The 1st EQAsia Matrix EQA on selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing Escherichia coli from cultures mimicking meat samples – 2021

Photo: Colourbox

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The 1st EQAsia Matrix EQA on selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from cultures mimicking meat samples – 2021

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1. Introduction

The EQAsia project was launched in 2020 aiming to strengthen the provision of External Quality Assessment (EQA) services across the One Health sector among National Reference Laboratories/ Centres of Excellence in South and Southeast Asia. EQAsia is supported by the Fleming Fund and strives to increase the quality of laboratory-based surveillance of WHO GLASS pathogens [1] and FAO priority pathogens [2].

The EQAsia Consortium includes the National Food Institute, Technical University of Denmark (DTU Food) as the Lead Grantee, the International Vaccine Institute (IVI) in South Korea, the National Institute of Health (NIH), Department of Medical Sciences in Thailand and the Faculty of Veterinary Science, Chulalongkorn University (CUVET) in Thailand.

EQAsia provides a state-of-the-art EQA program free of charge for the South and Southeast Asian region through existing local providers (NIH Thailand and CUVET Thailand). The program, referred to as a "One-Shop EQA program", is designed to enable the laboratories to select and participate in relevant proficiency tests of both pathogen identification and antimicrobial susceptibility testing (AST), in line with the requirements of the WHO GLASS [1]. The EQA program is supported by an informatics module where laboratories can report their results and methods applied.

As suggested by FAO and in alignment with the scope of WHO Tricycle, the EQA trials taking place in 2021 have included a Matrix-based specific EQA, carried out in August-October, aiming to assess the laboratories' ability to detect and isolate AmpC beta-lactamases (AmpC), extended-spectrum beta-lactamases (ESBL) and carbapenemase producing *Escherichia coli* from complex matrices, followed by identification and antimicrobial susceptibility testing. This Matrix EQA aims to monitor the capacity of the participating laboratories to perform isolation and AST of *E. coli* from matrices, and identify potential problems or focus areas for future training/education.

To prepare for the launch of this Matrix EQA, several preliminary studies were conducted at CUVET Thailand, using meat samples spiked with *E. coli* isolates presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes. However, due to the impossibility of shipping such samples from Thailand to the participating countries, another approach was attempted. Shortly, a portion of pork-minced meat was spiked with an *E. coli* strain, the meat sample was pre-enriched and bacterial growth was allowed. The resulting bacterial culture (a mixture of the different bacteria present in the meat sample) was then lyophilized and a culture mimicking the meat content obtained (see section 2.2).

All *E. coli* isolates used for spiking the meat samples were assessed by DTU Food and validated by CUVET Thailand. The assessment included both phenotypic minimum inhibitory concentration (MIC) determination by broth microdilution, and whole genome sequencing (WGS) to detect antimicrobial resistance (AMR) genes and chromosomal point mutations.

The evaluation of the participants' results is based on international guidelines, namely the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Interpretative criteria referring to both disk diffusion and MIC determination are listed in the Matrix EQA protocol (Appendix 1) and allow for the obtained results to be interpreted into categories as resistant or susceptible depending on the method used. Results in agreement with the expected interpretation are categorised as '1' (correct), while results deviating from the expected interpretation are categorised as '0' (incorrect). This standardized interpretation of results is necessary to allow comparison of performance between laboratories. No thresholds were set in advance to evaluate the

performance of the participating laboratories; thus, the results were evaluated case by case. Nevertheless, a laboratory performance of < 5% deviation from expected results would be considered ideal.

Evaluation of a result as "deviating from the expected interpretation" should be carefully analysed in a route cause analysis procedure performed by individual participants (self-evaluation) when the EQA results are disclosed. The methods applied have limitations in reproducibility, thus, on repeated testing, the same strain/antimicrobial combination can result in different MIC or Inhibition Zone Diameter values differing by one-fold dilution or ±3mm, respectively. If the expected MIC/Zone Diameter is close to the threshold for categorising the strain as susceptible or resistant, a one-fold dilution/±3mm difference may result in different interpretations. Since this report evaluates the interpretations of MIC/Zone Diameter and not the values, some participants may find their results classified as incorrect even though the actual MIC/Zone Diameter measured is only one-fold dilution/±3mm different from the expected MIC/Zone Diameter. In these cases, the participants should be confident about the good quality of their AST performance.

In this report, results from laboratories affiliated with the Human Health (HH) or the Animal Health (AH) Sectors are presented together. The laboratories are identified by codes and each code is known only by the corresponding laboratory and the organizers. The full list of laboratory codes is confidential and known only by the EQAsia Consortium.

This report is approved in its final version by a Technical Advisory Group composed by members of the EQAsia Consortium, and by the EQAsia Advisory Board members Navin Karan (Pacific Pathology Training Centre, New Zealand), Monica Lahra (WHO Collaborating Center for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia) and Ben Howden (The Peter Doherty Institute for Infection and Immunity, Australia).

2. Materials and Methods

2.1 Participants in EQAsia Matrix EQA 2021

A total of nine laboratories participated in the first EQAsia Matrix EQA trial of the EQAsia project: two laboratories belonging to the HH Sector and seven belonging to the AH Sector from Bangladesh, Indonesia, Pakistan, and Sri Lanka (**Figure 1**).

2.2 Samples preparation

Laboratories that registered for the Matrix EQA trial received five lyophilized cultures mimicking meat content for isolation of presumptive ESBL, AmpC- and carbapenemase-producing *E. coli*, including identification, and AST of the obtained isolates. The preparation of the cultures followed the official EURL-AR protocols [3]. The pre-testing and spiking of the meat samples are briefly described below:

Pre-testing

Firstly, pork minced meat samples were tested for the presence of ESBL-, AmpC- and carbapenemase-producing *E. coli* to ensure that the meat does not naturally contain these type of bacteria. Meat portions of 25 g were mixed with 225 mL of Buffered Peptone Water (BPW) and incubated at 37°C ± 1°C for 18-22 h (pre-enrichment step as referred in the EURL-AR protocols). A loopful of the pre-enriched culture was plated onto a MacConkey agar plate containing 1 mg/L of cefotaxime and incubated overnight to assure that the batch used was negative for ESBL/AmpC/Carbapenemase-producing *E. coli* and that contained some background flora.

Spiking of the meat samples

To prepare the five lyophilized cultures mimicking meat content, five 25 g pork minced meat portions from the same batch as in the pre-testing were used. Four of the portions were each spiked with an *E. coli* isolate, whereas the fifth portion was not spiked and, therefore, expected to be negative.

After spiking the meat with the *E. coli* isolates, all meat portions were mixed with BPW, incubated and plated on selective agar as described in the pre-testing. The grown colonies, consisting in a mixture of the different bacteria present in the meat sample were then scrapped and lyophilized.

