Protocol for the External Quality Assessment (EQA) for laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2022

PROTOCOL

1 INTRODUCTION

The European Antimicrobial Resistance Surveillance Network (EARS-Net), formerly known as the European Antimicrobial Resistance Surveillance System (EARSS), provides analyses of trends in antimicrobial resistance over time and between all EU Member States and two 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 administrated and coordinated by the European Centre for Disease Prevention and Control (ECDC).

Participation in External Quality Assessment (EQA) exercise promotes production of reliable laboratory results and compliance with ISO 15189:2012 (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.

In the 2022 EARS-Net EQA exercises, laboratories are requested to identify the species of the strains provided, and antimicrobial susceptibility test results of the bacterial strains covered by the EARS-Net surveillance by the methods routinely used in their settings. The results must be submitted through a password-protected web tool. After the submission deadline and validation of the data , the laboratories will receive an email stating that an evaluation report including the score will be available in the password-protected web tool for download. This report will also be shared with the National EARS-Net EQA Coordinator. Further, the National EARS-Net EQA Coordinator will receive a national summary report of results including a short conclusion on the capacity of participating laboratories and, if relevant, recommendations for improvement. Results from all participating laboratories are included as an Appendix to the national summary report. An annual report summarising results from all participating laboratories (anonymized) will be made publicly available in 2023.

In 2022, the EARS-Net EQA exercise of AST will take place in June-August and will include six bacterial isolates that enable the assessment of data reported as part of EARS-Net surveillance.

2 SCOPE AND OBJECTIVES

The scope of the EARS-Net EQA exercise is to provide external quality assessment for antimicrobial susceptibility testing of the microorganisms included in EARS-Net surveillance to all laboratories participating in EARS-Net.

The overall objectives are to assess the accuracy of quantitative antimicrobial susceptibility test results reported by participating individual laboratories, and to evaluate the overall comparability of routinely collected test results between laboratories and EU/EEA Member States.

The EQA exercise should provide important information on performance of EARS-Net participating laboratories and comparability of the reported test results between laboratories and countries. Thus, the laboratory work for this EARS-Net EQA should be performed using the quantitative or qualitative AST method routinely used in the participating laboratory, i.e. automated systems, broth microdilution, disk/tablet diffusion, gradient diffusion etc.

Results must be submitted no later than 15 August 2022.

3 ELIGIBILITY CRITERIA FOR PARTICIPATION

Laboratories are eligible to participate in the 2022 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 2022 data to EARS-Net.

4 OUTLINE OF THE EQA 2022

4.1 Shipping, receipt, and storage of strains

All participating laboratories will receive a parcel containing six swabs, each containing a pure culture of one of six bacterial isolates that enable the assessment of data reported as part of EARS-Net surveillance, from the National Food Institute, Technical University of Denmark. Please inspect packages for evidence of breakage and leakage and discard by autoclaving if this is evident.

Upon receipt of the parcel at the laboratory, open the parcel as soon as possible to confirm the contents of six swabs with different identification (2022 EARS-Net 1, 2022 EARS-Net 2, 2022 EARS-Net 3, 2022 EARS-Net 4, 2022 EARS-Net 5, 2022 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 prepare the cultures for storage in your strain collection (e.g. in a -80°C freezer) within 48 hours from receipt of the parcel.

We encourage you to store this set of cultures and the original swabs to serve as reference, for example if discrepancies are detected during the testing.

Subculture the test strains onto non-selective media, e.g. a nutrient agar plate or blood agar plate, as illustrated in Figure 1, by:

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.

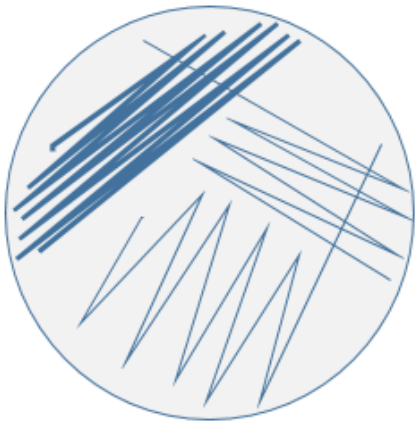

Figure 1: Plating of the test strains

4.2 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, thus, pose a challenge in the treatment of a potential human infection.

Note that it is the recipient laboratory's responsibility 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.

Thus, 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 (BSC). The use of needles, syringes, and other sharp objects should be strictly limited.

Hand washing is a primary safeguard against inadvertent exposure to biological agents. Always wash your hands before leaving the laboratory, even though you use gloves. Wash your hands after removing soiled protective clothing, before leaving the laboratory, and before eating, drinking, smoking, or using a restroom. Wash your hands periodically during the day at intervals dictated by the nature of your work. Wash with soap and running water, with hands held downward to flush

the contamination off the hands. Turn the tap off with a clean paper towel to prevent recontamination and dry your hands with clean towels.

4.3 Antimicrobial susceptibility testing

Participating laboratories should perform a quantitative or qualitative antimicrobial susceptibility test according to the laboratory's routine procedures, i.e. automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient diffusion etc. following EUCAST recommendations (https://www.eucast.org/ast_of_bacteria/).

