Funded by the European Union

Gran Agreement Number: EURL-PH-AMR - 101194806 - GAP-101194806

Project Title: EURL for public health in the field of Antimicrobial Resistance (AMR) in bacteria

Project Acronym: EURL-PH-AMR

EU4Health Programme

Protocol for the 2025 External quality assessment (EQA) exercise of performance of laboratories participating in European Antimicrobial Resistance Surveillance Network (EARS-Net)

Version n°: 1.0

Date: March 2025

OVERVIEW OF THE MOST IMPORTANT INFORMATION IN THIS PROTOCOL This table does not replace reading the full protocol.		
Strains included in this EQA	Six strains from bloodstream infections.	Page 5, 8
Handling instructions	Subculture and process the strains within 48 hours from receipt.	Page 6
Storage of strains	Freeze at -80° C for future analysis.	Page 6
Sharing of strains	Strains cannot be re-distributed by the recipient laboratories.	Page 6
Safety instructions	Strains are UN3373, Biological substance, category B. Handle in BSL2 facility.	Page 7
Antimicrobials included in this EQA	All included in ECDC AMR reporting protocol 2025.	Page 8 Annex 1
Recommendations for performing AST	Use the routine AST methods applied in your laboratory. Follow the most current EUCAST guidelines. Broth microdilution is the recommended method for most antimicrobials. Disk diffusion is the recommended method for cefiderocol (all species); cefoxitin and norfloxacin (*S. aureus*); oxacillin and norfloxacin (*S. pneumoniae*).	Page 8-10 Annex 1
Rules for reporting AST results	Follow the most current EUCAST guidelines. Report S/I/R results for all antimicrobials including screening agents. Do not use results from one antimicrobial to report results for other antimicrobial(s). For reporting results of gentamicin in *Enterococcus* spp. use: not-HLAR=S; HLAR=R.	Page 9-10
Link to the EARS-Net EQA website with the protocol, the test forms and the instructions for the webtool	https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net	Page 10
Link to the EARS-Net EQA webtool for submission of results	https://earsnet.eqa.dtu.dk	Page 10
Username and password for the webtool	All email addresses registered to each laboratory will receive an email with a link to the webtool, a username, and a description of how to create a webtool password.	Page 10
Deadline for submission of results	**4 August 2025**	Page 5, 10
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1 INTRODUCTION

Since 2005, the European Antimicrobial Resistance Surveillance Network (EARS-Net) has provided analyses of trends in antimicrobial resistance over time and between all European Union (EU) Member States and two European Economic Area (EEA) countries (Iceland and Norway). Data are based on routine antimicrobial susceptibility testing (AST) results collected from a network of clinical laboratories. At present, the pathogens included in the surveillance network are *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. EARS-Net is coordinated by the European Centre for Disease Prevention and Control (ECDC).

Participation in External Quality Assessment (EQA) exercises promotes production of reliable laboratory results and compliance with ISO 15189:2022 (Medical laboratories — Requirements for quality and competence) or ISO 17025:2017 (General requirements for the competence of testing and calibration laboratories) and provides important information on performance and comparability of the reported test results between participating laboratories and countries.

2 SCOPE AND OBJECTIVES

The scope of the EARS-Net EQA exercise is to provide external quality assessment of AST, for the microorganisms included in EARS-Net surveillance, to all clinical laboratories that have or intend to participate in EARS-Net surveillance.

The overall objectives are to assess the accuracy of AST results reported by participating individual clinical laboratories, and to evaluate the overall comparability of test results between laboratories and EU/EEA Member States.

These annual EQA exercises provide important information on the accuracy of AST, and the comparability of the AST test results reported to EARS-Net, by participating laboratories and countries. Therefore, the laboratory practices for this EARS-Net EQA should be the same as the AST method(s) routinely used in the participating laboratory, i.e. automated systems, broth microdilution, disk/tablet diffusion, gradient diffusion, or other method(s).

