Protocol for EQAsia EQA – 1st round

ID testing, serotyping and antimicrobial susceptibility of *Salmonella* and *E. coli* test strains

Changes from version 1 to version 2:
The disk diffusion value for ciprofloxacin in table 1 for *Salmonella* spp. is changed from ≤20 to ≤25.

1 INTRODUCTION

The overall aim of the EQAsia project is 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 AMR is offered to all the National Reference Laboratories/Centres of excellence in the region during 2021. The EQAS is organized by the consortium of EQAsia and supported by the Fleming Fund.

The EQAsia EQAs (1st round) includes identification of eight *Salmonella* spp. among eleven test strains, following serotyping and antimicrobial susceptibility of the *Salmonella* spp., and identification of eight *E. coli* strains among eleven test strains, following antimicrobial susceptibility of the *E. coli* strains. Moreover, antimicrobial susceptibility testing of the *Escherichia coli* ATCC 25922 (CCM 3954) reference strain for quality control (QC) in relation to antimicrobial susceptibility testing is included.

The QC reference strain supplied (ATCC 25922 (CCM 3954)) is an original CERTIFIED culture provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strain will not be included in the parcel related to future EQAS-iterations. Therefore, please take proper care of this strain. Handle and maintain it as suggested in the manual 'Subculture and Maintenance of QC Strains' available on the EQAsia website (see https://antimicrobialresistance.dk/eqasia.aspx).

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of serotyping (*Salmonella*), antimicrobial susceptibility testing and ID of pathogens, specifically *Salmonella* and *E. coli*. A further objective is to assess and improve the comparability of surveillance data on serotypes (*Salmonella*) and antimicrobial susceptibility reported by different laboratories. Therefore, the laboratory work for this EQAS should be performed using the methods routinely used in your laboratory.

3 OUTLINE OF THE EQAS 2021

3.1 Shipping, receipt and storage of strains

In February 2021, around 28 laboratories located in South- and South-East Asia will receive a parcel containing 11 test strains related to the *Salmonella* test and 11 test strains related to the *E. coli* test, as well as an *E. coli* ATCC 25922 reference strain. Only 8 of the 11 strains are in fact *Salmonella* and *E. coli*, respectively, and must be determined by ID-testing. All provided strains belong to UN3373, Biological substance category B. Extended Spectrum Beta Lactamase (ESBL)-, AmpC- or carbapenemase-producing strains could be included in the selected material.

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

The *Salmonella*, *E. coli* and the *E. coli* reference strain 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 mis-labelling or contamination), and also they can function as reference material available for reference at a later stage, when needed.

For reconstitution of the *Salmonella* and *E. coli* test strains, please see the document 'Instructions for opening and reviving lyophilised cultures – Human Health Labs' or 'Instructions for opening and reviving lyophilised cultures – Animal Health Labs' on the EQAsia website (see https://antimicrobialresistance.dk/eqasia.aspx).

For reconstitution of the *E. coli* reference strain, please see the document 'Instructions for opening and reviving lyophilised cultures' on the EQAsia website (see https://antimicrobialresistance.dk/eqasia.aspx).

3.2 Identification of *Salmonella* spp and *E. coli*

Three of the eleven test strains related to the *Salmonella* EQAS and *E. coli* EQAS, respectively, are not the target organism of the EQAS.
For identifying the 8 cultures of the target organism out of the eleven test strains, you should use the method routinely used in the laboratory for identification of the organism.

3.3 Serotyping of *Salmonella* spp. (voluntary)

The eight identified *Salmonella* strains should be serotyped by using the method routinely used in the laboratory. Also serogroup results will be evaluated, therefore, if you do not have all the necessary antisera for a serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

3.4 Antimicrobial susceptibility testing of *Salmonella* and *E. coli* test strains and *Escherichia coli* ATCC 25922

The strains identified as *Salmonella* and *E. coli* as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in your laboratory. Expected results are based on broth microdilution tests.

