EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST Subcommitee for Detection of Resistance Mechanisms

Christian G. Giske
Karolinska University Hospital and EUCAST

NEQAS, 29 November 2012

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

The background

- Guidance on methods of detection and characterization of resistance mechanisms are required to tie in with
  - The EUCAST MIC breakpoints
  - The EUCAST disk diffusion method
  - EUCAST Expert Rules
  - The ECDC requirements for update of the EARS-Net manual

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

The remit

- To develop practical guidelines for detection of specific antimicrobial resistance mechanisms of clinical and/or epidemiological importance.
- The guidance will include:
  - Definition of the mechanisms.
  - Explanation of the clinical and/or public health need for detection of the mechanisms.
  - An outline description of recommended methods of detection.
  - References to detailed descriptions of the methods.
- Draft guidelines will be subject to wide consultation via established EUCAST procedures and ECDC focal points

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Mechanisms and bacteria

- Methicillin-resistant *S. aureus*
- Vancomycin low-level resistance in *S. aureus* (VISA/heteroVISA)
- Vancomycin-resistant enterococci
- Penicillin non-susceptible *S. pneumoniae*
- Extended-spectrum β-lactamase producing Enterobacteriaceae
- Acquired AmpC-producing Enterobacteriaceae
- Acquired carbapenemases in Enterobacteriaceae

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Time schedule

- February 2012: recruiting of members
- March 2012: preparation of template
- April 2012 (at ECCMID): first (and only) meeting, sharing the work between members
- May and June 2012: preparation of first draft
- July 2012: preliminary report at EUCAST-meeting in Stockholm
- September 2012: revision of the first draft
- October 2012: revised draft presented to the steering committee
- November 2012: feedback from the EUCAST steering committee and the EARS-Net co-ordination group
- December 2012: open consultation (one month)
- January 2013: consultation closes, final revision
- February 2013: publication (following EUCAST SC-meeting)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Members of the SC

- Christian G. Giske (Chair; Sweden, EUCAST and EARS-Net)
- Luis Martinez-Martinez (Spain)
- Rafael Canton (Spain and EUCAST)
- Stefania Stefani (Italy)
- Robert Skov (Denmark)
- Youri Glupczynski (Belgium)
- Patrice Nordmann (France)
- Mandy Wootton (UK)
- Vivi Miriagou (Greece)
- Gunnar Skov Simonsen (Norway and EARS-Net)
- Helena Zemlickova (Czech republic and EARS-Net)
- James Cohen-Stuart (Netherlands)
- Marek Gniadkowski (Poland)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

What have we achieved?

- Drafts have been presented to the steering committee of EUCAST and are now being revised
- Preparation of a final draft for open consultation in December ongoing
- Highly probable that (annual?) revisions will be needed
- Publication in CMI is planned (spring 2013)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

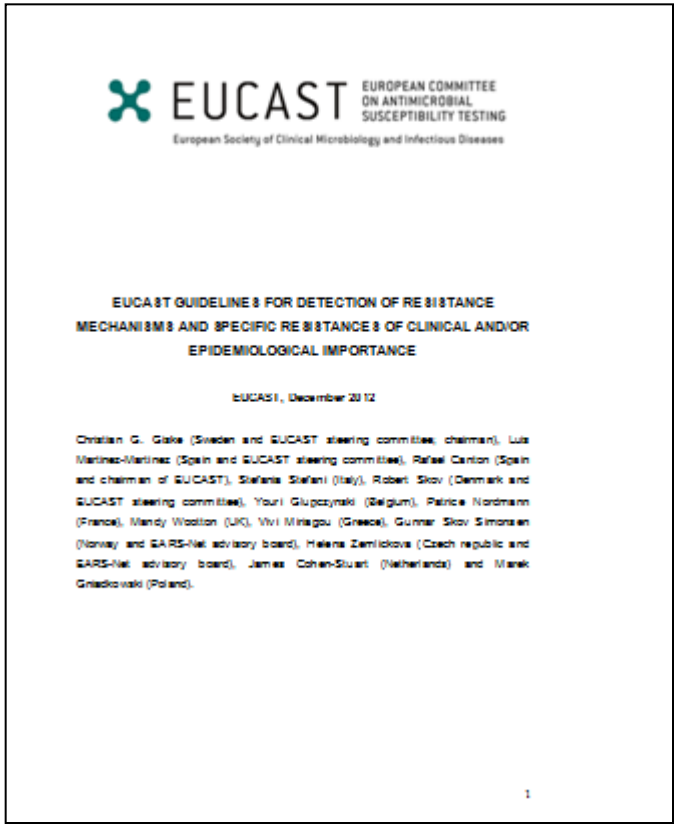
How has the work been carried out?