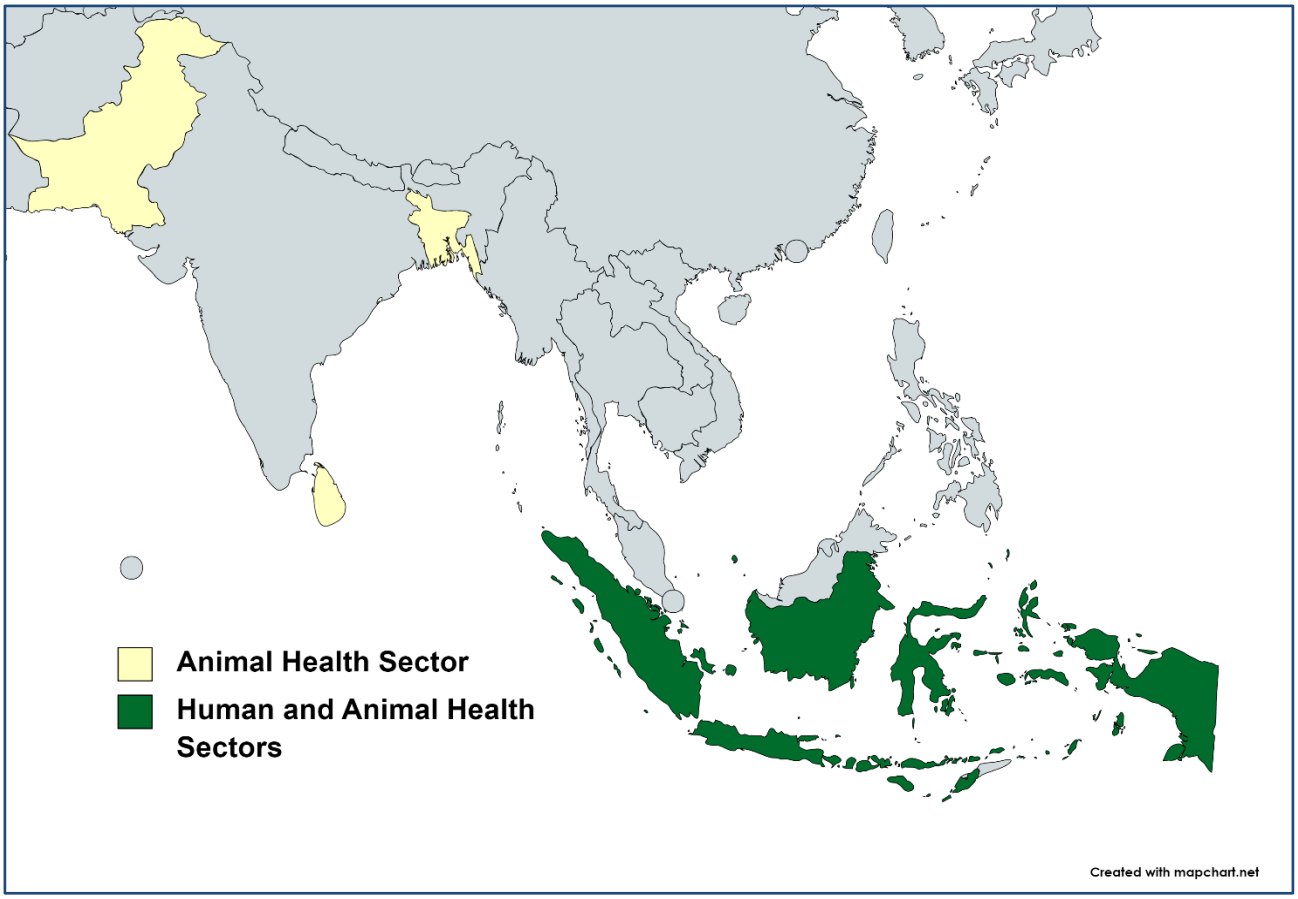

Figure 1: Countries participating in the first EQAsia Matrix EQA 2021. Color indicates sector affiliation of the participating laboratory as Animal Health laboratory (yellow) or both Human and Animal Health laboratories (green).

2.3 Isolation and identification of ESBL-, AmpC- and carbapenemase-producing *E. coli*

The *E. coli* isolates used for this EQA were tested at DTU Food and additionally verified by CUVET Thailand. Expected MIC values can be found on Appendix 2. The reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 were provided to all participants free of charge with instructions for storage and maintenance for quality assurance purposes and future EQA trials. The expected quality control ranges for the reference strain *E. coli* ATCC 25922 were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-31st Ed. [4], tables 4A-1 and 5A-1, and for *E. coli* NCTC 13846 from EUCAST in document "Routine and extended internal quality control for MIC determination and disk diffusion" [5] (Appendix 3).

The protocols for selective isolation and identification of the ESBL-, AmpC- and carbapenemase-producing *E. coli* isolates contained in the lyophilized cultures were briefly described in the Matrix EQA protocol (Appendix 1) and are based on the official EURL-AR protocols [3]. For bacterial identification, the participants were asked to perform the methods routinely applied in their laboratories. Information about the methods used for selective isolation and species identification were requested when submitting results in the informatics module.

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial are listed in the protocol (Appendix 1) and in **Table 1**. These antimicrobials correspond to several antimicrobial class representatives important for surveillance, as well as antimicrobials required for detection and confirmation of ESBL-, AmpC- and carbapenemase-producing phenotypes.

The reference values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current epidemiological cut-off values developed by EUCAST [6]. When not available, CLSI zone diameter and MIC breakpoint values were used instead. Cefotaxime/ clavulanic acid and ceftazidime/ clavulanic acid results were not scored, as these drug combinations are mostly important for confirmation of ESBL-, AmpC- and carbapenemase-producing phenotypes. Results for presumptive beta-lactam resistance mechanisms were interpreted according to the most recent EFSA (European Food Safety Authority) [7] recommendations also included in the Matrix EQA protocol (Appendix 1).

Participants were encouraged to test as many as possible of the antimicrobials listed.

2.4 Distribution

CUVET Thailand dispatched the lyophilized cultures in September 2021 to all the participating laboratories. The shipment (UN3373, biological substances category B) was sent according to International Air Transport Association (IATA) regulations. Participating laboratories received information on how to open, revive and store these lyophilized cultures.

Table 1. Panel of antimicrobials and respective abbreviations for AST of *E. coli* included in the EQAsia Matrix EQA 2021. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

Antimicrobials – *E. coli* AST
Ampicillin (AMP)
Azithromycin (AZI)
Cefepime (FEP)
Cefotaxime (FOT)
Cefotaxime/clavulanic acid (FOT/Cl)
Cefoxitin (FOX)
Ceftazidime (TAZ)
Ceftazidime/clavulanic acid (TAZ/Cl)
Chloramphenicol (CHL)
Ciprofloxacin (CIP)
Colistin (COL)
Ertapenem (ETP)
Gentamicin (GEN)
Imipenem (IMI)
Meropenem (MERO)
Nalidixic Acid (NAL)
Sulfamethoxazole (SMX)
Tetracycline (TET)
Tigecycline (TGC)
Trimethoprim (TMP)

2.5 Procedure

Protocols and all relevant information were available at the EQAsia website [8], to allow access to all the necessary information at any time. The participants were recommended to store the lyophilized samples in a dark, cool place until performing selective isolation and AST.

Participating laboratories were advised to perform identification and AST of the test strains according to the methods routinely applied in their laboratory.

Procedures as disk diffusion, gradient test, agar dilution and broth dilution were all valid. For the interpretation of results, only the categorisation as resistant/susceptible (R/S) was evaluated,

whereas MIC and Inhibition Zone Diameter values were used as supplementary information.

All participants were invited to enter the obtained results into an informatics module designed for this trial. The informatics module could be accessed through a secured individual login and password. After release of the results, the participants were invited to login to retrieve an individual database-generated evaluation report.

2.6 Data management

2.6.1 Adjusted data

Data analysis revealed a few instances of misinterpretation of results. Participating laboratories were recommended to interpret the obtained results using the tables provided in the Matrix EQA protocol (Appendix 1). To guarantee that all submitted data was interpreted according to the EQAsia guidelines, the data retrieved from the informatics module was revised and, when necessary, adjusted: supplementary MIC/ Inhibition Zone Diameter values reported by the participants were used for adjusting the interpretation (R/S) in accordance to the EQAsia interpretation tables. Adjusting the data allowed for an analysis of the submitted results, which more accurately reflects the laboratories' analytical performance.

In adition, antimicrobial susceptibility testing of the *E. coli* ATCC 25922 reference strain revealed a number of incorrect results. These deviations (results outside the acceptance interval) were caused by the method used for MIC determination. Briefly, MIC determination by broth microdilution using an automated system often tests for an antimicrobial concentration range above the acceptance interval. For example, the quality control range for cefepime for *E. coli* ATCC 25922 is 0.016-0.12, and the laboratories using 'MIC – broth microdilution' reported an MIC ≤ 1. The informatics module scores such result as '0' (incorrect). We are aware, however, that this is a method limitation and the laboratories cannot test for lower antimicrobial concentrations. For these specific occurrences, the score was changed to '1', as the reported values are not necessarily incorrect. **Table 2** summarizes all the situations where this change was applied.

2.6.2 Omitted data

Data analysis exposed a few incorrect results for some strain/antimicrobial combinations, caused by "MIC not possible to interpret issue":

- A "MIC not possible to interpret issue" is defined as a situation where the expected MIC and breakpoint are below the antimicrobial range tested by a laboratory. In this case, the laboratory cannot interpret the result as resistant or susceptible, as both interpretations could be possible. For example, if the expected MIC for ertapenem is 0.12 and ECOFF ≥ 0.06, the strain is categorized as resistant. However, if the result reported by the laboratory is MIC ≤ 0.50, the result cannot be interpreted.