3 ELIGIBILITY CRITERIA FOR PARTICIPATION

Laboratories are eligible to participate in the 2025 EARS-Net EQA exercise if they provide data following the EUCAST guidelines, and they either reported annually to EARS-Net and/or they intend to report 2025 data to EARS-Net.

4 OUTLINE OF THE EQA 2025

The processes for the 2025 EARS-Net EQA exercise are the same as for the 2024 EARS-Net EQA exercise.

4.1 Overview of the 2025 EARS-Net EQA process

In 2025, the EARS-Net EQA exercise will take place in June-August. Laboratories are requested to identify the species of the six provided strains, and to report AST results for the bacterial strains covered by the EARS-Net surveillance, using the routine method(s) in their settings, through a password-protected webtool. Results must be submitted no later than **4 August 2025**.

After the submission deadline, DTU Food, in their capacity as a consortium member of the European Union Reference Laboratory for public health in the field of Antimicrobial Resistance in bacteria (EURL-PH-AMR) validates all received data, with scores assigned to every submitted AST result according to the scoring system described in this protocol (see section 8). Participating laboratories will be informed by email when their evaluation report is available for download in the password-protected webtool.

Each year, the ECDC National Focal Point for Antimicrobial Resistance (AMR) in each EU/EEA country nominates a 'National EQA Coordinator' (NEC) for that year's EARS-Net EQA exercise. NECs support coordination of the EARS-Net EQA in their country and receive a copy of each individual evaluation report, for every participating laboratory in their country. Subsequently, NECs receive a national report that includes summary conclusions on the capacity of participating laboratories in their country for AST, and, if relevant, recommendations for improvement. The Appendix to the national report includes the results from all participating laboratories in their country.

In 2026, ECDC will publish an annual report, prepared with EURL-PH-AMR, summarising the results from every participating laboratory, with each laboratory anonymised.

4.2 Shipping, receipt, and initial processing of strains

For the 2025 EARS-Net EQA, all participating laboratories will receive a parcel containing six swabs from the NEC. Each swab contains a pure culture of a bacterial isolate.

Please inspect packages for evidence of breakage and leakage. If this is evident, discard by autoclaving, and contact the DTU Food to request a replacement package and inform the NEC by email. Upon receipt of the parcel at the laboratory, open the parcel as soon as possible to confirm the contents. The contents are six swabs, each with different identification (2025 EARS-Net 1, 2025 EARS-Net 2, 2025 EARS-Net 3, 2025 EARS-Net 4, 2025 EARS-Net 5, 2025 EARS-Net 6).

Store the swabs in a dark place at 5°C to 25°C until microbiological analysis.

We suggest that you subculture and process the strains within 48 hours from receipt of the parcel. Subculture the test strains onto non-selective media, e.g. a nutrient agar plate or blood agar plate, as illustrated in Figure 1:

1) Inoculate it on one side of the agar plate using the swab to apply material gently and densely;
2) Turn the plate and use a sterile loop to streak once through the area first inoculated, and allow further streaks to separate the culture, aiming to obtain single colonies;
3) Turn the plate and use a sterile loop to streak once through the second area inoculated, and allow further streaks to separate the culture, aiming to obtain single colonies.

It is recommended to store the strains in your strain collection (e.g. in a -80°C freezer), at least until you have reviewed your results from this EQA exercise. This will allow for repetition of species identification and AST, if needed, in light of your individual performance.