- Just one initial meeting at ECCMID
- Mostly e-mail contacts with circulation of drafts
- Systematic literature searches in addition to authors' personal experience
- Methods evaluated in multi-centre studies preferred
- Gram-positive and Gram-negative group, which have both reported to the chairman

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

The guidelines



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

EUCAST GUIDELINES FOR DETECTION OF RESISTANCE MECHANISMS AND SPECIFIC RESISTANCES OF CLINICAL AND/OR EPIDEMIOLOGICAL IMPORTANCE

EUCAST, December 2012

Christian G. Giske (Sweden and EUCAST steering committee, chairman), Luis Martinez-Martinez (Spain and EUCAST steering committee), Rafael Canton (Spain and chairman of EUCAST), Stefania Stefani (Italy), Robert Skov (Denmark and EUCAST steering committee), Youri Glupczynski (Belgium), Patrice Nordmann (France), Mandy Wootton (UK), Vivi Miriagou (Greece), Gunnar Skov Simonsen (Norway and EARS-Net advisory board), Helena Zemlickova (Czech republic and EARS-Net advisory board), James Cohen-Stuart (Netherlands) and Marek Gniadkowski (Poland).

1

2. CARBAPENEMASES IN ENTEROBACTERIACEAE

Importance of detection of resistance mechanism	
Required for antimicrobial susceptibility categorisation	No
Infection control	Yes
Public health	Yes

2.1 Definition

Carbapenemases are β-lactamases hydrolyzing penicillins, in most cases cephalosporins, and to varying degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo-β-lactamases).

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

When should screening be carried out?

Carbapenem	MIC (mg/L)		Disk diffusion zone diameter (mm)	
	S/I-Breakpoint	Screening cut-off	S/I-Breakpoint	Screening cut-off
Meropenem[1]	≤2	>0.125	≥22	<25[2]
Imipenem	≤2	>1	≥22	<23
Ertapenem[3]	≤0.5	>0.125	≥25	<25

[1]Meropenem offers the best balance between sensitivity and specificity in the detection of putative carbapenemase-producers.

[2]In rare cases OXA-48-producing Enterobacteriaceae have presented with zone diameters of 24-26 mm, for which reason 27 mm may be considered as a screening cut-off during outbreaks. It should be noted that this cut-off will bisect the wild-type.

[3]Ertapenem has high sensitivity, but low specificity for prediction of carbapenemase-producing Enterobacteriaceae, and is for this reason not recommended.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Phenotypic confirmation methods

β-lactamase	Synergy observed (mm increase of meropenem zone diameter)				
	DPA/EDTA	APBA/PBA	DPA+APBA	CLX	Temocillin > 32 mg/L
MBL	≥5	-	-	-	NA[1]
KPC	-	≥4	-	-	NA[1]
MBL+KPC[2]	Variable	Variable	≥5	-	NA[1]
OXA-48-like[3]	-	-	-	-	Yes
AmpC+porin loss	-	≥4	-	≥5	NA[1]
ESBL+porin loss	-	-	-	-	No

[1] Temocillin is only needed in cases where no synergy is detected, in order to differentiate between ESBL plus porin loss and OXA-48-like enzymes.

[2] There are no published reports with commercial disks or tablets containing double inhibitors (DPA or EDTA plus APBA or PBA), and in-house assays. This phenotype is so far rare outside of Greece, and will produce high-level resistance to carbapenems.

[3] For laboratories not testing temocillin, high-level resistance to piperacillin-tazobactam (MIC>32 mg/L) may be another phenotypic indicator of OXA-48, as ESBLs tend to have lower MICs.

3. EXTENDED-SPECTRUM β-LACTAMASES (ESBLs) IN ENTEROBACTERIACEAE

Importance of detection of resistance mechanism	
Required for antimicrobial susceptibility categorisation	No
Infection control	Yes
Public health	Yes

3.1 Definition

ESBLs are β-lactamases conferring resistance against oxyimino-β-lactam compounds (cefuroxime, third- and fourth-generation cephalosporins and aztreonam) and are resistant to most penicillins, cephalosporins (except for cephamycins) and monobactams. Most of ESBLs belong to the Ambler class A of β-lactamases and are inhibited by β-lactam inhibitors (clavulanate, sulbactam and tazobactam).