After examining each individual strain/antimicrobial combination, the EQAsia Consortium agreed to omit the following strain/antimicrobial combinations (only for the MIC – broth microdilution' method) from the general analysis, as these test results were not considered representative of the laboratories' capacity for performing AST:

- EQAsia 21.M2/ ETP; EQAsia 21.M3/ FEP; EQAsia 21.M4/ FEP.

Upon omisson of the abovementioned results, the laboratories' performance and deviations were recalculated and presented in this report.

Table 2. Adjusted scores for reported MIC values for *E. coli* ATCC 25922 reference strain. Adjustments were made due to the limitation of the broth microdilution method applied.

***E. coli* ATCC 25922**			
Antimicrobial	**MIC Quality Control Range**	**MIC reported by the labs**	**Score**
Cefepime	0.016-0.12	≤ 1	Changed to '1'
Cefotaxime	0.03-0.12	≤ 0.25	Changed to '1'
Ceftazidime	0.06-0.5	≤ 1	Changed to '1'
Ciprofloxacin	0.004-0.016	≤ 0.25	Changed to '1'
Ertapenem	0.004-0.016	≤ 0.5	Changed to '1'
Meropenem	0.008-0.06	≤ 0.25	Changed to '1'
Tigecycline	0.03-0.25	≤ 0.5	Changed to '1'

3. Results

3.1 Methods used by the participants

Participants were asked to indicate the methods used for selective isolation of the *E. coli* strains, as well as the method used for bacterial identification, during results' submission in the informatics module. **Figure 2** summarizes the methods reported by the participants.

Most laboratories (#03, #07, #21, #22, #30, #33, #37 and #41) reported that selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *E. coli* was performed exactly according to the protocol provided in the Matrix EQA protocol (Appendix 1), which is based in the official EURL-AR protocols [3], meaning that no changes in media, concentrations of antibiotics, etc. were referred (**Figure 2**, left column). The only difference reported was the use of peptone water (Luria Bertani broth not available) for reviving the lyophilized cultures (laboratory #40). Laboratory #37 also referred that besides using MacConkey agar plate containing 1 mg/L cefotaxime, CHROMagar™ ESBL plates were additionally used for selective isolation of ESBL-producing *E. coli*.

Regarding selective isolation of carbapenemase-producers (**Figure 2**, middle column), only laboratory #37 performed carbapenemase selective isolation using CHROMagar™ mSuperCARBA™ plates.

Confirmation of *E. coli* species identification (**Figure 2**, right column) was performed by the majority of the laboratories using biochemical tests, whereas laboratory #07 used chromogenic agar plating. One participant, laboratory #33, used VITEK® 2 Systems for bacterial identification.

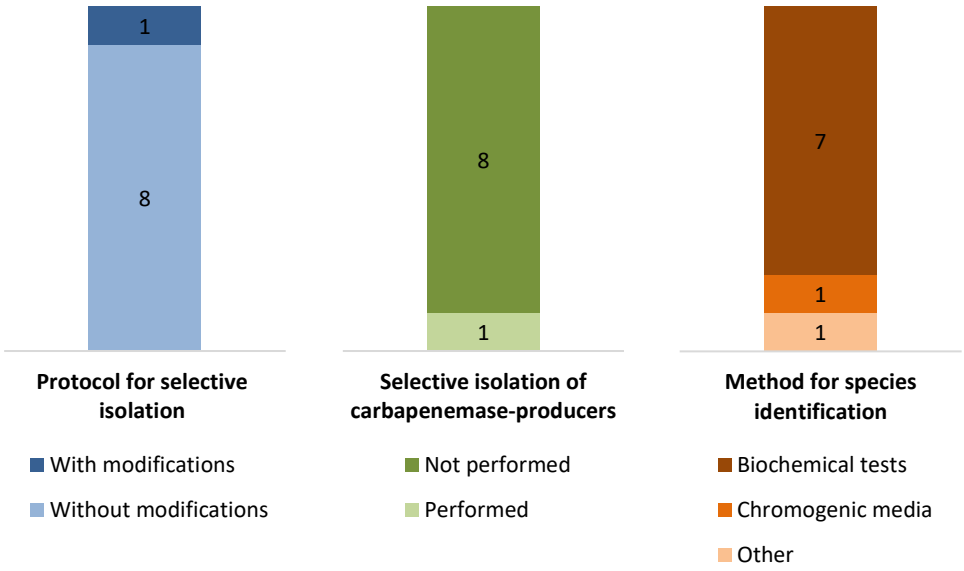

Figure 2. Methods used by the participants for selective isolation and confirmation of *E. coli* species identification.

3.2 ESBL-, AmpC- and carbapenemase-producing *E. coli* isolation and identification

Samples EQAsia 21.M1-M4 were spiked with different *E. coli* isolates and, therefore, expected to be positive for growth of presumptive ESBL-, AmpC- and carbapenemase-producing *E. coli*, whereas sample EQAsia 21.M5 was kept blank and thus expected to be negative. Participants were asked to describe the growth observed in the selective plates (**Table 3**). Eight of the participating laboratories described the growth observed, whereas laboratory #33 did not provide the requested information, even though the laboratory reported results for species identification.

For samples EQAsia 21.M1-M4, 75.0%, 100%, 87.5% and 75.0% of the laboratories, respectively, described the presence of typical *E. coli* colonies, either as a mixed or pure culture. For sample EQAsia 21.M5, only 50.0% of the laboratories reported the absence of typical *E. coli* colonies (**Table 3**).

Table 3. Expected and obtained results for ESBL/AmpC/Carbapenemase-producing *E. coli* isolation and identification. Number of obtained results (n) out of the total of reported results (N) is presented for each growth type and for each sample, as well as for the species identification. Obtained results in accordance with the expected result are shown in bold.

Growth observed on the selective plates	EQAsia 21.M1	EQAsia 21.M2	EQAsia 21.M3	EQAsia 21.M4	EQAsia 21.M5
Mixed culture containing typical *E. coli* colonies	**4/8 (50.0%)**	**3/8 (37.5%)**	**3/8 (37.5%)**	**3/8 (37.5%)**	3/8 (37.5%)
Mixed culture without typical *E. coli* colonies	1/8 (12.5%)	--	1/8 (12.5%)	--	**1/8 (12.5%)**
Pure culture of typical *E. coli* colonies	**2/8 (25.0%)**	**5/8 (62.5%)**	**4/8 (50.0%)**	**3/8 (37.5%)**	1/8 (12.5%)
Pure culture without typical *E. coli* colonies	1/8 (12.5%)	--	--	1/8 (12.5%)	**2/8 (25.0%)**
No growth	--	--	--	1/8 (12.5%)	**1/8 (12.5%)**
Confirmation of species identification	**Positive**	**Positive**	**Positive**	**Positive**	**Negative**
Positive	**7/9 (77.8%)**	**9/9 (100%)**	**8/9 (88.9%)**	**6/8 (75.0%)**	4/9 (44.4%)
Negative	2/9 (22.2%)	--	1/9 (11.1%)	2/8 (25.0%)	**5/9 (55.6%)**

(n/N) number of responses (n) out of the total of reported results (N)

Results confirming the species identification were reported by all nine laboratories (**Table 3** and **Figure 3**):

- EQAsia 21.M1: sample was confirmed as positive by all six laboratories reporting typical *E. coli* colonies and by laboratory #33. Laboratories #22 and #41 considered the sample negative;
- EQAsia 21.M2: sample was confirmed as positive by all nine laboratories;
- EQAsia 21.M3: sample was confirmed as positive by all seven laboratories reporting typical *E. coli* colonies and by laboratory #33. Laboratory #41 considered the sample negative;
- EQAsia 21.M4: sample was confirmed as positive by five of the laboratories reporting typical *E. coli* colonies and by laboratory #33. Laboratory #07, which observed growth as 'Pure culture of typical *E. coli* colonies' did not report results for species identification. Laboratories #40 and #41 considered the sample negative;
- EQAsia 21.M5: sample was confirmed as negative by two of the laboratories reporting no typical *E. coli* colonies/no growth and by laboratory #33. Laboratories #07 and #41, which observed growth as 'Pure/Mixed culture without typical *E. coli* colonies', respectively, left the identification field blank, which was considered as 'sample negative'. Laboratories #03, #21, #30 and #37 considered the sample positive.

