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EU4Health Programme

Protocol for the 2026 External quality assessment (EQA) exercise of performance of laboratories participating in European Antimicrobial Resistance Surveillance Network (EARS-Net)

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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 4, 8
Handling and safety instructions	Strains are UN3373, biological substance category B. Follow biosafety level 2 practices. Subculture and process the strains within 48 hours from receipt. Freeze at -80° C for future analysis.	Page 5, 6
Sharing of strains	Strains cannot be re-distributed by the recipient laboratories.	Page 6
Antimicrobials included in this EQA	All included in ECDC AMR reporting protocol 2026.	Page 7 Annex 1
Recommendations for performing AST and reporting AST results	Follow the most current EUCAST guidelines. Use the routine AST methods applied in your laboratory. Broth microdilution is the recommended method for most antimicrobials. Disk diffusion is the recommended method for: <ul style="list-style-type: none"> • Cefiderocol (all species) • Cefoxitin and norfloxacin (<i>S. aureus</i>) • Oxacillin and norfloxacin (<i>S. pneumoniae</i>). Use S/I/R to report results for all antimicrobials, including screening agents and antimicrobials with bracketed breakpoints. <u>Do not use results from one antimicrobial to report results for other antimicrobial(s).</u> For reporting results of: <ul style="list-style-type: none"> • Gentamicin in <i>Enterococcus</i> spp. use: not-HLAR=S; HLAR=R • Cefiderocol in <i>Acinetobacter</i> spp. use 'S', 'I' or 'R' as described in section 6. 	Page 8-9 Annex 1
EARS-Net EQA website with the relevant documents	https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net	Page 10
Link to webtool for submission of results	https://earsnet.eqa.dtu.dk	Page 10
Username and password	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	10 July 2026	Page 5, 10
Contact	earsnet-eqa@food.dtu.dk	Page 13

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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 across all European Union (EU) Member States and two European Economic Area (EEA) countries (Iceland and Norway). The 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 supports 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). It also provides important information on the performance and comparability of the reported test results between participating laboratories and countries.

The 2026 EARS-Net EQA is organised by the National Food Institute, Technical University of Denmark (DTU Food), in their capacity as a consortium member of the European Union Reference Laboratory for Public Health on Antimicrobial Resistance in Bacteria (EURL-PH-AMR). DTU Food is accredited by DANAK (no. 516) under ISO/IEC 17043:2023 to provide proficiency testing schemes including the scheme for EARS-Net EQA.

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 participated, or intend to participate, in EARS-Net surveillance.

The overall objectives are to assess the accuracy of AST results reported by individual participating 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 applied in this EARS-Net EQA should correspond to the AST method(s) routinely used in the participating laboratory, i.e. automated systems, broth microdilution, disk diffusion, gradient diffusion, or other method(s).

3 ELIGIBILITY CRITERIA FOR PARTICIPATION

Laboratories are eligible to participate in the 2026 EARS-Net EQA exercise if they provide data in accordance with EUCAST guidelines, and if they either reported annually to EARS-Net and/or intend to report 2026 data to EARS-Net.

4 OUTLINE OF THE 2026 EARS-Net EQA

The processes for the 2026 EARS-Net EQA exercise are the same as for the 2025 EARS-Net EQA exercise.

4.1 Overview of the 2026 EARS-Net EQA process

In 2026, the EARS-Net EQA exercise will take place in May-July. 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) applied in their laboratory, through a password-protected webtool. Results must be submitted no later than **10 July 2026**.

After the submission deadline, DTU Food validates all received data. Scores are 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 from the password-protected webtool.

Each year, the ECDC National Focal Point for Antimicrobial Resistance (AMR) in participating EU/EEA countries nominates a 'National EQA Coordinator' (NEC) for that year's EARS-Net EQA exercise. NECs support the 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 AST capacity of participating laboratories in their country, and, if relevant, recommendations for improvement. The annex to the national report includes the results from all participating laboratories in their country.