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

In relation to interpretation of the obtained results,

- Isolates from the genus ***Streptococcus*** should be considered as being obtained from **cerebrospinal fluid** from patients with clinical manifestations suggesting meningitis.

- Isolates from the genera *Staphylococcus, Enterococcus, Escherichia, Klebsiella, Pseudomonas* and *Acinetobacter* should be considered as being obtained from patients with a **bloodstream infection**.

Every strain in this EQA was tested at two internationally recognized reference laboratories using the same methodology. Specifically, the expected minimum inhibitory concentration (MIC) values for each antimicrobial and strain combination, and their respective interpretation, were determined by the consensus results from broth microdilution obtained by DTU Food and EUCAST. Subsequently, during the preparation of the test swabs, the test strains were confirmed by phenotypic testing by broth microdilution using Sensititre panels, and also detection of acquired antimicrobial resistance genes and chromosomal point mutations was achieved by whole-genome sequencing.

Note that, if using gradient tests, the obtained MIC values might not refer directly to a two-fold dilution concentration. Hence, to ensure the correct evaluation of the obtained results, you are advised to round up the values to the nearest upper two-fold dilution value. For example, an obtained MIC of 0.75 mg/L should be reported as 1 mg/L.

5 MANDATORY INCLUSION OF ANTIMICROBIALS

This year, all antimicrobials under surveillance in EARS-Net are included in the EARS-Net EQA exercise. These antimicrobials are considered mandatory for the purposes of this EQA (only). This is a more inclusive list than the panel of antimicrobials that are likely to be tested by a clinical microbiological laboratory during normal clinical practices. Laboratories that do not test the full panel of antimicrobials are still eligible to participate in the 2022 EARS-Net EQA and can report partial data.

These organism-antimicrobial combinations are listed in Table 8 of the EARS-Net reporting protocol[1] and in Annex 1 of this document. The 2022 EARS-Net EQA exercise does not include ofloxacin for *Staphylococcus aureus* nor norfloxacin for *Escherichia coli* and *Klebsiella pneumoniae* isolates, even though they are in the Table 8 of the EARS-Net reporting protocol. This is because they are not included in in the EUCAST Clinical Breakpoints v12.0, or because the breakpoint is only applicable to uncomplicated urinary tract infections.

The scoring system will apply a penalty if no result is provided when a result is expected for an organism-antimicrobial combination. However, there is an exception for missing results for colistin, in the relevant taxa, which will not be penalised.

The laboratory feedback reports will present the score for every organism-antimicrobial combination individually, and will not include a 'total' score, i.e. a sum of all individual scores.

6 REPORTING OF RESULTS

It is expected that results are reported for all organism-antimicrobial combinations included in this EARS-Net EQA exercise. This includes situations that, in routine clinical practice, would not be reported or would be subjected to special conditions, particularly:

- *Streptococcus pneumoniae*
  - Oxacillin results should be reported, regardless of its EUCAST status as 'screen only';
  - All β-lactam antimicrobials should be tested, regardless of results obtained for penicillin and oxacillin;
  - All macrolide antimicrobials should be tested, regardless of results obtained for erythromycin;
  - Norfloxacin results should be reported, regardless of its EUCAST status as 'screen only';
  - All fluoroquinolone antimicrobials should be tested, regardless of results obtained for norfloxacin.

[1] The EARS-Net reporting protocol is available from URL: https://www.ecdc.europa.eu/sites/default/files/documents/EARS-Net-reporting-protocol-2022.pdf

- *Staphylococcus aureus*
  - both oxacillin and cefoxitin should be tested;
  - oxacillin results should be reported, regardless of its EUCAST status as 'screen only';
  - cefoxitin results should be reported, regardless of its EUCAST status as 'screen only';
  - norfloxacin results should be reported, regardless of its EUCAST status as 'screen only';
  - all fluoroquinolone antimicrobials should be tested, regardless of results obtained for norfloxacin.
- *Enterococcus* spp.
  - for penicillins, it should be assumed that intravenous administration will take place;
  - amoxicillin should be tested, regardless of results obtained for ampicillin;
  - for gentamicin, it should be assumed the antimicrobial is going to be administered in combination with penicillins or glycopeptides.
- *Escherichia coli* and/or *Klebsiella pneumoniae*
  - for penicillins, it should be assumed that intravenous administration will take place; amoxicillin should be tested, regardless of results obtained for ampicillin;
  - for aminoglycosides and colistin, it should be assumed that the antimicrobials will be administered in combination with other agents;
  - breakpoints currently based on ECOFF values can be used for interpretation of results, if no others exist, when applicable.
- *Pseudomonas aeruginosa* and *Acinetobacter* spp.
  - for aminoglycosides and colistin, it should be assumed that the antimicrobials will be administered in combination with other agents;
  - breakpoints currently based on ECOFF values can be used for interpretation of results, if no others exist, when applicable.

We recommend that you write your results in the test forms that you can download from the website (https://antimicrobialresistance.dk/ears-net-eqa.aspx) before entering the results into the webtool. Read the description in Section 6 below carefully before entering your results in the web tool. The web tool will allow you to view and print a report with your reported results.