In summary (**Figure 3**), 4 laboratories reported all samples as positive for presumptive ESBL-, AmpC- or carbapenemase-producing *E. coli*. Only laboratory #33 correctly reported all five samples: EQAsia 21.M1-M4 samples as positive and EQAsia 21.M5 sample as negative.

As mentioned in section 3.1, laboratory #37 performed additional carbapenemase selective isolation that resulted in growth of sample EQAsia 21.M2 on the CARBA-selective plates. This observation is in line with the expected results and will be further discussed in section 3.4.

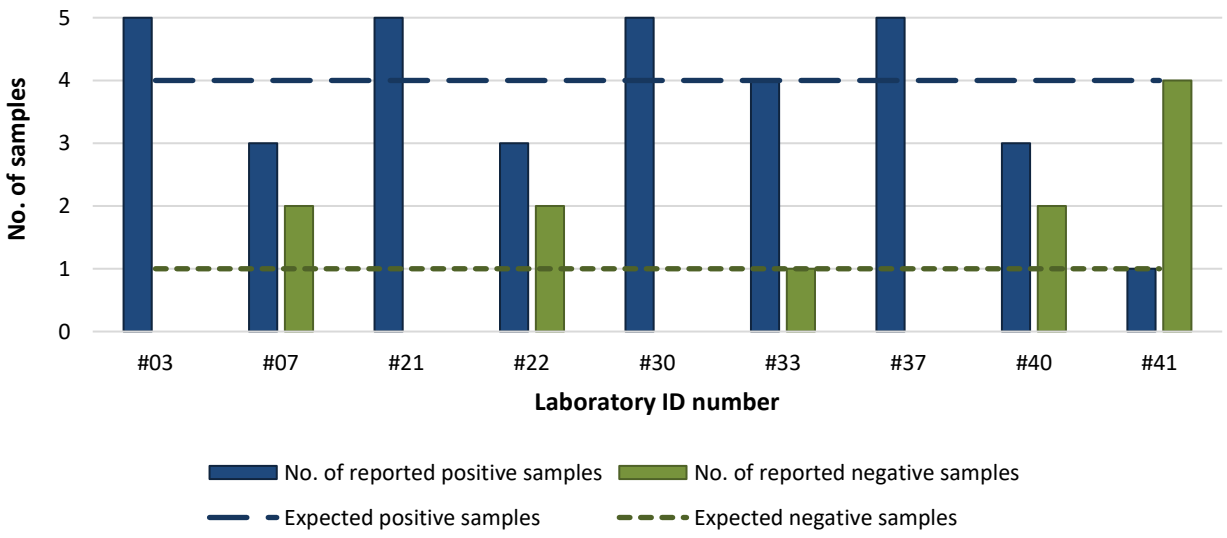

Figure 3. Expected and obtained results for confirmation of species identification by the participating laboratories.

3.3 Antimicrobial Susceptibility Testing

For the samples considered positive for ESBL- , AmpC- or carbapenemase-producing *E. coli*, one *E. coli* isolate per sample should be taken and further tested for susceptibility towards the antimicrobials suggested in the Matrix EQA protocol and outlined in **Table 1** (Appendix 1).

Of the nine laboratories submitting results, only six (#03, #21, #22, #30, #33 and #37) submitted results for AST. For sample EQAsia 21.M1 and respective *E. coli* isolate, only results from 5 laboratories were considered (laboratory #22 identified the isolated strain as *Salmonella*), and for samples EQAsia 21.M2-M4 (and respective *E. coli* isolates), results from all 6 laboratories were evaluated.

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R') or susceptible ('S') for each strain/antimicrobial combination. Only the categorisation was evaluated, whereas the Inhibition Zone Diameters/MIC values were used as supplementary information.

The Matrix EQA set-up allowed laboratories to choose the antimicrobials to be tested among the panel of suggested antimicrobials (**Table 1**). Among all tests, colistin was tested by only a couple of laboratories; the remaining antimicrobials were almost evenly tested by the participating laboratories (**Table 4**). Regarding the methodology applied, laboratories #21, #22 and #33 exclusively used disk diffusion for testing the antimicrobials and, therefore, reported Inhibition Zone Diameters; laboratory #30 applied broth microdilution for MIC determination; laboratory #03 applied broth microdilution for the majority of the antimicrobials, except for cefoxitin (disk diffusion); and finally laboratory #37 opted for disk diffusion for testing all antimicrobials apart from colistin, which was tested by the broth microdilution method (**Table 4**).

Table 4. Total of Antimicrobial Susceptibility Tests performed for each antimicrobial and by method applied.

Antimicrobial	ASTs performed		
	Disk Diff.	MIC	Total
AMP	15	8	23 (6.8%)
AZI	15	4	19 (5.6%)
FEP	15	1	16 (4.7%)
FOT	15	4	19 (5.6%)
FOX	19	--	19 (5.6%)
TAZ	15	8	23 (6.8%)
CHL	15	4	19 (5.6%)
CIP	15	8	23 (6.8%)
COL	--	8	8 (2.4%)
ETP	11	3	14 (4.1%)
GEN	15	8	23 (6.8%)
IMI	15	--	15 (4.4%)
MERO	15	8	23 (6.8%)
NAL	15	4	19 (5.6%)
SMX	11	4	15 (4.4%)
TET	15	4	19 (5.6%)
TGC	15	8	23 (6.8%)
TMP	15	4	19 (5.6%)
Total	**251**	**88**	**339 (100%)**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; MIC – MIC determination by broth microdilution

The AST performance of the laboratories can be analysed from a strain-, antimicrobial-, and laboratory-based perspective. From a strain-analysis point of view (**Figure 4**), the *E. coli* strain used to spike sample EQAsia 21.M2 presented the lowest deviation (8.0%), meaning that most of the susceptibility results obtained were in accordance with the expected (Appendix 2). The other three samples revealed more than 10% deviation, where the highest deviation (22.5%) was observed for the *E. coli* strain isolated from EQAsia 21.M3.

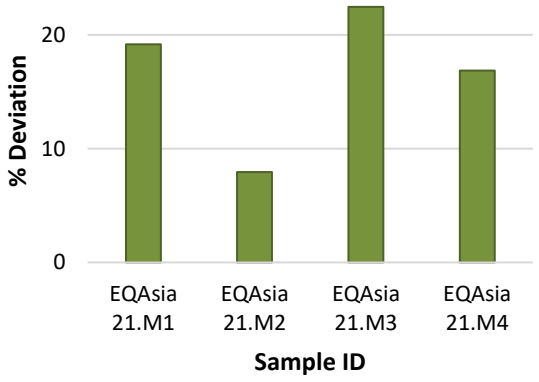

Figure 4. Percentage of deviation in the AST interpretation (R/S) per sample in the EQAsia Matrix EQA 2021.

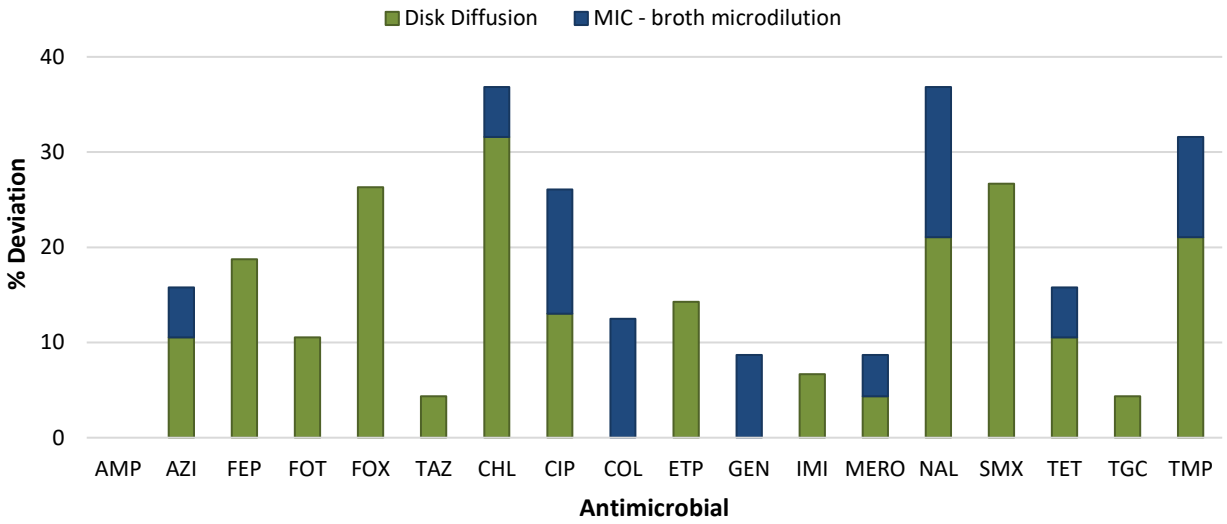

Figure 5. Percentage of deviation in the AST interpretation (R/S) among the matrix samples in EQAsia Matrix EQA 2021. The bars represents the total deviation for each antimicrobial agent, with the contribution of each of the methods applied (Disk diffusion and MIC – broth microdilution).