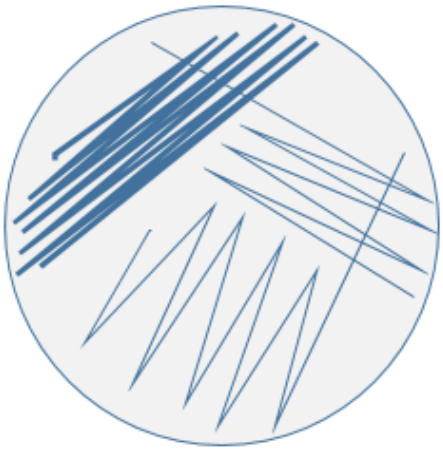

Figure 1: Plating of the test strains

Kindly note, due to the Material Transfer Agreements (MTAs) between DTU and the original providers of the isolates:

1. Strains received for the 2025 EARS-Net EQA cannot be re-distributed further by the recipient laboratories.
2. It is not possible for DTU or NECs to distribute strains to laboratories after the EQA exercise, e.g. for confirmatory, training, or reference purposes.

4.3 Safety instructions

All strains used in this iteration of the EARS-Net EQA are categorized as UN3373, Biological substance, category B. The EQA strains could potentially pose a risk to humans due to their resistance profile and pose a challenge in the treatment of a potential human infection.

Note that it is the responsibility of the recipient laboratory to comply with national regulations and guidelines regarding the correct handling of the provided bacterial cultures and to make use of the proper facilities, equipment, and protocols to handle these strains.

It is recommended to work with the strains in a BSL2 containment facility, using equipment and operational practices for work involving infectious or potentially infectious materials, and take the necessary precautions. It is recommended to wear protective clothing such as lab coat as well as gloves when direct skin contact with infected material is unavoidable. Eye protection must be used where there is a known or potential risk of exposure to splashes. Moreover, all procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited.

4.4 Antimicrobial susceptibility testing

4.4.1 Derivation of consensus results

As in 2021–2024, in order to permit the scoring of AST results reported by participating laboratories (see Table 1), the EURL-PH-AMR defined the expected AST results for each strain. These were the consensus of AST results from three reference laboratories. The laboratories were DTU Food (performed in triplicate); the EUCAST Development Laboratory, Sweden and the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), The Doherty Institute, Australia. All reference laboratories used the same AST methodology. Specifically, the expected minimum inhibitory concentration (MIC) values for each strain-antimicrobial combination, and their respective interpretation, were determined by broth microdilution and use of EUCAST clinical breakpoints tables v15.0 (https://www.eucast.org/clinical_breakpoints). The consensus AST results were reviewed and validated by ECDC and the ECDC EARS-Net Disease Network Coordination Committee.

After the AST by the three reference laboratories, the EURL-PH-AMR performed whole-genome sequencing and bioinformatics analyses of each EQA strain, to detect relevant acquired AMR genes and chromosomal point mutations.

During preparation of the test swabs for distribution, the EURL-PH-AMR performed confirmatory phenotypic testing of the test strains by broth microdilution, using Sensititre panels.

4.4.2 Instructions for participating laboratories

Participating laboratories should perform AST according to the laboratory's routine procedures, i.e. automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient diffusion, or other methods.

Apply the most recent EUCAST clinical breakpoints for the interpretation of the obtained AST results (https://www.eucast.org/clinical_breakpoints/). This allows for categorisation of the test results into the categories resistant (R), susceptible, increased exposure (I), and susceptible, standard dosing regimen (S).

All isolates included in the 2025 EARS-Net EQA should be considered as being obtained from patients with a bloodstream infection.

> ***Note:*** *if using gradient tests, the obtained MIC values might not refer directly to a two-fold dilution concentration. In this scenario, please be advised to round up the values to the nearest upper two-fold dilution value, to ensure the correct evaluation of the obtained results. For example, an MIC of "0.75 mg/L" according to a gradient test should be reported as "1 mg/L".*

5 INCLUDED ANTIMICROBIAL AGENTS

All organism-antimicrobial combinations under surveillance in EARS-Net are included in the 2025 EARS-Net EQA exercise. These organism-antimicrobial combinations are listed in Annex 1 of this document, which is adapted from Table 8 of the EARS-Net reporting protocol[1].

