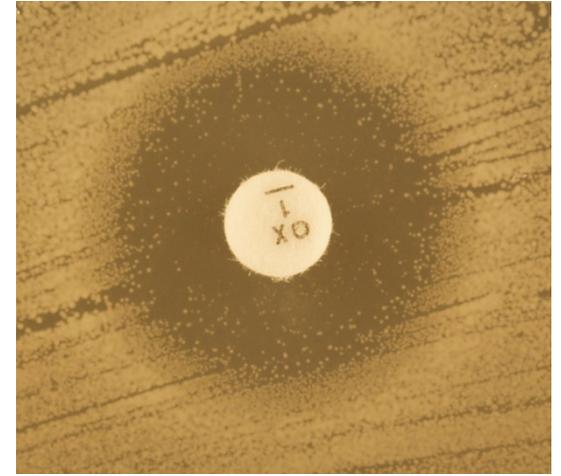
Penicillinase-hyperproducing *S. aureus*

S aureus 9848

oxacillin susceptible

(MIC 0.5-1 mg/L)

mecA-ve)



Method	Oxacillin		Cefoxitin	
	n	% I/R	n	% I/R
Automated	161	3.1	127	3.1
Disk diffusion	180	15.6	266	2.6
MIC	29	0	23	0
Multiple	55	5.4	61	3.2

Conclusions

- Performance good for most organism-agent combinations
- Performance can be linked to guidelines and methods used for some tests
- Discrepancies more common when:
 - Differences between guidelines
 - Failure to follow guidelines
 - Confusion over reporting
 - Susceptibility borderline