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

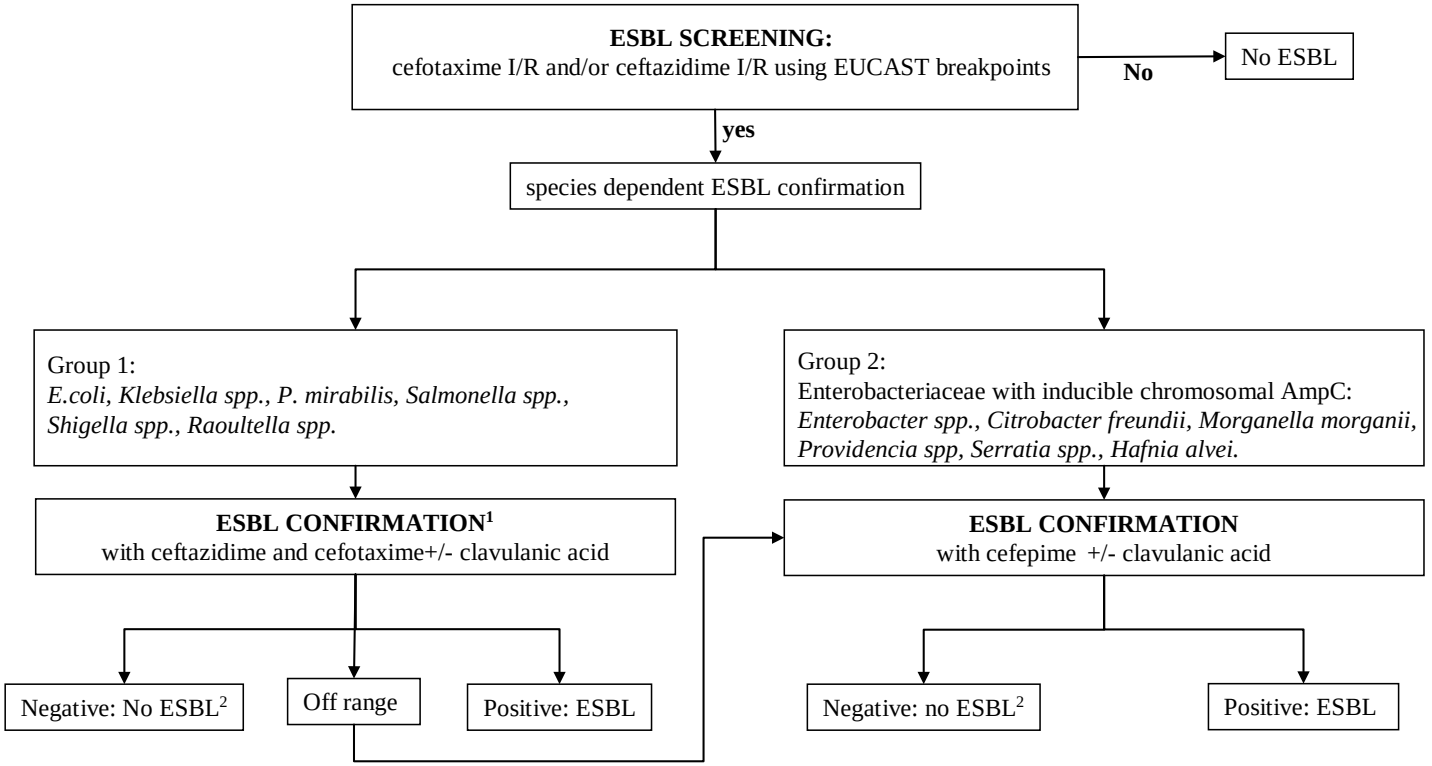
European Society of Clinical Microbiology and Infectious Diseases

When should ESBL-testing be done?

