Protocol for EQAsia EQAS – 2nd round

ID and antimicrobial susceptibility testing of *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* test strains

1 INTRODUCTION

The EQAsia project aims to strengthen the provision of External Quality Assessment (EQA) services across the One Health sector in South and Southeast Asia. Therefore, a comprehensive and high-quality EQA program for antimicrobial resistance (AMR) is offered to all the National Reference Laboratories/Centres of Excellence in the region during 2021. The EQA is organized by the consortium of EQAsia and supported by the Fleming Fund.

The 2nd iteration of EQAsia EQAS includes the antimicrobial susceptibility testing of eight *Klebsiella pneumoniae*, eight *Shigella* spp., eight *Acinetobacter* spp. and eight *Staphylococcus aureus* strains **identified** among a total of 11 test strains for each microorganism, which include three non-target species strains.

Additionally, antimicrobial susceptibility testing of five reference strains for quality control (QC) in relation to antimicrobial susceptibility testing is included. The QC reference strains supplied are: *Escherichia coli* ATCC 25922/CCM 3954, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC). These reference strains are original CERTIFIED cultures provided free of charge, and should be stored for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strains included in the

2nd EQA will not be included in the parcel related to future EQAS-iterations. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual 'Subculture and maintenance of quality strain' available on the EQAsia website.

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and, if necessary, improve the identification and antimicrobial susceptibility testing of pathogens, specifically *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus*. Therefore, the laboratory work for this EQAS should be performed using the methods routinely used in your own laboratory.

3 OUTLINE OF THE EQAS 2021

3.1 Shipping, receipt and storage of strains

In July/August 2021, it is expected that approximately 31 laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- 11 test strains of which eight are *Klebsiella pneumoniae*, in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 (if not already received for EQA1) and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains.

- 11 test strains of which eight are *Shigella* spp., in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 (if not already received for EQA1) and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains.

- 11 test strains of which eight are *Acinetobacter* spp., in addition to three non-target species strains. The *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 will be provided as reference strain.

- 11 test strains of which eight are *Staphylococcus aureus*, in addition to three non-target species strains. The *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC) will be provided as reference strains.

Please confirm receipt of the parcel through the confirmation form enclosed in the shipment

All strains are shipped lyophilized. The lyophilized strains must be stored in a dark, cool place. The strains must be sub-cultured and prepared for storage in your strain collection (e.g. in a -80°C freezer). This set of cultures should serve as reference if discrepancies are detected during the testing (e.g. they can be used to detect errors such as mislabelling or contamination), and they can function as reference material available for reference at a later stage, when needed.

For reconstitution of the test strains, please see the document 'Instructions for opening and reviving lyophilised cultures of test strains (Human health laboratories)' OR 'Instructions for opening and reviving lyophilised cultures of test strains (Animal health laboratories)' on the EQAsia website.

For reconstitution of the QC reference strains, please see the document 'Subculture and maintenance of quality strain' on the EQAsia website.

All provided strains belong to UN3373, Biological substance category B. These strains can potentially be harmful to humans and pose a risk due to their possible pan-resistant profile, therefore becoming a challenge in the treatment of a potential human infection. It is the recipient laboratory's responsibility to comply with national legislation, rules and regulations regarding the correct use and handling of the provided test strains, and to possess the proper equipment and protocols to handle these strains. Nevertheless, it is recommended to handle the strains in a BSL2 containment facility using equipment and operational practices for work involving infectious or potentially infectious materials. The containment and operational requirements may vary with the species, subspecies, and/or strains, thus, please take the necessary precautions.

Please consult the Pathogen Safety Data Sheets (PSDSs) produced by the Public Health Agency of Canada. The PSDSs of each pathogen can be found in the bottom of the page. These PSDSs are technical documents that describe the hazardous properties of human pathogens, and provide recommendations for the work involving these agents in a laboratory setting.

3.2 Identification of *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* test strains

For each test species, three out of the 11 test strains related to each bacterial species does not belong to the target species of the EQAS. To identify the eight cultures of the correct target species among the 11 test strains, you should use the method routinely used in your own laboratory for **identification** of the organism.

3.3 Antimicrobial susceptibility testing of *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* test strains, and of the reference strains

The strains identified as *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus*, as well as the appropriate reference strains should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test forms and in **Tables 1-4**. Should it however not be possible to test all antimicrobials, the optional antimicrobials are marked with [a]. Please use the methods routinely used in your own laboratory.

The reference values used in this EQAS for interpreting MIC and disk diffusion results are in accordance with current epidemiological cut-off values or clinical breakpoint values developed by EUCAST. When not available, CLSI zone diameter and MIC breakpoint values are used instead. The epidemiological cut-off values or clinical breakpoint values for *Klebsiella pneumonia*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* can be found in **Tables 1-4**, respectively. **Make sure to use the correct table for the interpretation**.

Interpretation of MIC or disk diffusion results will lead to categorization of the result into one of two categories: **resistant** (R) or **susceptible** (S). In the evaluation report you receive upon the submission deadline, you can find that obtained interpretations in accordance with the expected interpretation will be evaluated as '1' (correct), whereas obtained interpretations not in accordance with the expected interpretation will be evaluated as '0' (incorrect).