Antimicrobials with highest deviations from the expected result were chloramphenicol (36.8%), nalidixic acid (36.8%) and trimethoprim (31.6%), whereas ampicillin revealed no deviation from the expected results (**Figure 5**). Chloramphenicol, for example, should be interpreted as resistant if MIC ≥ 32; the *E. coli* isolate from sample EQAsia 21.M1 has an expected MIC value of 16, and the *E. coli* isolates from samples EQAsia 21.M3 and M4 have an expected MIC value of 32. In both situations, the expected result is around the breakpoint, meaning that a one-fold dilution/±3mm difference (a limitation in the method reproducibility) can result in a different interpretation, which can explain the high deviation observed for this antimicrobial (**Figure 5**).

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for only one participant, laboratory #03 (**Figure 6**). In average, the deviation was 16.3% (ranging from 0.0 to 41.2%).

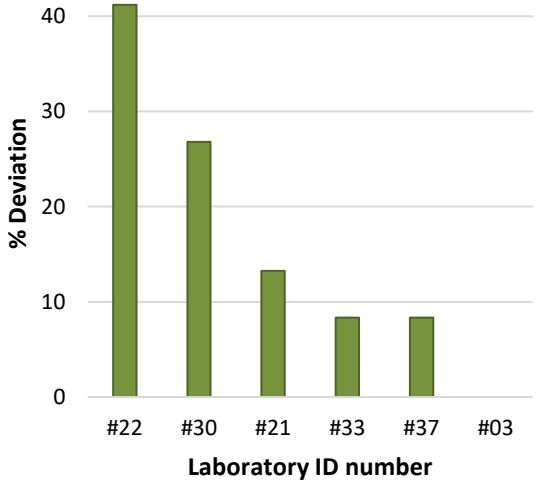

Figure 6. Percentage of deviation in the AST interpretation (R/S) among the matrix samples in EQAsia Matrix EQA 2021. Results are categorized by laboratory ID number.

Laboratory #22 presented several deviations from the expected results. As an example, the deviations observed for the *E. coli* strain used to spike sample EQAsia 21.M2 were entirely reported by this laboratory (**Figures 4 and 6**). The laboratory also seemed to have difficulties testing some antimicrobials: for instance, all strains were reported as highly resistant to

ciprofloxacin, nalidixic acid and trimethoprim, whereas the exact opposite was expected.

Laboratory #30' deviations all consisted in higher than expected MIC values for certain antimicrobials that led to interpretations as resistant, although the expected result was susceptible.

Most of the deviations observed for laboratories #22, #33 and #37 are within the method deviation (±3mm difference between the Inhibition Zone Diameter reported and the threshold), which should not be seen by the participants as a poor AST performance.

Laboratory #03 tested fewer antimicrobial coumpounds compared to the other laboratories (9 out of 20), but all of the submitted results were in accordance with the expected results.

3.4 ESBL, AmpC and carbapenemase phenotypic testing

Four lyophilized samples mimicking meat content were included in this Matrix EQA. The sample EQAsia 21.M1 contained an *E. coli* isolate expressing AmpC phenotype (**Table 5**). Only two laboratories (#03 and #33) correctly classified the phenotype; laboratory #30 considered the isolate as susceptible (no ESBL, AmpC or carbapenemase), even though they found the strain to be resistant to both cefotaxime and ceftazidime; laboratory #21 classified the strain as ESBL + AmpC phenotype as they observed synergy between cefotaxime alone and in combination with clavulanic acid (a 5 mm increase of diameter for FOT/Cl compared to FOT alone); laboratory #37 reported an ESBL phenotype, as they also observed synergy and reported the strain susceptible to cefoxitin.

The *E. coli* isolate from sample EQAsia 21.M2 expresses a carbapenemase phenotype (**Table 5**). Again, only two laboratories reported the correct phenotype (laboratories #03 and #37); laboratories #21 and #33, even though reported the wrong phenotype, also reported the strain resistant to meropenem and commented in the informatics module that the strain could also be classified as a carbapenemase producer; laboratory #22 observed synergy and resistance towards cefoxitin and, thus, classified the strain as ESBL + AmpC phenotype; laboratory #30 reported ESBL phenotype, although the strain was found to be resistant to meropenem.

Samples EQAsia 21.M3 and EQAsia 21.M4 were both spiked with *E. coli* isolates expressing ESBL phenotype (**Table 5**). In both situations, the same four laboratories (#03, #30, #33 and #37) obtained the correct phenotype; laboratories #21 and #22 classified both strains as ESBL + AmpC phenotype, due to finding the strains resistant to cefoxitin.

In summary, among the six participating laboratories, only laboratories #03 and #33 correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the four *E. coli* strains.

Table 5. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *E. coli* strains used to spike the matrix samples. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold.

Sample ID		EQAsia 21.M1	EQAsia 21.M2	EQAsia 21.M3	EQAsia 21.M4
Expected results		AmpC	Carbapenemase	ESBL	ESBL
Obtained results (n/N)	ESBL	1/5 (20.0%)	1/6 (16.7%)	**4/6 (66.7%)**	**4/6 (66.7%)**
	AmpC	**2/5 (40.0%)**	1/6 (16.7%)	--	--
	ESBL + AmpC	1/5 (20.0%)	2/6 (33.3%)	2/6 (33.3%)	2/6 (33.3%)
	Carbapenemase	--	**2/6 (33.3%)**	--	--
	Susceptible*	1/5 (20.0%)	--	--	--

*no AmpC, ESBL and carbapenemase; (n/N) number of responses (n) out of the total of reported results (N)

3.5 Quality control strains

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge to all participating laboratories to be used as reference strains. Antimicrobial susceptibility test results for the quality control strains were submitted by eight of the participating laboratories (#03, #07, #22, #30, #33, #37, #40 and #41).

The participants used different methodologies for testing the reference strain: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by broth microdilution. The highest proportion of test results outside of the expected range were observed for cefotaxime, whereas no deviations were observed for ertapenem, nalidixic acid and tigecycline (**Table 6**). In addition, there seemed to be more deviations when the antimicrobials were tested by disk diffusion then by broth microdilution. In fact, only one deviation (colistin) was reported by laboratory #33.

The laboratories' performance is summarized in **Figure 7**. Laboratories #03, #07 and #30 presented no deviation, and strikingly, all of them applied broth microdilution for MIC determination. Inversely, laboratories' #33 and #40 deviations were as high as 60.0% and 42.9%, respectively.

Laboratory #40 obtained 6 out of 14 results outside of range, but all of them were just slightly (1-2mm) below the acceptance interval (except sulfamethozaxole, which was 2mm above). A similar scene is observed for laboratory #33, whereas most of the deviations (total of 9 out of 15 test results) were 1-2mm above the acceptance interval.

Laboratories #22, #37 and #41 obtained 1, 3 and 2 deviations, respectively, all of them just 1-2mm outside of range.