Results must be submitted no later than 15 August 2022.

7 HOW TO SUBMIT RESULTS VIA THE WEB TOOL

7.1 Login to the web tool

- An email with a link to the web tool will be sent to all registered email addresses. A laboratory may have registered more than one email address.
- A separate email with personal **username and password** will afterwards be sent to all registered email addresses. Contacts that participated in the 2021 EARS-Net EQA will receive an email with the same username and password as was provided in 2021.

7.2 Guidelines

The '2022 EARS-Net EQA Webtool guideline' is available for download directly from the EARS-Net EQA website (https://antimicrobialresistance.dk/ears-net-eqa.aspx). Please follow the guideline carefully. Test forms are also available for download from the EARS-Net EQA website.

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

8 EVALUATION OF RESULTS

No later than 2 December 2022, you will receive an email stating that evaluation reports including the score will be available in the password-protected web tool for download. This year a new scoring system will be implemented in the evaluation of interpretated results. The scoring will take "level of difficulty" and "severity of error" into account for each organism-antimicrobial combination.

The level of difficulty will indicate the magnitude of the risk of getting the categorisation wrong. It will consist of two levels: easy and difficult. "Easy" will be results far from the breakpoint, where the categorisation is obvious and therefore the error will be considered severe. "Difficult" will be results that are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added. The categorisation will be difficult and therefore the error will be considered mild. The scoring of a result will reflect the level of difficulty.

The severity of error is divided into three levels: very major error (VME), major error (ME) and no error. Both VME and ME will be penalised. VME is reporting false susceptibility – expecting an R but obtaining an S or I. If the only categories are I and R, then reporting I is also a VME. ME is reporting false resistance – expecting an S or I but obtaining an R. The scoring of a result will reflect the severity of an error.

Moreover, this year a penalty will be given if results on mandatory antimicrobials are omitted. Please see an overview of mandatory antimicrobials in Annex 1. Table 1 shows the scoring system

according to difficulty of result, category of error, and mandatory or not mandatory reporting of antimicrobials.

Table 1. Scoring system of the 2022 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 (mandatory antimicrobials)	-4	-4	-4	-1	-1	-1
	Not reported (colistin)	0	0	0	0	0	0

Note: R= resistant, I= susceptible, increased exposure, S= susceptible, standard dosing regimen; (VME): very major error, (ME): major error.

Upon receipt of the evaluation report, we encourage all participating laboratories to perform a self-evaluation regarding the accuracy, adequacy and reliability of the methods used, and assessing if there is need for corrective actions related to the AST procedures in use. The evaluation reports will also be shared with the National EQA Coordinator. A country summary report presenting data from all participating laboratories will also be shared with the National EQA Coordinator; an anonymous version of the reports with laboratory codes will be shared with ECDC.

A report summarising 2022 EARS-Net EQA results from all participating laboratories will be made publicly available in 2023.

9 CONTACT

Do not hesitate to contact the EARS-Net EQA management team by e-mail earsnet-eqa@food.dtu.dk, explaining any issues you encounter when entering your results or accessing the web tool. In your communication with the EARS-Net EQA management team, please write in English.

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ANNEX 1

Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2022: Microorganism and antimicrobial agent combinations under surveillance by EARS-Net (isolates from blood and/or cerebrospinal fluid). Available at: https://www.ecdc.europa.eu/sites/default/files/documents/EARS-Net-reporting-protocol-2022.pdf

As indicated in the text preceding the table, "*When, according to the EUCAST guidelines, a specific type of test is to be used, the method is indicated next to the antimicrobial*."

Testing of ofloxacin for *Staphylococcus aureus* and testing of norfloxacin for *Escherichia coli* and *Klebsiella pneumoniae* isolates are included in the original table but are not part of the 2022 EARS-Net EQA exercise. This is due to the lack of a breakpoint in the EUCAST Clinical Breakpoints v12.0 or due to the breakpoint only being applicable to uncomplicated urinary tract infections, respectively.

Microorganism	Antimicrobial agent
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
Staphylococcus aureus **(STAAUR)**	Cefoxitin (FOX) – Disk diffusion Oxacillin (OXA)* – MIC test

Microorganism	Antimicrobial agent
	Levofloxacin (LVX) Ciprofloxacin (CIP) Norfloxacin (NOR) – Disk diffusion Vancomycin (VAN) – MIC test Rifampin (RIF) Linezolid (LNZ) Daptomycin (DAP) – MIC test
Enterococcus faecalis **(ENCFAE)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
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) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Tigecycline (TGC)

Microorganism	Antimicrobial agent
	Colistin (COL) - Broth microdilution
***Klebsiella pneumoniae* (KLEPNE)**	Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Colistin (COL) - Broth microdilution
***Pseudomonas aeruginosa* (PSEAER)**	Piperacillin/Tazobactam (TZP) Piperacillin (PIP) Ceftazidime (CAZ) Cefepime (FEP) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
***Acinetobacter* species (ACISPP)**	Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution