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EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
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EUCAST Subcommittee for Detection of Resistance Mechanisms (ESDReM)

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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

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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 that have been subject to wide consultation via established EUCAST procedures and ECDC focal points

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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

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Timeline

- February 2012: recruiting of members
- April 2012 (at ECCMID): first (and only) meeting, sharing the work between members
- December 2012: open consultation (one month)
- January 2013: consultation closes
- February-April 2013: revision of the document
- May 2013: final consultation
- June 2013: revision
- July 2013: approval by the steering committee

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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)

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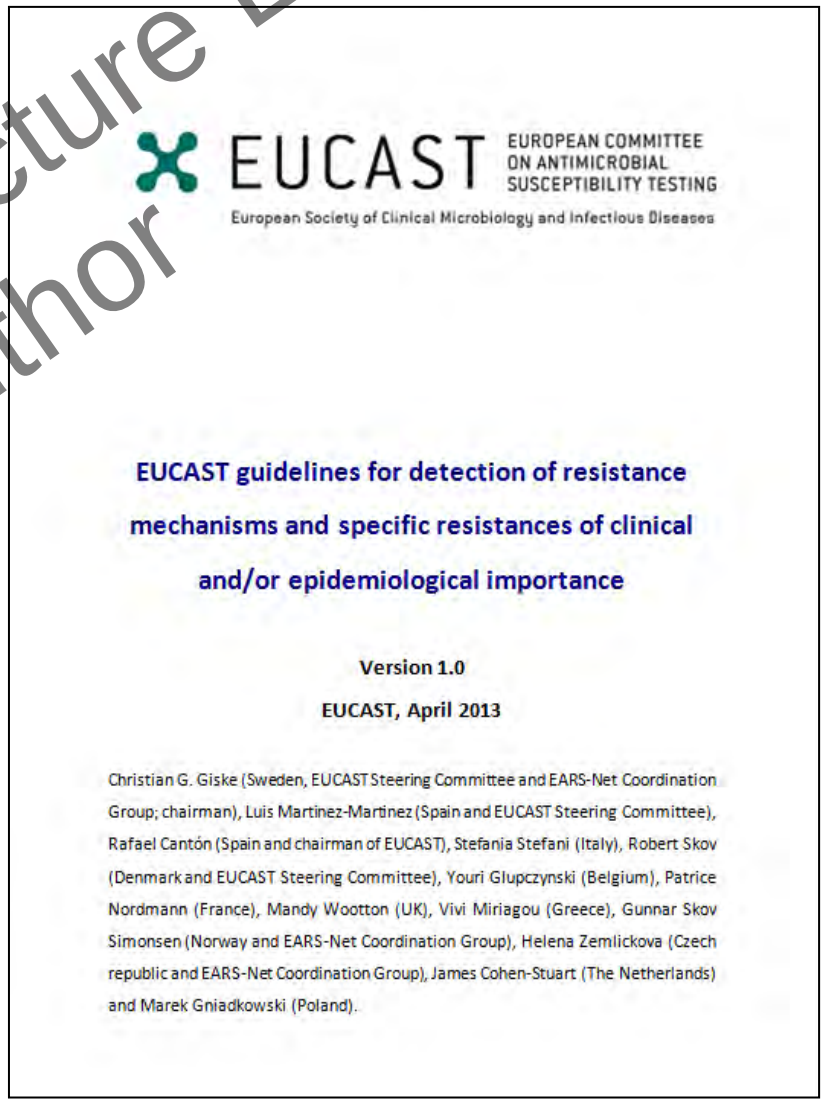
The guidelines



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EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

Version 1.0

EUCAST, April 2013

Christian G. Giske (Sweden, EUCAST Steering Committee and EARS-Net Coordination Group; chairman), Luis Martinez-Martinez (Spain and EUCAST Steering Committee), Rafael Cantón (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 Coordination Group), Helena Zemlickova (Czech republic and EARS-Net Coordination Group), James Cohen-Stuart (The Netherlands) and Marek Gniadkowski (Poland).

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).

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Q1 and Q2

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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.

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Q3

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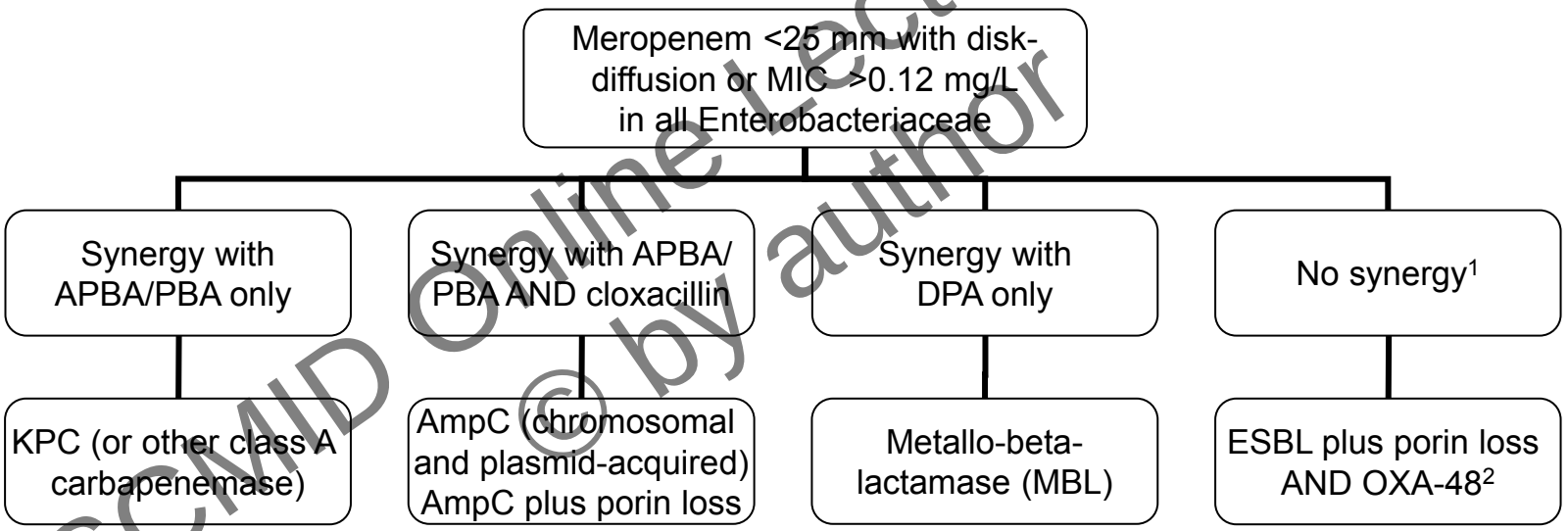
Phenotypic confirmation methods