Table 6. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMP	1/5	0/3	**1/8**
FEP	1/4	0/1	**1/5**
FOT	3/5	0/1	**3/6**
FOX	1/5	--	**1/5**
TAZ	2/5	0/3	**2/8**
CHL	1/5	0/1	**1/6**
CIP	2/5	0/2	**2/7**
COL	--	1/3	**1/3**
ETP	0/2	0/1	**0/3**
GEN	2/5	0/3	**2/8**
IMI	1/5	--	**1/5**
MERO	2/5	0/2	**2/7**
NAL	0/5	0/1	**0/6**
SMX	1/3	0/1	**1/4**
TET	2/5	0/1	**2/6**
TGC	0/3	0/3	**0/6**
TMP	1/5	0/1	**1/6**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; MIC – MIC determination by broth microdilution

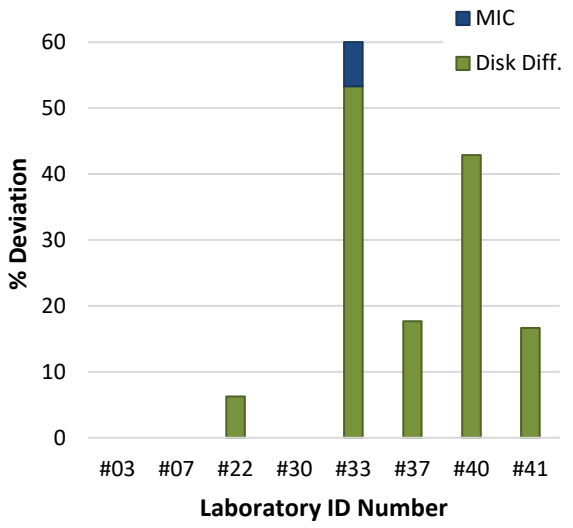

Figure 7. Percentage of deviation in the AST of the quality control strains.

4. Discussion

A total of nine laboratories from both the HH and AH Sectors participated in the 1st EQAsia Matrix EQA on selective isolation of presumptive ESBL-AmpC- and carbapenemase-producing *E. coli* from cultures mimicking meat samples. In general, the participants used the recommended methods for selective isolation, which are based in the official EURL-AR protocols [3]. Only one laboratory had the means to perform carbapenemase selective isolation using specific selective plates. Bacterial identification was mostly achieved by the use of biochemical tests.

Samples EQAsia 21.M1-M4 were expected to be positive for growth of presumptive ESBL-, AmpC- or carbapenemase-producing *E. coli*, whereas sample EQAsia 21.M5 was expected to be negative. Regarding samples EQAsia 21.M1-M4, at least 75% of the laboratories correctly identified the samples as positive for the presence of *E. coli* colonies. Sample EQAsia 21.M5 seemed to cause more problems as several laboratories reported the sample as positive (44.4%) instead of negative. In fact, only one laboratory correctly identified all the samples as positive/negative, which demonstrates the need for more education and training in the selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing bacteria from complex matrices.

Of the nine laboratories submitting results, only six submitted results for antimicrobial susceptibility testing. The AST performance was assessed from different perspectives to better identify deviations from the expected results. Hence, the strain-based analysis revealed that the *E. coli* strain isolated from sample EQAsia 21.M2 presented the lowest deviation from the expected results in comparison to the other three isolates. One possible explanation could be the high level of resistance of this strain towards cefotaxime (MIC > 64), which assured the growth of this strain on the selective MacConkey agar plate containing 1 mg/L cefotaxime. The other isolates, which are less resistant to cefotaxime (Appendix 2) may be overgrown by contaminating bacteria and more challenging to isolate. Nevertheless, it is not completely clear if the submitted results regarding the other *E. coli* results are due to the isolation and testing of other bacteria than the one used to spike the samples or due to performance issues. For instance, the deviations in the AST results from the *E. coli* isolate contained in sample EQAsia 21.M1 were mostly caused by reported Inhibition Zone Diameter values being close to the cut-off epidemiological values and leading to the wrong interpretation. Only one laboratory reported results very different from the expected.

On the other hand, AST results from the *E. coli* isolates from samples EQAsia 21.M3 and M4 revealed several deviations caused by three different laboratories, which could suggest that the obtained results originated from isolates other than the ones used to spike the meat samples.

In summary, these results led to an average deviation of 16.3% in the laboratories' AST performance, with only one laboratory showing no deviation from the expected results.

The high number of incorrect results reported by the laboratories also led to inaccurate classification of the *E. coli* phenotypes into ESBL, AmpC or carbapenemase production. Still, some laboratories seem to have issues in classifying the isolates as carbapenemase producers when the strain is found to be resistant to meropenem. In addition, only half of the laboratories (3 out of 6) performed confirmatory testing, meaning testing the strains towards cefotaxime/ceftazidime in combination with the β-lactamase inhibitor clavulanic acid to determine synergy/no-synergy. These observations demonstrate the need for capacity building within this area.

Lastly, AST of the quality control strains demonstrated that the laboratories' performance was somehow methodology-dependent, as almost no deviations were reported by the

laboratories applying broth microdilution for MIC determination. For the laboratories reporting Inhibition Zone Diameters determined by disk diffusion, the majority of the results outside the quality control range were just slightly (1-2mm) below/above the acceptance interval. It demonstrates possible technical problems in performing AST, but may also suggest problems in maintenance of the quality control strains.

5. Conclusion

This report presented the results of the first EQAsia Matrix EQA trial 2021. This EQA assessed the performance in 1) isolation and identification of presumptive ESBL-, AmpC-, and carbapenemase-producing *E. coli* from cultures mimicking meat content, 2) AST determination and interpretation and 3) detection and classification of β-lactam resistance phenotypes mediated by ESBL, AmpC and carbapenemase enzymes.

The goal of the EQAsia Matrix EQAs is to have all participating Human and Animal Health laboratories being able to provide quality data to be used for the comparability of surveillance data on ESBL-, AmpC- or carbapenemase-producing *E. coli* reported by different laboratories.

This first Matrix EQA trial allowed the EQAsia Consortium to have an overview of the laboratories capacity for a complete participation in such a proficiency test. Firstly, only nine laboratories participated in the trial, even though 15 laboratories initially signed-up. The reason for not participating was mainly the lack of essential resources, such as selective media/plates. Secondly, not all of the participating laboratories submitted results for all the components. For example, only six laboratories reported AST results. Thirdly, there seems to be still some confusion on the classification of the resistance phenotypes, which certainly requires more training in the subject. Lastly, the laboratories lack resources required for this type of proficiency test, such as cefotaxime/clavulanic acid or ceftazidime/clavulanic acid combination required for confirmatory testing.

On a final note, even though this trial was initially meant for laboratories of the Animal Health Sector, since ESBL-, AmpC- and carbapenemase-producing *E. coli* continue to spread in food producing animals, we were pleased to witness the insterest from the Human Health laboratories in participating, aligned with the concept of the WHO, FAO, OIE tripartite Tricycle project. In fact, the increasing number of this type of strains is concerning and it is of high importance to support all type of laboratories with technical guidance and capacity building. In future EQAs, the EQAsia Consortium therefore aims to offer samples from other complex matrices that could be more relevant for the Human Health Sector.

6. References

[1] Annex 8: Pathogen-antimicrobial combinations under GLASS-AMR surveillance. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

[2] FAO. 2019. Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption. Regional Antimicrobial Resistance Monitoring and Surveillance Guidelines – Volume 1. Bangkok.

[3] EURL-AR protocols: https://www.eurl-ar.eu/protocols.aspx

[4] Clinical and Laboratory Standards Institute;

M100. Performance standards for Antimicrobial Susceptibility Testing 31st edit, January 2021.

[5] The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org.

[6] EUCAST Website: https://www.eucast.org/

[7] 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.

[8] EQAsia Website: https://antimicrobialresistance.dk/eqasia.aspx

7. Appendices

Appendix 1: Matrix EQA 2021 Protocol

Protocol for EQAsia Matrix EQAS

Selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from cultures mimicking meat samples

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 EQAsia consortium and supported by the Fleming Fund.