In 2027, 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 2026 EARS-Net EQA, all participating laboratories will receive a parcel from the NEC containing a cover letter and six swabs. Each swab contains a pure culture of a bacterial isolate. The cover letter includes safety and handling instructions.

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

The laboratories are advised to subculture and process the strains within 48 hours from receipt of the parcel. Subculture the test strains onto non-selective media, such as a nutrient agar plate or blood agar plate, as illustrated in Figure 1:

- 1) Inoculate 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 first inoculated area, followed by additional streaks to further separate the culture, aiming to obtain single colonies
- 3) Turn the plate and use a new sterile loop to streak once through the second inoculated area, followed by additional streaks to further 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 allows for repetition of species identification and AST, if needed, in light of your individual performance.

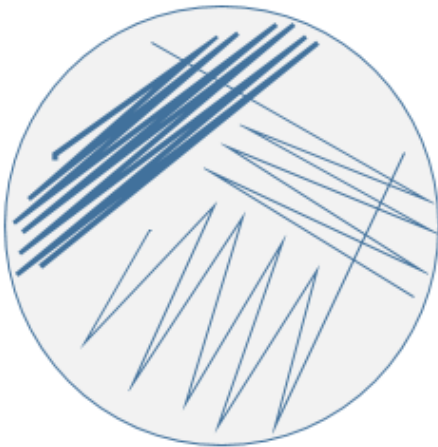


Figure 1: Plating of the test strains

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

1. Strains received for the 2026 EARS-Net EQA cannot be redistributed by the recipient laboratories
2. It is not possible for DTU or NECs to distribute strains to laboratories after the EQA exercise, for example for confirmatory, training, or reference purposes.

4.3 Determination of expected results

To enable scoring of AST results reported by participating laboratories, the EURL-PH-AMR defined the expected AST results for each strain. These results were established as a consensus of AST results generated by three reference laboratories: DTU Food, the EUCAST Development Laboratory, Växjö, Sweden, and the EUCAST Development Laboratory, Karlskrona, Sweden. All reference laboratories used the same AST methodology.

Specifically, the expected minimum inhibitory concentration (MIC) values for each strain-antimicrobial combination were determined by broth microdilution following EUCAST recommendations (i.e. following ISO 20776-1:2019 and using MH-F broth for fastidious organisms). For antimicrobials for which disk diffusion is the recommended method, disk diffusion was performed according to the EUCAST disk diffusion manual (<https://www.eucast.org/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/>). All results were interpreted according to the EUCAST clinical breakpoints tables v16.0 (<https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/>).

The consensus AST results were subsequently reviewed and validated by ECDC and the ECDC EARS-Net Disease Network Coordination Committee.

Following AST by the three reference laboratories, the EURL-PH-AMR performed whole-genome sequencing (WGS) 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 also performed confirmatory AST of the strains to ensure there were no alterations to the test strains.

5 INCLUDED ANTIMICROBIAL AGENTS

All organism-antimicrobial combinations under surveillance in EARS-Net are included in the 2026 EARS-Net EQA exercise. These combinations are listed in annex 1 of this document, which is adapted from Table 8 of the EARS-Net reporting protocol (<https://www.ecdc.europa.eu/en/publications-data/reporting-protocol-antimicrobial-resistance-amr>).

Note: *the list in annex 1 is more inclusive than the panel of antimicrobials likely to be tested by clinical microbiological laboratories during routine clinical practice. Laboratories that do not test the full panel of antimicrobials are still eligible to participate in the 2026 EARS-Net EQA and may report partial data.*

In this EQA, the organism-antimicrobial combinations are not ranked according to their importance for clinical practice, as there are no definitive criteria that would allow an

appropriate or universally applicable ranking across all countries that are eligible to participate in this EQA.