Table 1. Interpretive criteria for *Klebsiella pneumoniae* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK[a]	≥ 16	< 18
Ampicillin, AMP	≥ 16	< 14
Azithromycin, AZI[a]	≥ 32*	≤ 12*
Cefepime, FEP[a]	≥ 0.50	< 24
Cefotaxime, FOT	≥ 0.50	< 21
Cefotaxime, FOT + clavulanic acid	NA	NA
Cefoxitin, FOX	≥ 16	< 19
Ceftazidime, TAZ	≥ 1	< 20
Ceftazidime, TAZ + clavulanic acid	NA	NA
Chloramphenicol, CHL	≥ 32*	≤ 12*
Ciprofloxacin, CIP	≥ 0.25	< 22
Colistin, COL	≥ 4	NA
Ertapenem, ETP[a]	≥ 0.06	< 24
Gentamicin, GEN	≥ 4	< 17
Imipenem, IMI	≥ 2	< 23
Meropenem, MERO	≥ 0.25	< 25
Nalidixic acid, NAL	≥ 16	< 16
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 16*	≤ 11*
Tigecycline, TIG[a]	≥ 4	< 18
Trimethoprim, TMP	≥ 4	< 18

Reference values are based on *K. pneumoniae* and/or *E. coli* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on Enterobacterales breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

Beta-lactam and carbapenem resistance

The following tests for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes for ***Klebsiella pneumoniae*** are recommended.

- Reduced susceptibility to cefotaxime (FOT) and/or ceftazidime (TAZ): it indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype. These strains should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests.

- Confirmatory test for ESBL production: it requires the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone, as well as in combination with a β-lactamase inhibitor (clavulanic acid). Synergy can be determined by broth microdilution methods, E-test or Disc Diffusion. It is defined as a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC FOT : FOT/Cl or TAZ : TAZ/Cl ratio ≥ 8). A positive synergy testing for Disc Diffusion is defined as ≥ 5 mm increase of diameter of FOT or TAZ in combination with clavulanic acid (FOT/Cl or TAZ/Cl) compared to testing them alone. The presence of synergy indicates ESBL production.

- Detection of AmpC-type beta-lactamases: it can be performed by testing the bacterial culture for susceptibility to cefoxitin (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

- Confirmatory test for carbapenemase production: it requires the testing of meropenem (MERO). Resistance to MERO indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on the adaptation of the most recent EFSA recommendations (**Figure 1** below) – The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA Journal 2020;18 (3). https://doi.org/10.2903/j.efsa.2020.6007

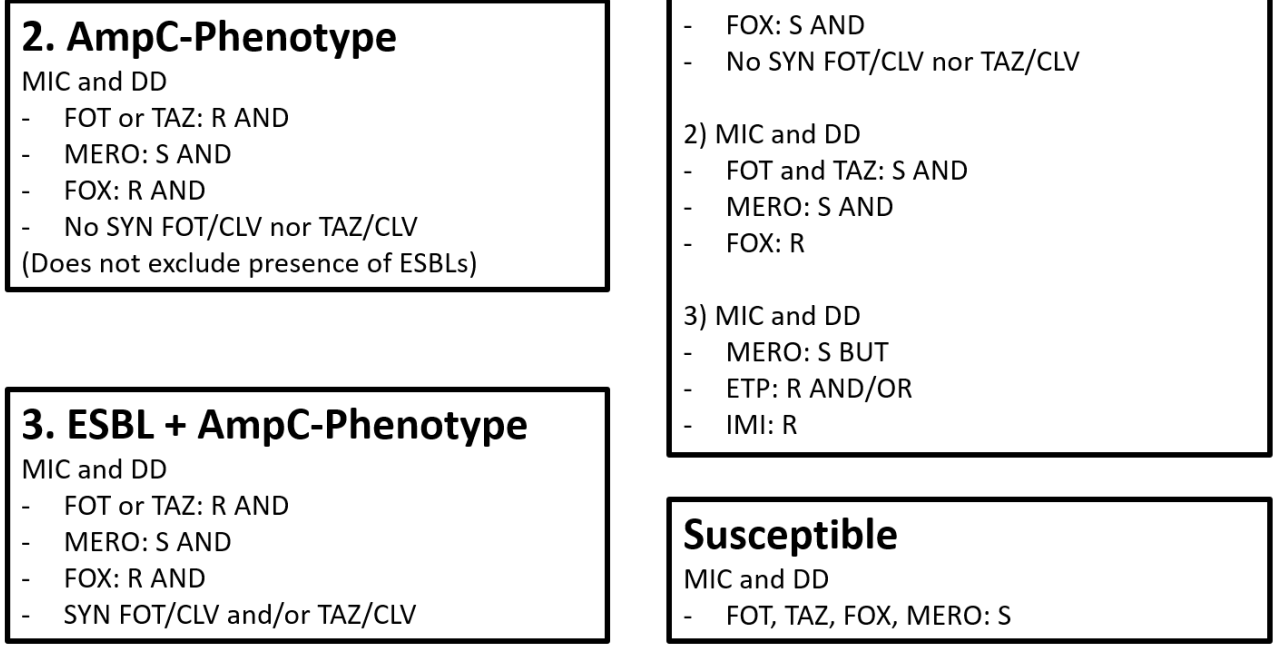

Figure 1: Adapted from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018.

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but it is not requested as part of this EQAS.

Table 2. Interpretive criteria for *Shigella* spp. antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK[a]	≥ 16	< 17
Ampicillin, AMP	≥ 16	< 14
Azithromycin, AZI[a]	≥ 32*	≤ 12*
Cefepime, FEP[a]	≥ 0.50	< 28
Cefotaxime, FOT	≥ 0.50	< 21
Cefoxitin, FOX	≥ 16	< 17
Ceftazidime, TAZ	≥ 1	< 20
Chloramphenicol, CHL	≥ 32*	≤ 12*
Ciprofloxacin, CIP	≥ 0.12	< 25
Colistin, COL	≥ 4	NA
Ertapenem, ETP[a]	≥ 0.06	< 24
Gentamicin, GEN	≥ 4	< 17
Imipenem, IMI	≥ 1	< 24
Meropenem, MERO	≥ 0.12	< 25
Nalidixic acid, NAL	≥ 16	< 19
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 16*	≤ 11*
Tigecycline, TIG[a]	≥ 1	< 18
Trimethoprim, TMP	≥ 4	< 20

Reference values are based on *E. coli* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on Enterobacterales breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

Table 3. Interpretive criteria for *Acinetobacter* spp. antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK	≥ 16	< 19
Cefotaxime, FOT	≥ 64*	≤ 14*
Ceftazidime, TAZ	≥ 32*	≤ 14*
Ciprofloxacin, CIP	≥ 2	< 21
Colistin, COL	≥ 4	NA
Doripenem, DOR	≥ 4	< 22
Gentamicin, GEN	≥ 8	< 17
Imipenem, IPM	≥ 8	< 21
Levofloxacin, LVX	≥ 2	< 20
Meropenem, MERO	≥ 16	< 15
Minocycline, MIN	≥ 16*	≤ 12*
Piperacillin/tazobactam, P/T4	≥ 128/4*	≤ 17*
Tigecycline, TGC	≥ 1	NA
Tobramycin, TOB	≥ 8	< 17

Reference values are based on *Acinetobacter* spp. clinical breakpoint values from www.eucast.org on June 2021.
*Reference values are based on *Acinetobacter* spp. breakpoint values from CLSI M100, 30th Ed.

Table 4. Interpretive criteria for *Staphylococcus aureus* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Cefoxitin, FOX	≥ 8	< 22
Chloramphenicol, CHL	≥ 32	< 18
Ciprofloxacin, CIP	≥ 2	< 20
Clindamycin, CLI	≥ 0.50	< 22
Erythromycin, ERY	≥ 2	< 21
Fusidate, FUS[a]	≥ 1	< 24
Gentamicin, GEN	≥ 4	< 18
Kanamycin, KAN[a]	≥ 16	< 18
Linezolid, LZD	≥ 8	< 19
Mupirocin, MUP[a]	≥ 2	< 30
Penicillin, PEN	≥ 0.25	< 26
Quinupristin/Dalfopristin, SYN[a]	≥ 2	< 21
Rifampin, RIF	≥ 0.03	< 25
Streptomycin, STR[a]	≥ 32	NA
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 2	< 22
Tiamulin, TIA[a]	≥ 4	NA
Trimethoprim, TMP	≥ 4	< 19
Vancomycin, VAN	≥ 4	NA

Reference values are based on *S. aureus* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on *S. aureus* breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

4 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the web database. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The web database will allow you to view and print a report with your reported results. The scores for the results will be released after the result submission deadline where you will be able to access the evaluation of your results. Results in agreement with the expected interpretation are categorised as '1' (correct), while results deviating from the expected interpretation are categorised as '0' (incorrect).

Results must be submitted no later than September 15th 2021.

If you have trouble entering your results, please contact the EQAsia Project Manager directly, explaining the issues that you encountered:

Rikke Braae
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK
E-mail: rikb@food.dtu.dk

Direct communication with the EQAsia Project Manager must be in English.

5 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

The 'Guideline for reporting results in the EQAsia Informatics Module' is available for download directly from the EQAsia website. Please follow the guideline carefully.

Access the webtool using this address. See below how to login to the webtool.

When you submit your results, remember to have by your side the completed test forms (template available for download from the EQAsia website).

Do not hesitate to contact us if you have troubles with the webtool.

Before finally submitting your input for all the organisms, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once!** 'Final submit' blocks data entry.

Login to the webtool:

When first given access to login to the webtool, your **personal loginID and password** is sent to you by email.

Note that the primary contact person for a participating institution is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact rikb@food.dtu.dk

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