The 2nd iteration of EQAsia EQAS includes a selective isolation of presumptive extended spectrum beta-lactamase (ESBL)-, AmpC- or carbapenemase-producing *Escherichia coli*, as well as antimicrobial susceptibility testing (AST) of obtained isolates from five cultures mimicking meat content. These consist of five lyophilized bacterial cultures obtained from 25g samples of pork minced meat. Briefly, 25g of pork minced meat were spiked with an *E. coli* strain. The meat sample was then pre-enriched by the addition of 225 ml of Buffered Peptone Water (BPW, **Appendix 1**) and incubated at 37°C ± 1°C for 18-22 h (pre-enrichment step as referred in the official EURL-AR protocols). A loopfull of the pre-enriched culture was plated and incubated overnight. The grown

cells (a mixture of the different bacteria present in the meat sample) were then scrapped and a lyophilized culture prepared. These lyophilized cultures may contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes.

The procedures described here, on how to perform the selective bacterial isolation, follow the EU recommended methods published on the EURL-AR website.

Additionally, antimicrobial susceptibility testing of the reference strains *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) for quality control (QC) in relation to antimicrobial susceptibility testing is included. 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. 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 quality of results obtained in the selective isolation of presumptive ESBL-, AmpC- or carbapenemase-producing isolates from minced meat samples. A further objective is to assess and improve the comparability of surveillance data on ESBL-, AmpC- or carbapenemase -producing *E. coli* reported by different laboratories. Therefore, the laboratory work for this EQAS should be performed using the methods routinely applied in your laboratory. Additional methodology for selective isolation is provided in section 3.2.

3 OUTLINE OF THE EQAS 2021

3.1 Shipping, receipt and storage of strains

In July/August 2021, participating laboratories located in South and Southeast Asia will receive a parcel from Dr. Rungtip Chuanchuen, Chulalongkorn University, Thailand containing five lyophilized cultures obtained from meat samples. The lyophilized cultures obtained from spiked matrix samples of pork meat content will be distributed in separate tubes labelled from M.1 to M.5. Participants should expect that ESBL-, AmpC- and/or carbapenemase-enzymes producing strains will be included in some of the lyophilized cultures.

Upon arrival, the lyophilized cultures must be stored in a dark, cool place until microbiological analysis. This should be initiated as soon as possible after receipt in the laboratory.

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

All strains used in the spiking of samples are categorised as 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 Selective isolation of ESBL, AmpC or carbapenemase producing *E. coli* from the samples

Testing of meat samples requires a pre-enrichment step as referred in the official EURL-AR protocols. As the provided samples are lyophilized cultures mimicking meat content, no pre-enrichment step is required (it has already been done by us). Instead, the lyophilized cultures should be revived before proceding to the selective isolation. The steps are briefly described here (detailed information can be found on the official EURL-AR protocols):

1- Add 0.5 mL of sterile Luria Bertani broth into the lyophilized cells. Mix gently and carefully to avoid creating aerosols. Then, transfer all re-suspended cells into 5mL fresh Luria Bertani broth. For instructions on how to open and handle the ampoules, please see the document 'Instructions for opening and reviving lyophilised cultures of test strains (Animal health laboratories)' on the EQAsia website.

2- After mixing gently the culture, subculture one loopful (10µL loop) by applying a single streak onto a MacConkey agar plate containing 1 mg/L of cefotaxime (**Appendix 1**). From this streak, further two streaks are made using either the same loop or a 1µL loop to ensure growth of single colonies. Incubate the plates at 44°C ± 0.5°C for 18-22 h.

3- Based on colony morphology (presumptive ESBL-/AmpC producing *E. coli* colonies will usually be red/purple on the MacConkey agar plates containing 1 mg/L cefotaxime – see **Figure 1**), subculture individual colonies onto MacConkey agar containing 1 mg/L cefotaxime to maintain the selective pressure. Up to three colonies should be individually subcultured. Incubate at 37°C ± 1°C for 18-22 h. Subsequently, select one of these subcultures for species identification (ID). In case the first subculture is not identified as *E. coli*, the second and eventually the third subculture shall be tested.

4- One confirmed *E. coli* isolate presumptively producing ESBL- /AmpC shall be re-subcultured to avoid contamination and to confirm the growth in presence of 1 mg/L cefotaxime. This is

performed by picking one single colony from the subculture and streaking it on a new plate of the relevant selective agar, which is then incubated at 37°C ± 1°C for 18-22 h. This re-subcultured bacterial isolate should be stored under appropriate conditions 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.

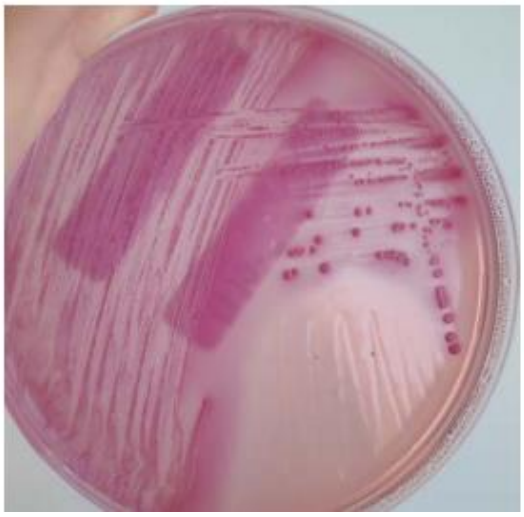

Figure 1: Typical appearance of *E. coli* on MacConkey agar supplemented with 1 mg/L cefotaxime.

The participants are responsible for assuring the validity of the plates by testing a positive (a known ESBL-/AmpC producing *E. coli*) and a negative (ESBL-/AmpC **non**-producing *E. coli*) control. A protocol for 'Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC producing *E. coli* in meat and animals' is available on the EURL-AR webpage.

3.3 Antimicrobial susceptibility testing

If the sample is considered positive for ESBL- , AmpC- or carbapenemase -producing *E. coli*, one *E. coli* isolate per sample should be taken further and tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test forms and in **Table 1**. However, in the impossibility of testing them all, 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 developed by EUCAST. When not available, CLSI zone diameter and MIC breakpoint values are used instead.

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 *E. coli* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Ampicillin, AMP	≥ 16	< 14
Azithromycin, AZI[a]	≥ 32*	≤ 12*
Cefepime, FEP[a]	≥ 0.50	< 28
Cefotaxime, FOT	≥ 0.50	< 21
Cefotaxime, FOT + clavulanic acid	NA	NA
Cefoxitin, FOX	≥ 16	< 17
Ceftazidime, TAZ	≥ 1	< 20
Ceftazidime, TAZ + clavulanic acid	NA	NA
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.

Beta-lactam and carbapenem resistance

The following tests are for the confirmation of ESBL-, AmpC-, and carbapenemase-producing *E. coli* isolates:

- 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 2** 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