Method	Antibiotic	Conduct ESBL-confirmation if
Broth dilution	cefotaxime	MIC > 1 mg/L
	ceftazidime	MIC > 1 mg/L
Agar dilution	cefotaxime	MIC > 1 mg/L
	ceftazidime	MIC > 1 mg/L
Disk diffusion	cefotaxime (5 µg)	Inhibition zone < 21 mm
	ceftriaxone (30 µg)	Inhibition zone < 23 mm
	ceftazidime (10 µg)	Inhibition zone < 22 mm
Automated systems	cefotaxime	MIC > 1 mg/L
	ceftazidime	MIC > 1 mg/L

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases



1: If cefoxitin MIC > 8 mg/L, perform cefepime+/- clavulanic acid confirmation test

2: Genotypic or phenotypic confirmation of acquired AmpC is recommended in group 1 Enterobacteriaceae isolates with negative ESBL confirmation test. .

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Group I Enterobacteriaceae (see Table 1)			
Method	**Antibiotic**	**Disk/tablet load**	**Confirmation is positive if**
Etest ESBL	Cefotaxime +/- clavulanic acid	-	MIC ratio[1] ≥ 8 or deformation ellipse / phantom zone present
	Ceftazidime +/- clavulanic acid	-	MIC ratio[1] ≥ 8 or deformation ellipse / phantom zone present
Combination disk diffusion test (CDT)	Cefotaxime +/- clavulanic acid	Cefotaxime 30 ug +/- clavulanic acid 10 ug	≥ 5 mm increase in inhibition zone[2]
	Ceftazidime +/- clavulanic acid	Ceftazidime 30 ug+/- clavulanic acid 10 ug	≥ 5 mm increase in inhibition zone[2]
Broth microdilution	Cefotaxime +/- clavulanic acid	-	MIC ratio[1] ≥ 8
	Ceftazidime +/- clavulanic acid	-	MIC ratio[1] ≥ 8
Double disk synergy test (DDST)	Cefepime +/- clavulanic acid	-	MIC ratio[1] ≥ 8
	Cefotaxime, ceftazidime and cefepime	-	Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Group II Enterobacteriaceae (see Table 1)			
Method	**Antibiotic**		**Screening is positive if**
Etest ESBL	Cefepime +/- clavulanic acid	-	MIC ratio[1] ≥ 8 or deformation ellipse / phantom zone present
Combination disk diffusion test	Cefepime +/- clavulanic acid	Cefepime 30 ug Clavulanic acid 10 ug	≥ 5 mm increase in inhibition zone[2]
Broth microdilution	Cefepime +/- clavulanic acid	-	MIC ratio[1] ≥ 8
Double disk synergy test (DDST)	Cefotaxime, ceftazidime, cefepime	-	Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc

5. DETECTION OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* (MRSA)

Importance of detection of resistance	
Required for antimicrobial susceptibility categorisation	Yes
Infection control	Yes
Public health	Yes

5.1 Definition

S. aureus isolates with an auxiliary penicillin binding protein 2 (PBP2a or the recently discovered PBP2c) to which β-lactam agents, except for the novel class of cephalosporins having anti-MRSA activity, have little or no affinity.

5.4 Recommended methods for detection

Methicillin/oxacillin resistance can be detected both phenotypically by MIC or disk diffusion methods, or genotypically using PCR.

5.4.1 Detection with MIC determination or disk diffusion

The heterogeneous expression of resistance particularly affects MICs of oxacillin. Cefoxitin is a very sensitive and specific marker of *mecA/mecC* mediated methicillin resistance and is the substance of choice for disk diffusion. Disk diffusion using oxacillin is discouraged and interpretive zone diameters are no longer presented in the EUCAST breakpoint table. Strains with increased MIC to oxacillin but sensitive to cefoxitin are uncommon. If oxacillin is tested and gives a different interpretation than cefoxitin the interpretation should be as shown below. It is recommended to subject such strains to phenotypic or genotypic investigations of MecA or MecC.

Table 1. Interpretation of discrepant oxacillin and cefoxitin results.

	Cefoxitin MIC or disk diffusion	
	S	R
Oxacillin MIC R	Report as R	Report as R
Oxacillin MIC S	Report as S	Report as R

6. NON-SUSCEPTIBILITY TO GLYCOPEPTIDES IN *STAPHYLOCOCCUS AUREUS*

Importance of detection of resistance	
Required for antimicrobial susceptibility categorisation	Yes
Infection control	Yes
Public health	Yes

6.1 Definition

GRSA: Glycopeptide resistant *S. aureus*

S. aureus isolates with high-level resistance to vancomycin (MIC > 8 mg/L).

GISA: glycopeptide intermediate *S. aureus*

S. aureus isolates with low-level resistance to vancomycin (MIC 4 - 8 mg/L).

hGISA: Heterogeneous glycopeptide intermediate *S. aureus*.