6 PERFORMING AND REPORTING AST RESULTS FOR THIS EQA EXERCISE

6.1 Performing AST

Participating laboratories should perform AST according to their routine laboratory procedures, e.g. automated systems, broth microdilution, agar dilution, disk diffusion, gradient diffusion, or other methods.

Note: When using gradient tests, the obtained MIC values may not correspond directly to a two-fold dilution concentration. In such case, the values should be rounded up to the nearest upper two-fold dilution concentration to ensure the correct evaluation of the results. For example, an MIC value of 0.75 mg/L obtained using gradient test should be reported as 1 mg/L.

All isolates included in the 2026 EARS-Net EQA should be considered as originating from patients with a bloodstream infection.

6.2 Reporting AST results

The 2026 EARS-Net EQA allows laboratories to submit AST results for organism-antimicrobial combinations that may not normally be reported by clinical laboratories during routine procedures, or that may be reported using terminology other than the S/I/R categorisation.

For the purpose of this EQA exercise, all test results must be reported using the categories resistant (R), susceptible, increased exposure (I), and susceptible, standard dosing regimen (S). Follow the instructions below when reporting AST results, including specific adjustments to EUCAST reporting guidelines:

- Apply the **most recent EUCAST clinical breakpoints** for interpretation of the AST results (<https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/>).
- **Do not use results obtained for one antimicrobial to report results for other antimicrobial(s). Only report results for antimicrobials that were tested for this EQA.**
For example:
 - Do not use results from ampicillin to report results for amoxicillin
 - Do not use results from norfloxacin to report results for other fluoroquinolones
 - Do not use results from erythromycin to report results for other macrolides

- Do not use results from carbapenems to report results to carbapenems in combination with beta-lactamase inhibitors.
- Use the **S/I/R notation** to report the results for antimicrobials described by EUCAST as ‘**screen only**’, such as oxacillin, ceftioxin and norfloxacin.
- Use the **S/I/R notation** to report the results for antimicrobials with **bracketed breakpoints**, such as colistin and aminoglycosides. These breakpoints are based on ECOFF values, reflecting a lack of clinical evidence for use as monotherapy.
- EUCAST currently recommends using **disk diffusion for testing of cefiderocol**. Consult the **EUCAST Warnings page** for more information (<https://www.eucast.org/bacteria/methodology-and-instructions/warnings/>).
- EUCAST has issued a Warning regarding the performance of **gradient test for testing of penicillin** in *S. pneumoniae*. Consult the **EUCAST Warnings page** for more information (<https://www.eucast.org/bacteria/methodology-and-instructions/warnings/>).
- **Table 1** provides additional **organism-specific instructions** that must be followed when reporting results for this EQA.

Table 1. Additional organism-specific reporting instructions, by eligible species

Organism	Notes for reporting
<i>Enterococcus</i> spp.	For penicillins (amoxicillin and ampicillin): <ul style="list-style-type: none"> • Assume that intravenous administration will take place For gentamicin: <ul style="list-style-type: none"> • Report isolates that do not contain aminoglycoside-modifying enzymes as ‘S’, i.e. the isolates that do not present high-level aminoglycoside resistance (HLAR) and that can be considered wild type for gentamicin • Report isolates testing positive for aminoglycoside-modifying enzymes as ‘R’, i.e. those presenting HLAR
<i>Escherichia coli</i>	For penicillins (amoxicillin and ampicillin): <ul style="list-style-type: none"> • Assume that intravenous administration will take place
<i>Acinetobacter</i> spp.	For cefiderocol: <ul style="list-style-type: none"> • Report isolates that are mostly devoid of resistance mechanisms as ‘S’ • Report isolates with some acquired resistance mechanisms that may still be a target for this agent as ‘I’ • Report likely resistant isolates with acquired resistance mechanisms as ‘R’

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

To report AST results, participating laboratories are recommended to follow the sequential steps below:

1. Carefully read the instructions for the webtool in section 7 below
2. First record your results on the 'test forms' for this EQA (available from: <https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net>)
3. Transfer the results from the test forms into the webtool and submit your results (see section 7). **Results must be submitted in the webtool no later than 10 July 2026.**

The webtool allows laboratories to view and print a report containing the submitted results.

