Protocol for the External Quality Assessment (EQA) for laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2021

PROTOCOL

For antimicrobial susceptibility testing of *Escherichia coli* and *Klebsiella pneumoniae*

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 susceptibility over time and between all EU Member States and two EEA countries (Iceland and Norway). Data are based on routine antimicrobial susceptibility test (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) exercises is useful for ensuring production of reliable laboratory results of consistently good quality and for meeting the demands of 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 EARS-Net EQA iterations, laboratories are requested to test the bacterial strains by the methods routinely used and the results should then be submitted through a web tool (password-protected) which subsequent to the deadline will generate an evaluation report. Further, all participating laboratories will receive an individual report through the national EQA coordinator together with a national summary report. An EU/EEA summary report (anonymised) will be published on the ECDC website.

In 2021, the EARS-Net EQA exercise of antimicrobial susceptibility testing will take place in June-August and will include three isolates each of the two species, *Escherichia coli* and *Klebsiella pneumoniae*. It is anticipated that the participating laboratories already have access to an original CERTIFIED stock of the *Escherichia coli* reference strains ATCC 25922 for which antimicrobial susceptibility testing should also be performed for quality control purposes.

2 OBJECTIVES

The EARS-Net EQA exercise aims to support laboratories providing surveillance data for the EARS-Net surveillance by assessing the quality and accuracy of the quantitative or qualitative antimicrobial susceptibility test results reported by participating individual laboratories, in the 2021 iteration of the EARS-Net EQA exercise with special focus on *Escherichia coli* and *Klebsiella pneumoniae*. Further, based on the submitted EQA results, the objectives are to evaluate the overall comparability of routinely collected test results between laboratories and EU/EEA countries reported to ECDC by the different EARS-Net laboratories. Thus, the laboratory work for this iteration of the EARS-Net EQA exercise should be performed using the quantitative or qualitative

antimicrobial susceptibility test method routinely used in your own laboratory i.e. automated systems, broth micro dilution, disk/tablet diffusion, gradient-diffusion etc.

3 OUTLINE OF THE EQA 2021

3.1 Shipping, receipt and storage of strains

All laboratories, who have signed-up using the online registration tool to participate in the EARS-Net exercise, will receive a parcel containing six swabs (Amies agar gel with charcoal; Copan Transystem™) each containing a pure culture of one of the two species: 3 cultures of *Escherichia coli* and 3 cultures *Klebsiella pneumoniae* from the National Food Institute, Technical University of Denmark. The content of the swabs can be seen below. Please inspect packages for evidence of breakage and leakage and discard by autoclaving if this is evident.

Code	Microorganism
EARS-NET 2021 EC.1	*Escherichia coli*
EARS-NET 2021 EC.2	*Escherichia coli*
EARS-NET 2021 EC.3	*Escherichia coli*
EARS-NET 2021 KPN.1	*Klebsiella pneumoniae*
EARS-NET 2021 KPN.2	*Klebsiella pneumoniae*
EARS-NET 2021 KPN.3	*Klebsiella pneumoniae*

Upon reception of the parcel at the laboratory, open the parcel as soon as possible to confirm the contents are as listed in the table above. Store the swabs in a dark place at 5°C to 25°C until microbiological analysis.

We suggest that you sub-culture and prepare the cultures for storage in your strain collection (e.g. in a -80°C freezer) within 48 hours from reception 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; e.g. they can be used to detect potential errors.

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) Inoculating it in 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.

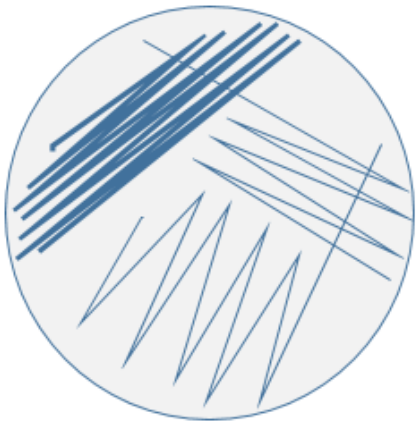

Figure 1: Plating of the test strains

Please note that test strain EARS-NET 2021 EC.3 might show variable degrees of hemolytic activity if cultured on a blood agar plate. The observed variation has been shown not to affect the antimicrobial resistance profile of the strain.