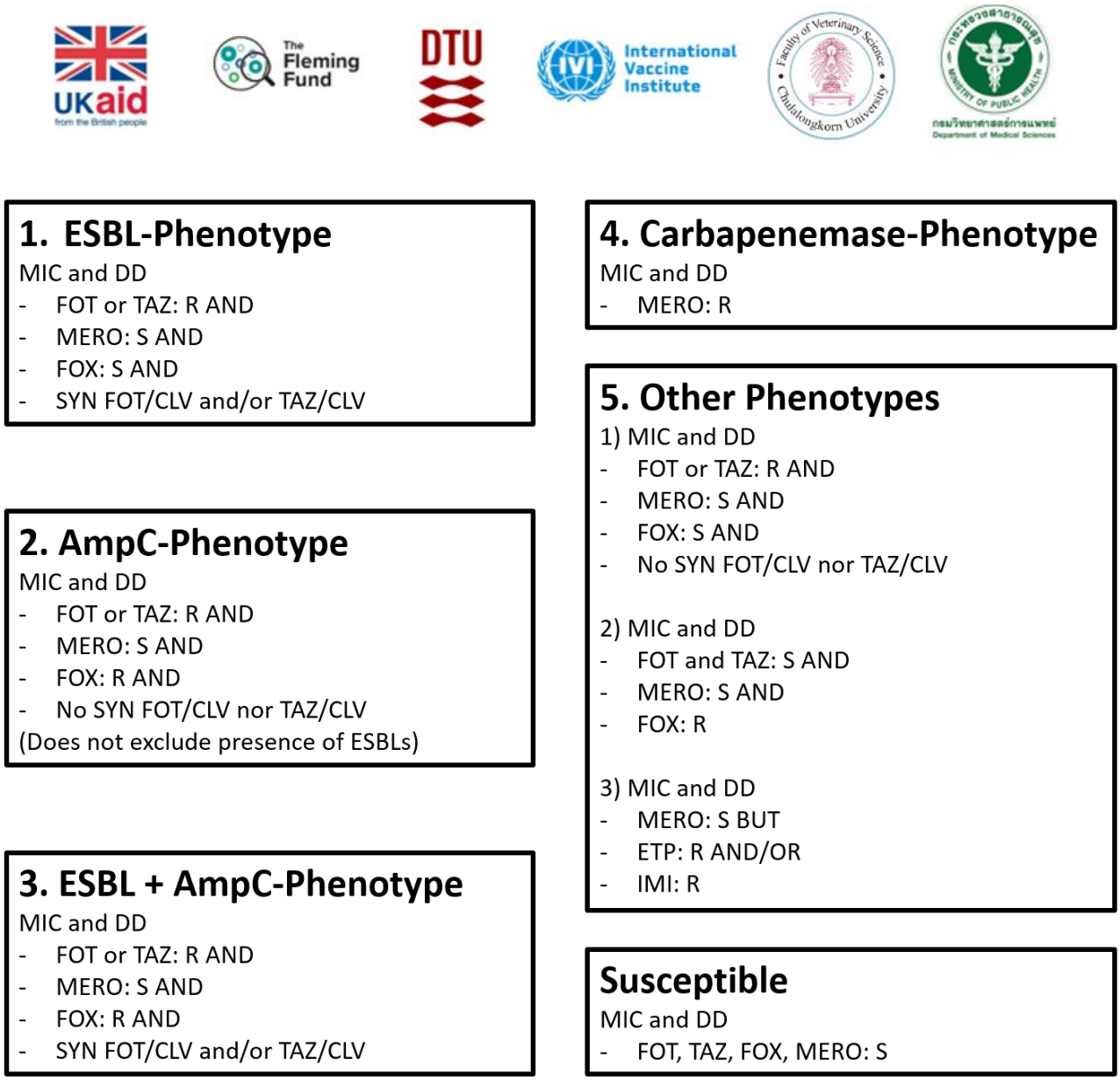

Figure 2: Adapted from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018.

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 is not requested as part of this EQAS.

Even though this protocol for monitoring ESBL- and AmpC-producing *E. coli* has the potential to detect also most variants of carbapenemases produced in *E. coli*, as these normally confer reduced susceptibility to third-generation cephalosporins, an exception is represented by OXA-48 and OXA-48-like producers, which will be undetected by using the ESBL/AmpC monitoring protocol unless they simultaneously co-produce an ESBL or an AmpC enzyme. Therefore, to specifically isolate carbapenemase-producing *E. coli* (including strains producing OXA-48 and OXA-48-like enzymes)

from the cultures mimicking meat samples, it may be required to choose selective agar plates that have been validated with regard to specificity and sensitivity of detection of carbapenemase-producing *E.coli*. For example, commercially available chromogenic agar for isolation of carbapenemase-producing *E. coli* (including isolates producing only OXA-48 and/or OXA-48-like enzymes) can be used. A protocol for 'Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*' is available on the EURL-AR webpage. We encourage you to perform the validation, but it is optional and not 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 '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 in 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 trouble 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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APPENDIX 1

Composition and preparation of culture media and reagents (available on EURL-AR protocols)
The Buffered Peptone Water (BPW), MacConkey agar media and reagents are available from several companies. The composition of the dehydrated media given below is an example and may vary slightly among the different manufacturers. Of note, the media should be prepared according to the manufacturer's instructions, if they differ from the description given here.

Buffered peptone water (Example)

Formula	g/L
Enzymatic digest of casein	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9.0
Potassium dihydrogen phosphate (KH_2PO_4)	1.5
pH 7.0 +/- 0.2 @ 25°C	

Dissolve the components in water by heating if necessary. Adjust the pH so that after sterilization it is 7.0+/- 0.2 at 25°C. Dispense the medium into flasks of suitable capacity to obtain the portions necessary for the test. Sterilize by autoclaving at 121°C for 15 minutes.

MacConkey agar (Example)

Formula	g/L
Pancreatic Digest of Gelatin	17.0
Peptones (meat and casein)	3.0
Lactose	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5
pH 7.1 +/- 0.2@ 25°C	

Suspend 50 g in 1 L of distilled water (Optional: Add 6.5 g agar to increase the hardness of the agar plates). Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

Selective Supplements

Formula	mg/mL
Cefotaxime sodium salt stock solution prepared in bi-distilled water	1

It is important to take into account the potency of the drug to ensure that 1 mg/mL active compound is used. Aliquots of aqueous cefotaxime stock solution (concentration 1 mg/mL) can be stored at -20°C.
Example: If the manufacturer has stated a potency of 50%, 2 mg of antibiotic powder should be added to 1 mL of sterile dH2O (or the solvent routinely used) to reach a final concentration of 1 mg/mL of active compound.

Appendix 2: Reference values (MIC) – *Escherichia coli*

Reference values (MIC values and interpretation) – *Escherichia coli*

	Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		FOT+clav F/C	Cefoxitin FOX		Ceftazidime TAZ		TAZ+clav T/C	Chloramphenicol CHL		Ciprofloxacin CIP	
EQAsia 21.M1	> 64	R	> 64	R	0.25	S	2	R	2/4	64	R	8	R	8/4	16	S	0.03	S
EQAsia 21.M2	> 64	R	4	S	> 32	R	> 64	R	> 64/4	> 64	R	> 128	R	128/4	≤ 8	S	0.03	S
EQAsia 21.M3	> 64	R	8	S	1	R	4	R	≤ 0.06/4	4	S	8	R	≤ 0.12/4	32	R	≤ 0.015	S
EQAsia 21.M4	> 64	R	4	S	0.5	R	4	R	≤ 0.06/4	2	S	16	R	≤ 0.12/4	32	R	≤ 0.015	S

R, Resistant; S, Susceptible

	Colistin COL		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Meropenem MERO		Nalidixic acid NAL		Sulfamethoxazole SMX		Tetracycline TET		Tigecycline TGC		Trimethoprim TMP	
EQAsia 21.M1	≤ 1	S	0.03	S	≤ 0.5	S	≤ 0.12	S	≤ 0.03	S	8	S	> 1024	R	4	S	0.5	S	> 32	R
EQAsia 21.M2	≤ 1	S	2	R	2	S	8	R	8	R	≤ 4	S	16	S	≤ 2	S	≤ 0.25	S	≤ 0.25	S
EQAsia 21.M3	≤ 1	S	≤ 0.015	S	1	S	0.25	S	≤ 0.03	S	≤ 4	S	> 1024	R	≤ 2	S	≤ 0.25	S	0.5	S
EQAsia 21.M4	≤ 1	S	≤ 0.015	S	≤ 0.5	S	≤ 0.12	S	≤ 0.03	S	≤ 4	S	> 1024	R	64	R	0.5	S	≤ 0.25	S

R, Resistant; S, Susceptible

Appendix 3: Quality control ranges for reference strains

Quality Control ranges for reference strains

E. coli ATCC 25922		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Ampicillin, AMP	2-8	15-22
Azithromycin, AZI	--	--
Cefepime, FEP	0.016-0.12	31-37
Cefotaxime, FOT	0.03-0.12	29-35
Cefotaxime + clavulanic acid, F/C	--	--
Cefoxitin, FOX	2-8	23-29
Ceftazidime, TAZ	0.06-0.5	25-32
Ceftazidime + clavulanic acid, T/C	--	--
Chloramphenicol, CHL	2-8	21-27
Ciprofloxacin, CIP	0.004-0.016	29-38
Ertapenem, ETP	0.004-0.016	29-36
Gentamicin, GEN	0.25-1	19-26
Imipenem, IMI	0.06-0.25	26-32
Meropenem, MERO	0.008-0.06	28-35
Nalidixic acid, NAL	1-4	22-28
Sulfamethoxazole, SMX	8-32	15-23
Tetracycline, TET	0.5-2	18-25
Tigecycline, TGC	0.03-0.25	20-27
Trimethoprim, TMP	0.5-2	21-28

MIC ranges and disk diffusion ranges are according to CLSI M100 31st edition, Tables 4A-1 and 5A-1

E. coli NCTC 13846		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Colistin, COL	2-8	--

MIC range in accordance to “The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org.”

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