S. aureus isolates with incomplete low-level resistance to vancomycin (MICs ≤ 2mg/L) but with minority populations (1 in 10^{-6} cells) with vancomycin MIC > 2 mg/L by population analysis.

6.4 Recommended methods for detection

Disk diffusion can **NOT** be used to test for either hGISA or GISA.

6.4.1 MIC determination

BMD using EUCAST methodology is the gold standard (ISO 20776-1), but MICs may also be determined by gradient strips, agar dilution or automated systems. It should be noted that the results using gradient strips are 0.5 - 1 dilution steps higher than the results obtained by BMD (7). The EUCAST breakpoint for resistance to vancomycin in *S. aureus* is MIC > 2 mg/L.

6.4.2 Specific tests for hGISA

GISA is detected by measuring the MIC, but this is not the case for hGISA. Detection of hGISA has proven difficult. Detection is therefore divided into screening and confirmation. For screening a number of specialised methods have been designed.

7. DETECTION OF VANCOMYCIN RESISTANCE IN *ENTEROCOCCUS FAECALIS* AND *ENTEROCOCCUS FAECIUM*

Importance of detection of resistance	
Required for antimicrobial susceptibility categorisation	Yes
Infection control/public health	Yes
Public health	Yes

7.1 Definition

Enterococcus faecalis or *Enterococcus faecium* with resistance to vancomycin (VRE) (vancomycin MIC > 4 mg/L).

7.2 Mechanism of resistance

Clinically relevant resistance is most often mediated by plasmid-encoded VanA and VanB ligases that confer replacement of D-Ala in the peptidoglycan with D-Lac.

	VanA	VanB
Vancomycin MIC	64-1024 mg/L	4-1024mg/L
Teicoplanin MIC	16-512mg/L	0.06-1mg/L

7.4 Recommended methods for detection

Vancomycin resistance can be detected by MIC-determination, disk diffusion and the breakpoint agar method. For all three methods it is essential that plates are incubated for a full 24hrs in order to capture inducible resistance.

All three methods readily detect *vanA*-mediated resistance. Detection of *vanB*-mediated resistance is more challenging. MIC determination using agar or broth dilution works with high accuracy but is seldom used in routine laboratories. Reports show that detection of *vanB*-mediated resistance is problematic for automated methods (Swenson, Endtz, Klare). Disk diffusion using a 5µg vancomycin disk performs well provided the guidelines for reading as specified by EUCAST are followed meticulously (data from EUCAST Reference Laboratory, Växjö, Sweden).

8. DETECTION OF PENICILLIN NON-SUSCEPTIBILITY IN *STREPTOCOCCUS PNEUMONIAE*

Importance of detection of resistance	
Required for antimicrobial susceptibility categorisation	Yes
Infection control	No
Public health	Yes

8.1 Definition

S. pneumoniae isolates with reduced susceptibility (non-wild-type MICs) to penicillin due to the presence of modified penicillin binding proteins (PBPs) with lower affinity to β-lactams.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Zone diameter (mm) with oxacillin (1µg)	Antimicrobial agents	Further testing and/or interpretation
≥ 20 mm	All beta-lactam agents for which clinical breakpoints are listed (including those with "Note")	Report susceptible irrespective of clinical indication.
< 20 mm*	Benzylpenicillin (meningitis) and phenoxymethylpenicillin (all indications)	Report resistant.
	Ampicillin and amoxicillin (without and with beta-lactamase inhibitor), cefepime, cefotaxime and ceftriaxone	Oxacillin zone diameter ≥ 8 mm: Report susceptible.
		Oxacillin zone diameter < 8 mm: determine the MIC of the beta-lactam agent intended for clinical use but for ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor) infer susceptibility from the MIC of ampicillin.
	Other beta-lactam agents (including benzylpenicillin for infections other than meningitis)	Test by an MIC method for the agent considered for clinical use and interpret according to the clinical breakpoints

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Conclusions

- Pan-European guidelines are soon to be available
- Scrutiny and constructive feedback from national methodology committees is pivotal to ensure that the guidelines are improved over time
- My prediction: there will be a need for this work also beyond January 2013
- European standardization may be helpful for EARS-Net, but hopefully even more so for laboratories, patients and infection control