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 username, and instructions on how to create a password. Each laboratory can have more than one registered email address.

Contacts who participated in the previous EARS-Net EQA, as registered by DTU, will use the same username as in previous years.

Users wishing to reset the webtool password may 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 '2026 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 when entering your results.

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

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

8 EVALUATION OF SUBMITTED EQA RESULTS

8.1 Scoring system

The webtool for the 2026 EARS-Net EQA will apply the same scoring system that was used in 2023 - 2025 EARS-Net EQA.

During the first step in the EQA, if an isolate is identified with the correct species, the interpretation of AST results will be evaluated using the scoring system. Conversely, if the species identification is incorrect, 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 implemented scoring system for the evaluation of interpreted results takes the 'level of difficulty' and the 'severity of error' into account for each organism-antimicrobial combination. The level of difficulty reflects the likelihood of misclassification and is categorized as 'difficult' or 'easy'.

'Difficult' situations include cases where:

- An AST result with a one-fold dilution difference from the expected MIC value, or with a zone diameter difference of ± 3 mm from the expected diameter, would result in a different S/I/R interpretation; and/or
- The expected MIC value or diameter falls within the area of technical uncertainty (ATU); and/or
- The relevant EUCAST clinical breakpoint had been recently changed or newly introduced in the latest EUCAST breakpoint table.

'Easy' situations include cases where:

- An AST result with a one-fold dilution difference from the expected MIC value or within ± 3 mm of the expected diameter would lead to the same S/I/R interpretation; and/or
- The EUCAST clinical breakpoint had not been recently changed or added.

Errors are classified into three categories: very major error (VME), major error (ME) and no error. Both VME and ME are penalised in the scoring system. A VME is defined as reporting false susceptibility, i.e. the expected result is R, but the reported result is S or I; and a ME is defined as reporting false resistance, i.e. the expected results is S or I, but the reported result is R.

Table 2 shows the 2026 EARS-Net EQA scoring system. 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.

The scoring system does not rank or group organism-antimicrobial combinations according to 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 instead be performed at (sub-)national level using the database of national results that is sent to NECs.

Table 2. Scoring system of the 2026 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

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 2026, the laboratory contact person(s) will receive an email informing them that an evaluation report for their laboratory, including the score, is available for download from the password-protected webtool. 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 are only relevant for the small subset of participating laboratories that are expected, as part of their routine practice, to perform AST for all of the organism-antimicrobial combinations in annex 1.

Note: All participating laboratories are encouraged to perform a self-evaluation of the accuracy, adequacy, and reliability of the AST methods and procedures used, and to assess whether corrective actions are needed.

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 the laboratory feedback reports for every participating laboratory in their country, as well as 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 2027, ECDC will publish an annual report summarising 2026 EARS-Net EQA results from all participating laboratories.

9 CONTACT

If you require 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 encounters issues when entering results or accessing the webtool, please provide a description of the issue that is sufficiently detailed to ensure efficient follow-up.

EARS-Net EQA Team

earsnet-eqa@food.dtu.dk

ANNEX 1 - Antimicrobials included in the 2026 EARS-Net EQA

Adapted from Table 8 of the EARS-Net reporting protocol 2026: ‘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
<i>Acinetobacter</i> species (ACISPP)	Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
<i>Enterococcus faecalis</i> (ENCFAE) and <i>Enterococcus faecium</i> (ENCFAI)	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
<i>Escherichia coli</i> (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
<i>Klebsiella pneumoniae</i> (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
<i>Pseudomonas aeruginosa</i> (PSEAEER)	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
<i>Staphylococcus aureus</i> (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
<i>Streptococcus pneumoniae</i> (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