[1] Combination of KPC and MBL can also produce no synergy. Normally these isolates will have very high resistance levels to carbapenems. They are easiest to detect with molecular methods.

[2] High-grade temocillin resistance (>32 mg/L, temocillin (30 µg) zone diameter ≤11 mm) is a phenotypic indicator of OXA-48.

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).

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When should ESBL-testing be done?

Method	Antibiotic	Conduct ESBL-testing if
Broth or agar dilution	Cefotaxime	MIC > 1 mg/L
	Ceftazidime	MIC > 1 mg/L
	Cefpodoxime	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
	Cefpdoxime (10 µg)	Inhibition zone < 21 mm
Automated systems	Cefotaxime	MIC > 1 mg/L
	Ceftazidime	MIC > 1 mg/L

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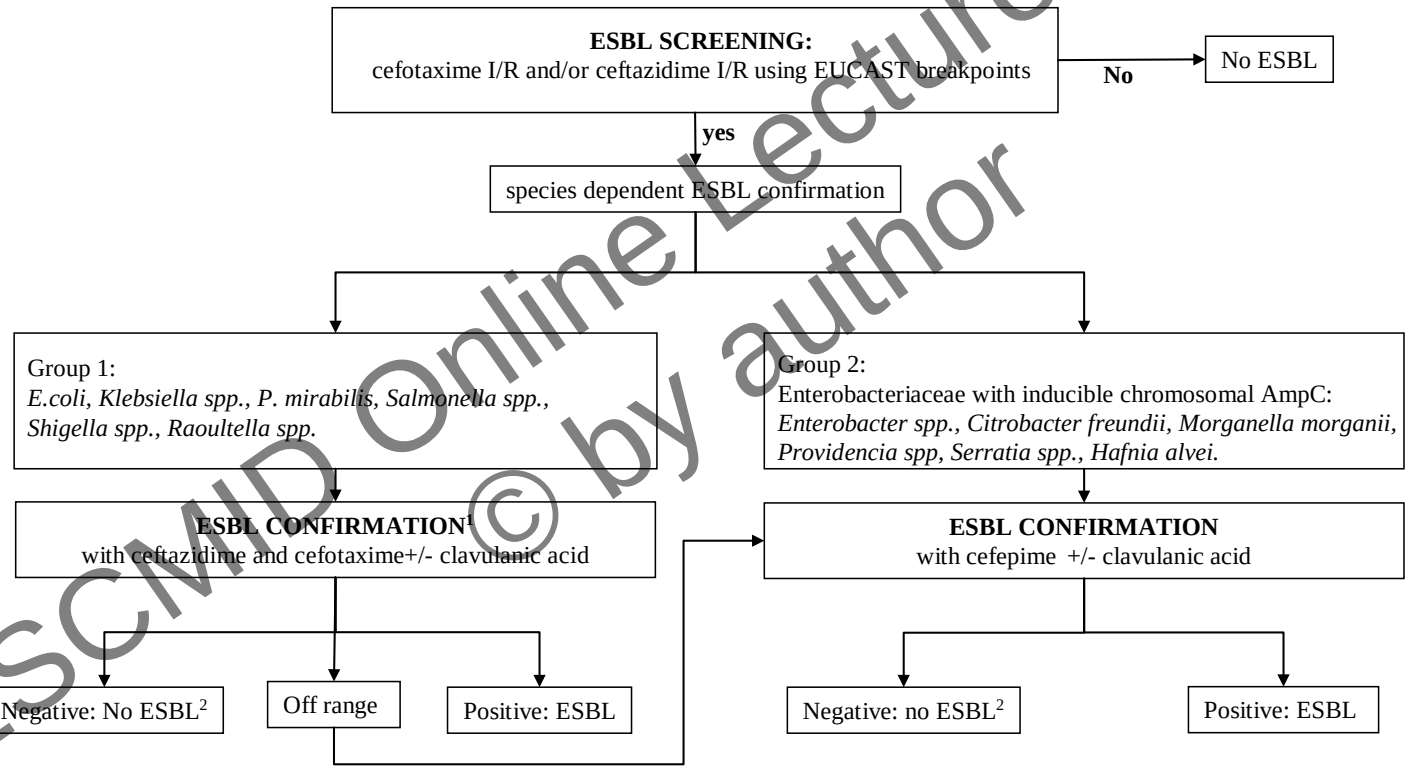

1: If cefoxitin MIC > 8 mg/L, perform cefepime+/- clavulanic acid confirmation test

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

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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

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Q4

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.

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.

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Q5 and Q6

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.

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Q7

	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.

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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 July 2013
- European standardization may be helpful for EARS-Net, but hopefully even more so for laboratories, patients and infection control