3.2 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. More specifically, the manufacturer of the Copan Transystem™ swabs recommends the following precautions when handling the swabs: *Swab sample processing should be performed inside a protective safety cabinet or protective hood. Protective laboratory clothing and eyeglasses should be worn at all times when processing culture swab samples.*

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 rest room. 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.

Please consult the Pathogen Safety Data Sheets (PSDSs) produced by the Public Health Agency of Canada (PHAC). The PSDSs are technical documents that describe the hazardous properties of human pathogens. Thus, provide recommendations for the work involving these agents in a laboratory setting. These documents have been prepared as educational and information resources for laboratory personnel working with infectious substances:

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/klebsiella.html

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enterohemorrhagic.html

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enteroinvasive.html

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enteropathogenic.html

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enterotoxigenic.html

3.3 Antimicrobial susceptibility testing

Participating laboratories should perform a quantitative or qualitative antimicrobial susceptibility test according to the laboratory's applied 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 EUCAST clinical breakpoints (https://www.eucast.org/clinical_breakpoints/) for the interpretation of the antimicrobial susceptibility test results obtained. 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, consider all isolates as the results of an invasive infection.

The strains were tested at two internationally recognized reference laboratories using the same methodology for concordance. Subsequently, during the preparation of the test swabs, the test

strains were confirmed by phenotypic testing using Sensititre broth microdilution including also the detection of acquired antimicrobial resistance genes and chromosomal point mutations by whole genome sequencing. The expected reference values applied for evaluation of the submitted results and scoring of the individual laboratories' results were assigned based on data generated by the EARS-Net EQA provider.

Interpretation of quantitative and qualitative antimicrobial susceptibility test results will lead to categorization of the result into one of three categories: resistant (R), 'susceptible, increased exposure' (I), or 'susceptible, standard dosing regimen' (S).

Note that if using gradient tests the obtained MIC-values might not refer directly to a 2-fold dilution MIC-value, 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 i.e. an obtained MIC-value at 0.75 mg/L should be reported as 1 mg/L.

In the evaluation report available subsequent to the submission deadline, obtained interpretations in accordance with the expected interpretation will be evaluated as 'correct' (indicated with a score of '1'), whereas obtained interpretations not in accordance with the expected interpretation will be evaluated as 'incorrect' (indicated with a score of '0').

4 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the test forms you can download from the website: (https://antimicrobialresistance.dk/ears-net-eqa.aspx). Read carefully the below description in paragraph 5 before entering your results in the webtool. The webtool will allow you to view and print a report with your reported results. After the submission deadline, you will receive an email with the evaluation report attached. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

Upon release of the evaluation report, we encourage you to perform self-evaluation which implies that each participant should evaluate the accuracy, adequacy and reliability of the used methods by carefully examining the evaluation report received upon submission of results and assessing if there is need of corrective actions related to the applied Antimicrobial Susceptibility Testing procedure.

An individual feedback on results will be sent to the National EQA coordinator for further distribution by 15th November 2021. A country summary report presenting data from all participating laboratories will be sent to the national EQA coordinator; an anonymous version of the report with laboratory codes will be shared with ECDC.

Results must be submitted no later than 24th August 2021.

The overall EARS-Net EQA results will be summarised in a report which will be publicly available from the website of ECDC.

5 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

Login to the webtool

- An email with a link to the webtool will be sent to all registered email addresses. A laboratory may have registered more than one email address.
- A separate email with **personal loginID and password** will afterwards be sent to all registered email addresses.

Guidelines

The '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.

When you submit your results, remember to have by your side the completed test forms. Test forms are also available for download from the EARS-Net EQA website.

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

Contact

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

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