EURL-PH-AMR

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Document overview

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Authors	Oskar Ekelund, Gunnar Kahlmeter, EDL
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Index

Abbreviations

AST	Antimicrobial susceptibility testing
CRAb	Carbapenem-resistant Acinetobacter baumannii
CRPa	Carbapenem-resistant Pseudomonas aeruginosa
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological cut-off values
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EDL	EUCAST Development Laboratory, Sweden
EURGen-Net	European Antimicrobial Resistance Genes Surveillance Network
EURL-PH-AMR	EU Reference Laboratory for Public Health on Antimicrobial Resistance in Bacteria
MIC	Minimum inhibitory concentration
S	Susceptible microorganism at standard dosing regimen
I	Susceptible microorganism at increased exposure
R	Resistant microorganism

Purpose

The purpose of this document is to describe the phenotypic testing for carbapenem-and colistin-resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* for National Reference Laboratories and National Expert Laboratories in European countries that participate in EURGen-Net. The document also aims to support clinical laboratories in their identification of isolates to include in European-level genomic surveillance activities for *Acinetobacter* spp. and *P. aeruginosa*, particularly in ECDC standardised surveys and outbreak investigations of carbapenem-resistant isolates.

All recommendations in this document are compliant with relevant parts of current EUCAST technical guidance for AST, and are not intended to replace current EUCAST guidance (https://www.eucast.org/ast_of_bacteria).

Protocol 1: EUCAST (European Committee on Antimicrobial Susceptibility Testing) – disk diffusion for carbapenem susceptibility testing

For more detailed information, consult the regularly updated EUCAST Manuals available at https://www.eucast.org/ast_of_bacteria and in EUCAST breakpoint tables (https://www.eucast.org/clinical_breakpoints) for respective species and the instructive videos organised by EUCAST with subtitles in several languages (https://www.eucast.org/videos_and_online_seminars).

Preparation of media. Prepare Mueller-Hinton (MH) agar according to manufacturer's instructions and EUCAST recommendations.

Preparation of inoculum. Prepare the inoculum according to EUCAST instructions and recommendations for *A. baumannii* and *P. aeruginosa*.

Inoculation of agar plates. Use the adjusted inoculum suspension within 15 minutes of preparation in accordance with EUCAST instructions and apply disks within 15 minutes.

Application of antimicrobial disks. Apply in accordance with EUCAST instructions.

Incubation of plates. Incubate plates within 15 minutes of disk application.

Examination of plates after incubation. A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth.

Measurement of zones and interpretation of susceptibility. For all agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye. For clinical categorization S, I and R, interpret zone diameters by reference to breakpoint tables: http://www.eucast.org/clinical_breakpoints (summarised in table 1).

When carbapenem-resistance is detected by phenotypic methods, report according to EUCAST recommendations and when appropriate, characterize further using sequencing or other available tests (e.g. lateral flow tests).

Protocol 2: EUCAST – broth microdilution for carbapenem susceptibility testing

For more information, consult the EUCAST recommendation for broth microdilution and media preparation at https://www.eucast.org/ast_of_bacteria.

Minimum inhibitory concentration (MIC) determination is performed by broth microdilution according to ISO standard 20776-1. The medium, inoculum, incubation and reading of plates is evident from EUCAST. Quality control: *P. aeruginosa* ATCC 27853.

For a complete and yearly updated list of breakpoints and screening recommendations, consult the EUCAST breakpoint table at http://www.eucast.org/clinical_breakpoints.

For updates of MIC and zone diameter ECOFFs, consult the MIC and zone distribution programme at https://mic.eucast.org.

Pseudomonas aeruginosa and carbapenem resistance

EUCAST recommends the use of meropenem for screening for carbapenem resistance. In contrast to in Enterobacterales, ertapenem cannot be used due to insufficient activity against *Pseudomonas* spp. The S/I breakpoint (MIC and/or disk diffusion) corresponds to the ECOFF and will therefore screen for all epidemiologically interesting resistance (table 1). Commercial semi-automated devices running on clinical breakpoints should therefore be able to detect isolates of epidemiological interest.

Table 1. EUCAST breakpoints for *P. aeruginosa* and carbapenems (v 15.0, 2025).

Carbapenems	Clinical breakpoints MIC (mg/L)		Clinical breakpoints zone diameter (mm)	Cutoff values for screening for carbapenem resistance (ECOFFs)	
	S ≤	R >	S ≥ / R <	Meropenem MIC (mg/L)	Meropenem disk diffusion (10 μg)
Meropenem (other than meningitis)	2	8	20 / 14	> 2 mg/L	< 20 mm
Meropenem (meningitis)	2	2	20 / 20		
Imipenem	0.001	4	50 / 20	N/A	

Acinetobacter baumannii and carbapenem resistance

EUCAST recommends the use of meropenem for screening for carbapenem resistance. In contrast to in Enterobacterales, ertapenem cannot be used due to insufficient activity against Acinetobacter spp. The S/I breakpoint (MIC and/or disk diffusion) corresponds to the ECOFF and will therefore screen for all epidemiologically interesting resistance (table 2). Commercial semi-automated devices running on clinical breakpoints should therefore be able to detect isolates of epidemiological interest.

Table 2. EUCAST breakpoints for *A. baumannii* and carbapenems (v 15.0, 2025).

Carbapenems	Clinical breakpoints MIC (mg/L)		Clinical breakpoints zone diameter (mm)	Cutoff values for screening for carbapenem resistance (ECOFFs)	
	S ≤	R >	S ≥ / R <	Meropenem MIC (mg/L)	Meropenem disk diffusion (10 μg)
Meropenem (other than meningitis)	2	8	21 / 15	> 2 mg/L	< 21 mm
Meropenem (meningitis)	2	2	21 / 21		
Imipenem	2	4	24 / 21	N/A	

Protocol 3: EUCAST – colistin broth microdilution testing

For more information, consult the EUCAST recommendation for broth microdilution and media preparation at https://www.eucast.org/ast_of_bacteria/mic_determination and the specific document related to colistin testing (Recommendations_for_MIC_determination_of_colistin_March_2016.pdf).

EUCAST recommendation

Reference testing of *P. aeruginosa* and *A. baumannii* follows the EUCAST recommended ISO-standard broth microdilution method (20776-1). Disk diffusion and gradient tests are not relevant for testing Cation-adjusted MH broth is used. Additives shall not be included in any part of the testing process (do not include polysorbate-80 or other surfactants). Trays must be made of plain polystyrene and not treated in any way before use. Sulphate salts of polymyxins must be used (the methanesulfonate derivative of colistin must not be used - it is an inactive pro-drug that breaks down slowly in solution).

The testing procedure is available on the EUCAST website.

Quality control: *P. aeruginosa* ATCC 25922 and for colistin, add *E. coli* NCTC 13846 with a colistin MIC target value of 4 mg/L (values should mostly be 4 mg/L, but may occasionally be 2 or 8 mg/L). For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables (https://www.eucast.org/ast_of_bacteria/quality_control).

For a complete and yearly updated list of breakpoints and screening recommendations, consult the EUCAST breakpoint table at http://www.eucast.org/clinical_breakpoints.

For updates of MIC and zone diameter ECOFFs, consult the MIC and zone distribution programme at https://mic.eucast.org.

Colistin resistance in *P. aeruginosa* and *A. baumannii*

EUCAST clinical colistin breakpoints are identical to the respective ECOFFs (*P. aeruginosa 4 mg/L* and *A. baumannii* 2 mg/L, see table 3 and 4). The sensitivity for detection of colistin resistance using clinical breakpoints is therefore absolute. Provided the phenotypic test method is properly controlled (see https://www.eucast.org/ast_of_bacteria) it is not necessary to confirm the phenotypic result by genome sequencing. However, the latter may be deemed necessary for epidemiologic reasons.

For other colistin phenotypic test methods, consult the EUCAST Warning No. 3 (https://www.eucast.org/ast-of-bacteria/warnings) which in summary excludes gradient test and disk diffusion as valid methods.

Table 3. EUCAST MIC breakpoints for *P. aeruginosa* and colistin (v 15.0, 2025)

	MIC breakpoints (mg/L)	
	S ≤	R >
Colistin	4	4

Table 4. EUCAST MIC breakpoints for *A. baumannii* and colistin (v 15.0, 2025)

	MIC breakpoints (mg/L)	
	S ≤	R >
Colistin	2	2

General aspects on alternative testing and therapy for carbapenem resistant, often multidrug-resistant, *P. aeruginosa* and *A. baumannii*

P. aeruginosa and *A. baumannii* resistant to carbapenems are often resistant also to many other agents. The efficiency of colistin is considered poor, also when the organism is formally susceptible. The agent is now only recommended as part of combination therapy. Nevertheless, in some settings, colistin remains the only available agent.

In recent years, several new beta-lactam antibiotics active against *P. aeruginosa* have been introduced to the market, including ceftazidime-avibactam, ceftolozane-tazobactam, cefiderocol, aztreonam-avibactam, imipenem-relebactam and meropenem-vaborbactam. Many of these agents are potentially useful for treatment of otherwise multi-resistant bacteria and more agents are under development. For *A. baumannii*, there are fewer alternative betalactam agents, even though sulbactam-durlobactam (under development) seems promising.

EUCAST has issued testing recommendations for most of these agents and the EURL-PH-AMR, can offer help in the form of advice (at eurl@kronoberg.se) on susceptibility testing when needed.

Breakpoints, Expected phenotypes and Expert rules.

Pertinent and additional information may be found in breakpoint tables, tables of expected phenotypes and expert rules related to specific species or agents.

Assessing the needs for future amendments

Breakpoints and cut-off values for screening for resistance are under regular review by EUCAST. As of 3 December 2025, the committee does not plan to change breakpoints or screening cut-off values for carbapenems, carbapenems with inhibitor molecules, or colistin, in either of the species discussed in this document.