The breakpoints used in this EQAS for interpreting MIC and disk diffusion results are in accordance with current epidemiological cut-off values developed by EUCAST (www.eucast.org). The breakpoints for *Salmonella* can be found in Table 1. The breakpoints for *E. coli* can be found in Table 2. Interpretation of MIC or disk diffusion results will lead to categorization of the result into one of two categories: resistant (R) and susceptible (S). In the evaluation report you receive upon submission deadline, you can find that obtained interpretations in accordance with the expected interpretation will be evaluated as 'correct', whereas obtained interpretations not in accordance with the expected interpretation will be evaluated as 'incorrect'.

Testing of gentamicin susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* should not be reported as susceptible to aminoglycosides.

Table 1. Interpretive criteria for *Salmonella* spp. antimicrobial susceptibility testing

Antimicrobials	**Reference value, MIC (µg/mL)**	**Reference value, Disk diffusion (mm)**
	Resistant	**Resistant**
Ampicillin, AMP	≥16	<18
Azithromycin, AZI	≥32	<12
Cefepime, FEP	≥16*	≤18*
Cefotaxime, FOT	≥1	<20
Cefotaxime, FOT + clavulanic acid	N/A	N/A
Cefoxitin, FOX	≥16	<21
Ceftazidime, TAZ	≥4	<20
Ceftazidime, TAZ + clavulanic acid	N/A	N/A
Chloramphenicol, CHL	≥32	<19
Ciprofloxacin, CIP	≥0.125	≤25*
Colistin, COL	≥4*	N/A
Ertapenem, ETP	≥2*	≤18*
Gentamicin, GEN	≥4	<17

Imipenem, IMI	≤19*	≥2*
Meropenem, MERO	≥4*	<27
Nalidixic acid, NAL	≥16	N/A
Sulfamethoxazole, SMX	≥512*	≤12*
Tetracycline, TET	≥16	<17
Tigecycline, TIG	N/A	<16
Trimethoprim, TMP	≥4	<23

Reference values are based on epidemiological cut off values from www.eucast.org.

*Reference values are based on CLSI M100, 30th Ed.

Table 2. Interpretive criteria for *E. coli* antimicrobial susceptibility testing

Antimicrobials	**Reference value, MIC (µg/mL)**	**Reference value, Disk diffusion (mm)**
	Resistant	**Resistant**
Ampicillin, AMP	≥16	<14
Azithromycin, AZI	≥32*	≤12*
Cefepime, FEP	≥0.50	<28
Cefotaxime, FOT	≥0.50	<21
Cefotaxime, FOT + clavulanic acid	≥0.50	≤27*
Cefoxitin, FOX	≥16	<17
Ceftazidime, TAZ	≥1	<20
Ceftazidime, TAZ + clavulanic acid	≥1	≤22*
Chloramphenicol, CHL	≥32	≤12*
Ciprofloxacin, CIP	≥0.125	<25
Colistin, COL	≥4	N/A
Ertapenem, ETP	≥0.06	<24
Gentamicin, GEN	≥4	<17
Imipenem, IMI	≥1	<24

Meropenem, MERO	≥0.25	<25
Nalidixic acid, NAL	≥16	≤13*
Sulfamethoxazole, SMX	>512*	≤12*
Tetracycline, TET	≥16	≤11*
Tigecycline, TIG	≥1	<18
Trimethoprim, TMP	≥4	<20

Reference values are based on epidemiological cut off values from www.eucast.org.

*Reference values are based on CLSI M100, 30th Ed.

Beta-lactam and carbapenem resistance

The following tests for detection of ESBL-, AmpC-, and carbapenamase-producing phenotypes for *Salmonella* spp. and *E. coli* are optional. This component is relevant when MIC-values are available for analysis.

If choosing to participate in this component of the EQAS, all strains displaying reduced susceptibility to cefotaxime (FOT) and/or ceftazidime (TAZ) should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests. Reduced susceptibility to any of the above-mentioned antimicrobials indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype.

Confirmatory test for ESBL production requires the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone, and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) by microbroth dilution methods or E-test; 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). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases 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 requires the testing of meropenem (MERO). Reduced susceptibility to MERO indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (Annex A) (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 (Annex A)).

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 is not a requested as part of this EQAS.

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. 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 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

Results must be submitted no later than 31st March 2021.

If you experience difficulties in entering your results, please contact the EQAS Coordinator 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 EQAS organiser must be in English.

5 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

The 'guideline for submission of results via webtool' is available for download directly from the EQAsia website (https://antimicrobialresistance.dk/eqasia.aspx). Please follow the guideline carefully.

Access the webtool using this address: https://EQASIA-pt.dtu.dk. About login to the webtool, see below.

When you submit your results, remember to have by your side the completed test forms (template available for download from https://antimicrobialresistance.dk/eqasia.aspx).

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting your input for *Salmonella* and *E. coli*, respectively, 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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Annex A

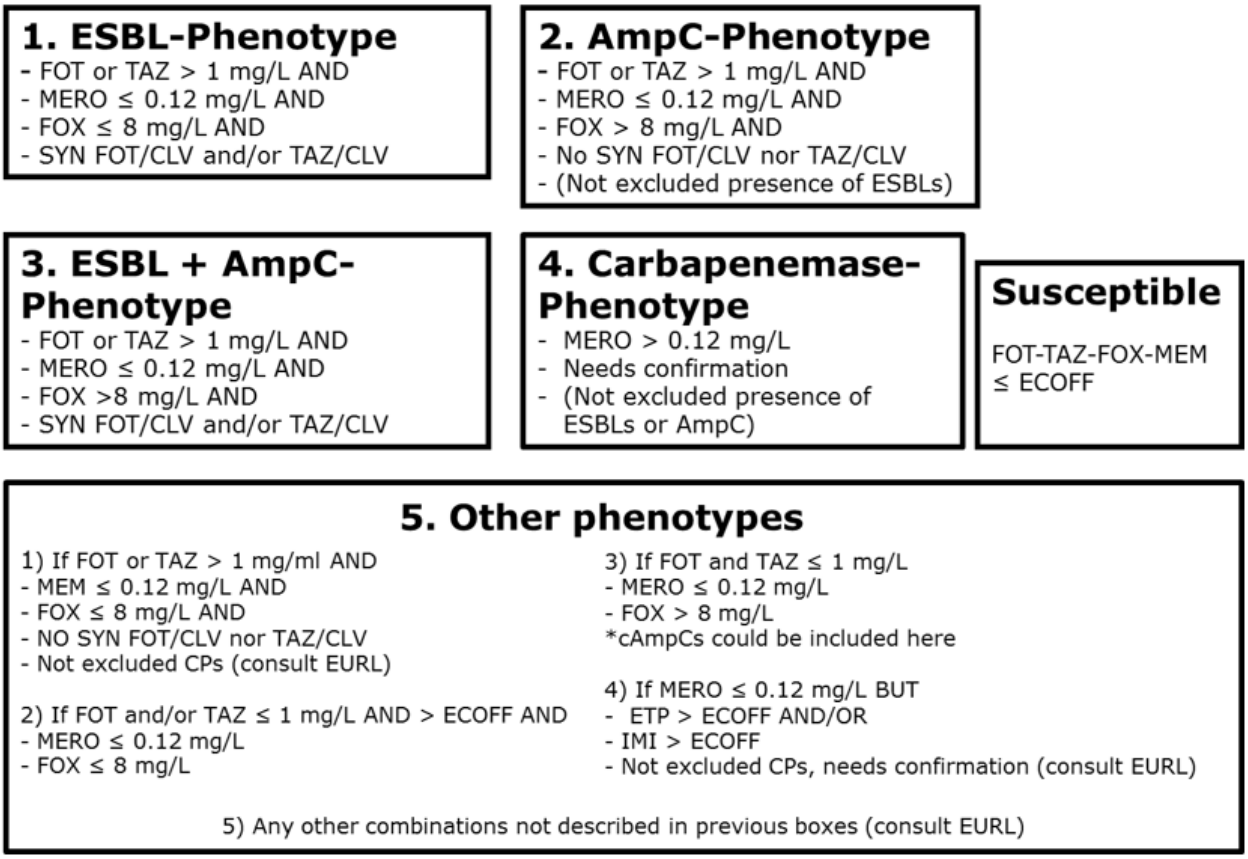

Figure 1: EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018.