> ***Note:*** *the list in Annex 1 is more inclusive than the panel of antimicrobials likely to be tested by a clinical microbiological laboratory during normal clinical practice. Laboratories that do not test the full panel of antimicrobials are still eligible to participate in the 2025 EARS-Net EQA and can report partial data.*

In this EQA, the organism-antimicrobial combinations are not ranked by their level of importance to clinical practice, because there are no definitive criteria for ranking that are appropriate or applicable for all countries that are eligible to participate in this EQA.

[1] The EARS-Net surveillance protocol is available from the webpage: https://www.ecdc.europa.eu/en/publications-data/reporting-protocol-antimicrobial-resistance-amr

6 REPORTING AST RESULTS FOR THIS EQA EXERCISE

AST results can be reported for all organism-antimicrobial combinations included in this EARS-Net EQA exercise (see section 5 and Annex 1).

To report AST results, we recommend that you follow these sequential steps:

1. Carefully read the instructions for the webtool in Section 7 below;
2. First write your results on the 'test forms' for this EQA (available from: https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net).
3. Transfer your results from the test forms into the webtool, and submit your results (see Section 7). **Results must be submitted in the web tool no later than 4 August 2025.** The webtool allows you to view and print a report that contains your reported AST results.

As in 2024, **the 2025 EARS-Net EQA exercise permits laboratories to report AST results for organism-antimicrobial combinations that might not be directly reported by clinical laboratories during routine procedures.** Table 1 provides the description of these situations.

Table 1. Notes for reporting AST results in the EARS-Net EQA 2025, by eligible pathogen

Pathogen	Notes for reporting
Enterococcus spp.	- For penicillins (amoxicillin and ampicillin), assume that intravenous administration will take place. - Amoxicillin should be tested, regardless of results obtained for ampicillin. - For gentamicin, assume administration in combination with penicillins or glycopeptides. - For gentamicin, report isolates not presenting high-level aminoglycoside resistance (HLAR) as susceptible (S). Report isolates presenting HLAR as resistant (R).
Escherichia coli and *Klebsiella pneumoniae*	- For penicillins (amoxicillin and ampicillin), assume that intravenous administration will take place. - Amoxicillin should be tested, regardless of results obtained for ampicillin. - For colistin and aminoglycosides (amikacin, gentamicin and tobramycin), assume administration in combination with other agents. - Breakpoints currently based on ECOFF values can be used for interpretation of results, when applicable, if no other relevant EUCAST clinical breakpoints exist. - Currently, EUCAST recommends using disk diffusion for testing of cefiderocol, but only after consulting the EUCAST Warnings! page (Warning 12) about certain media and disks (https://www.eucast.org/ast-of-bacteria/warnings).
Pseudomonas aeruginosa and *Acinetobacter* spp.	- For colistin and aminoglycosides (amikacin, gentamicin and tobramycin), assume administration in combination with other agents. - Breakpoints currently based on ECOFF values can be used for interpretation of results, when applicable, if no other relevant EUCAST clinical breakpoints exist.

	- Currently, EUCAST recommends using disk diffusion for testing of cefiderocol, but only after consulting the EUCAST Warnings! page (Warning 12) about certain media and disks (https://www.eucast.org/ast-of-bacteria/warnings).
Staphylococcus aureus	- Oxacillin, cefoxitin and norfloxacin results should all be reported, regardless of their EUCAST status as 'screen only'. - Oxacillin and cefoxitin should both be tested. - All fluoroquinolone antimicrobials (ciprofloxacin, levofloxacin and norfloxacin) should be tested, regardless of results obtained for norfloxacin. - Breakpoints currently based on ECOFF values can be used for interpretation of results, when applicable, if no other relevant EUCAST clinical breakpoints exist.
Streptococcus pneumoniae	- Oxacillin and norfloxacin results should be reported, regardless of their EUCAST status as 'screen only'. - All β-lactam antimicrobials (penicillin, oxacillin, cefotaxime and ceftriaxone) should be tested, regardless of results obtained for penicillin and oxacillin. - All macrolide antimicrobials (azithromycin, clarithromycin and erythromycin) should be tested, regardless of results obtained for erythromycin. - All fluoroquinolone antimicrobials (levofloxacin, moxifloxacin and norfloxacin) should be tested, regardless of results obtained for norfloxacin.

7 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

7.1 Login to the webtool

All email addresses registered by the NEC will receive an email from earsnet-eqa@food.dtu.dk containing a link to the webtool, a webtool username, and a description of how to create a webtool password. Each laboratory can have more than one registered email address.

Contacts that participated in the previous EARS-Net EQA, provided by DTU, will have the same username as in previous years.

Users wishing to reset the webtool password may also consult the document 'User guide to reset the EARS-Net EQA webtool password' available on the EARS-Net EQA website (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net).

7.2 Submitting results in the webtool

The '2025 EARS-Net EQA Webtool guide' is available for download directly from the EARS-Net EQA website (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net). Please follow the guide carefully.

Before submitting your input for each of the strains, please ensure that you have filled in all the relevant fields as **you can only submit once per strain. Clicking on the button 'Final submit' blocks further data entry.**

Note: *Final submission must be performed for each strain individually*.

8 EVALUATION OF SUBMITTED EQA RESULTS

8.1 Scoring system

The webtool for the 2025 EARS-Net EQA will apply the same scoring system that was used in 2024 EARS-Net EQA.

During the first step in the EQA, if the species of the isolate is identified correctly, then the interpretation of AST results will be evaluated using the scoring system. Conversely, if the species is not identified correctly, the AST results for that isolate will not be evaluated further.

During the second step, the scoring system assesses the reported interpretations of AST results.

The scoring system considers the 'level of difficulty' and 'severity of error' of every permitted organism-antimicrobial combination. The level of difficulty, classified as 'Difficult' or 'Easy', reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table. The scoring of a result reflects the level of difficulty, with errors in 'difficult' results being considered mild and errors in 'easy' results being considered severe.

The severity of error is divided into three levels: very major error (VME), major error (ME) and no error. VME is reporting false susceptibility – expecting an R but obtaining an S or I. ME is reporting false resistance – expecting an S or I but obtaining an R. The scoring system penalises VMEs more severely for 'easy' results than for 'difficult' results, and does not penalise MEs if the test is considered 'difficult'. The classification of 'no error' includes situations where one susceptibility category (S or I) is expected, but the other susceptibility category is reported. However, this results in a lower score than if the expected susceptibility category is reported.

Table 2 shows the 2025 EARS-Net EQA scoring system

The scoring system does not rank or group organism-antimicrobial combinations by their level of importance to clinical practice. This is because there are no definitive criteria for ranking or grouping that are appropriate and applicable to all participating countries. Such analyses can be performed (sub-)nationally using the database of national results that is sent to NECs.

Table 2. Scoring system of the 2025 EARS-Net EQA exercise

		Difficulty of result and expected interpretation					
		Easy			Difficult		
		R	I	S	R	I	S
Obtained interpretation	R	1	-3 (ME)	-3 (ME)	4	0 (ME)	0 (ME)
	I	-4 (VME)	1	-1	-1 (VME)	4	2
	S	-4 (VME)	-1	1	-1 (VME)	2	4
	Not reported	-	-	-	-	-	-

Legend: R: resistant; I: susceptible, increased exposure; S: susceptible, standard dosing regimen; ME: major error; VME: very major error; - : no data.

8.2 Laboratory feedback reports

By October 2025, the laboratory contact person(s) will receive an email stating that an evaluation report for their laboratory, including the score, is available in the password-protected webtool for download. This report will also be shared with the NEC for their country. The reports contain scores for every organism-antimicrobial combination that can be reported.

Upon receipt of the evaluation report, laboratories are recommended to review the score for each organism-antimicrobial combination individually.

Laboratory feedback reports do not provide the total score (i.e. the sum of scores for every organism-antimicrobial combination listed in Annex 1). This is because total scores will only be relevant to the small subset of participating laboratories that are expected to provide, as standard practice, AST for all of the organism-antimicrobial combinations in Annex 1.

> ***Note:*** *We encourage all participating laboratories to perform a self-evaluation regarding the accuracy, adequacy and reliability of the AST methods and procedures used, assessing if there is need for corrective actions.*

8.3 Data sharing

Participating laboratories will receive data for their laboratory in the laboratory feedback reports (see section 8.2).

NECs will receive copies of laboratory feedback reports for every participating laboratory in their country, and a national-level report containing EQA results and recommendations for their country.

ECDC will also receive the national-level reports and EQA results for all participating laboratories, but without any laboratory identifiers, except for an anonymised laboratory codes and the country name. In 2026, ECDC will publish an annual report summarising 2025 EARS-Net EQA results from all participating laboratories.

9 CONTACT

If you would like any support, or have any questions or suggestions, please do not hesitate to contact the EARS-Net EQA management team by e-mail earsnet-eqa@food.dtu.dk. In your communication with the EARS-Net EQA management team, please write in English. If your laboratory is encountering an issue entering results, or accessing the webtool, please provide a description of the issue sufficient to ensure efficient follow-up.

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ANNEX 1 - Antimicrobials included in the 2025 EARS-Net EQA

Adapted from Table 8 of the EARS-Net reporting protocol 2025: 'Microorganism and antimicrobial agent combinations under surveillance by EARS-Net (isolates from blood and/or cerebrospinal fluid)' (Available at: https://www.ecdc.europa.eu/en/publications-data/reporting-protocol-antimicrobial-resistance-amr). As indicated in the text preceding that table, "*According to the EUCAST guidelines, when a specific type of test is to be used, the method is indicated next to the antimicrobial.*"

Microorganism	Antimicrobial agent
***Acinetobacter* species (ACISPP)**	Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
***Enterococcus faecalis* (ENCFAE) and *Enterococcus faecium* (ENCFAI)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
***Escherichia coli* (ESCCOL)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (CZA) Ceftriaxone (CRO) Cefepime (FEP) Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Ertapenem (ETP) Tigecycline (TGC) Aztreonam-avibactam (AZA) Colistin (COL) - Broth microdilution
***Klebsiella pneumoniae* (KLEPNE)**	Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ)

Microorganism	Antimicrobial agent
	Ceftazidime-avibactam (CZA) Ceftriaxone (CRO) Cefepime (FEP) Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Ertapenem (ETP) Aztreonam-avibactam (AZA) Colistin (COL) - Broth microdilution
Pseudomonas aeruginosa **(PSEAER)**	Piperacillin/Tazobactam (TZP) Piperacillin (PIP) Ceftazidime (CAZ) Ceftazidime-avibactam (CZA) Cefepime (FEP) Cefiderocol (FDC) Ceftolozane-tazobactam (CZT) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Colistin (COL) - Broth microdilution
Staphylococcus aureus **(STAAUR)**	Cefoxitin (FOX) – Disk diffusion Oxacillin (OXA)* – MIC test Levofloxacin (LVX) Ciprofloxacin (CIP) Norfloxacin (NOR) – Disk diffusion Vancomycin (VAN) – MIC test Rifampin (RIF) Linezolid (LNZ) Daptomycin (DAP) – MIC test
Streptococcus pneumoniae **(STRPNE)**	Oxacillin (OXA) – Disk diffusion Penicillin (PEN) – MIC test Clarithromycin (CLR) – MIC test Erythromycin (ERY) Azithromycin (AZM) – MIC test Levofloxacin (LVX) Moxifloxacin (MFX) Norfloxacin (NOR) – Disk diffusion Cefotaxime (CTX) – MIC test Ceftriaxone (CRO) – MIC test