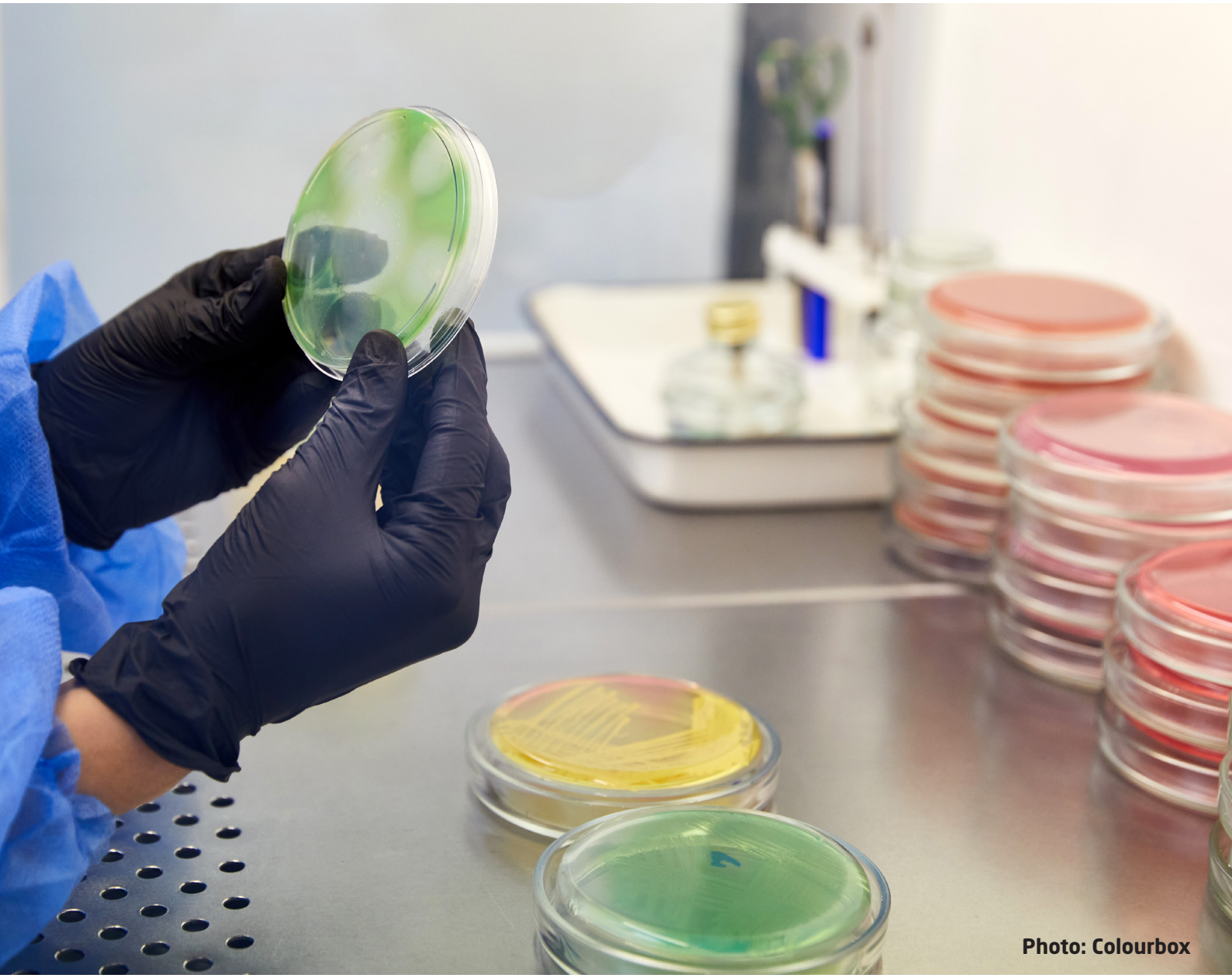
The 1st EQAsia External Quality Assessment trial: *Escherichia coli* and *Salmonella* spp. - 2021



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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 and FAO priority pathogens.

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) in Thailand and the Faculty of Veterinary Science, Chulalongkorn University (CU) 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 CU 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. The EQA program is supported by an informatics module where laboratories can report their results and methods applied.

Three EQA trials are taking place during Feb 2021 – Feb 2022. The EQA trials focus on the WHO GLASS pathogens and FAO priority pathogens (see Section 7. References): *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Campylobacter* (*C. coli* and *C. jejuni*), Enterococci (*E. faecium* and *E. faecalis*), *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*. In addition, a Matrix EQA is offered, aligning with the scope of WHO Tricycle and suggested from FAO, aiming to assess the veterinary laboratories' ability to detect ampC beta-lactamases (ampC), extended-spectrum beta-lactamases (ESBL) and carbapenemase producing *E. coli* from animal caeca samples and food matrices.

For a given organism, candidate strains are assessed and validated by DTU and the external partner (United States Food and Drug Administration, FDA). The validation includes 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 test strains are then selected based on the phenotypic AMR profile to include a heterogeneous panel, allowing for strain variation from almost pan-resistant to fully susceptible isolates.

Each EQA trial encompasses the testing of a total of 11 test strains of a given organism. Of these, eight of the test strains are of the organism in focus (target organism), whereas three test strains are different from the targeted species (reported as non-[organism], e.g. non-*Salmonella*). For each of the 11 test strains, participants are requested to report which eight strains belong to the expected target organism. For the three organisms different from the expected, no further testing is required. For the remaining eight test strains of the target organism, results in relation to AST and serotyping (if relevant) are requested.

This report contains results from the first EQA trial of the EQAsia project carried out in February-April 2021. This first EQA trial includes serotyping of *Salmonella* spp., as well as identification and AST of *Salmonella* spp. and *Escherichia coli*. The aim of this EQA trial is to monitor the quality of AST results produced by the participating laboratories and identify underperforming laboratories in need of

assistance to improve their performance in AST.

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 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. Laboratory performance is considered acceptable if there are < 5% deviation from expected results.

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 separately. 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 EQA1

A total of 23 laboratories participated in the first EQA survey of the EQAsia project: 13 laboratories belonging to the HH Sector and 10 belonging to the AH Sector.

For the *E. coli* trial, laboratories originated from the following countries: Bangladesh, Bhutan, Brunei Darussalam, Lao's People Democratic Republic, Indonesia, Maldives, Nepal, Pakistan, Philippines, Sri Lanka and Timor-Leste (**Figure 1**, top panel). For the *Salmonella* trial, laboratories originated from Bhutan, Lao's People Democratic Republic, Indonesia, Maldives, Nepal, Pakistan, Philippines and Sri Lanka (**Figure 1**, bottom panel).

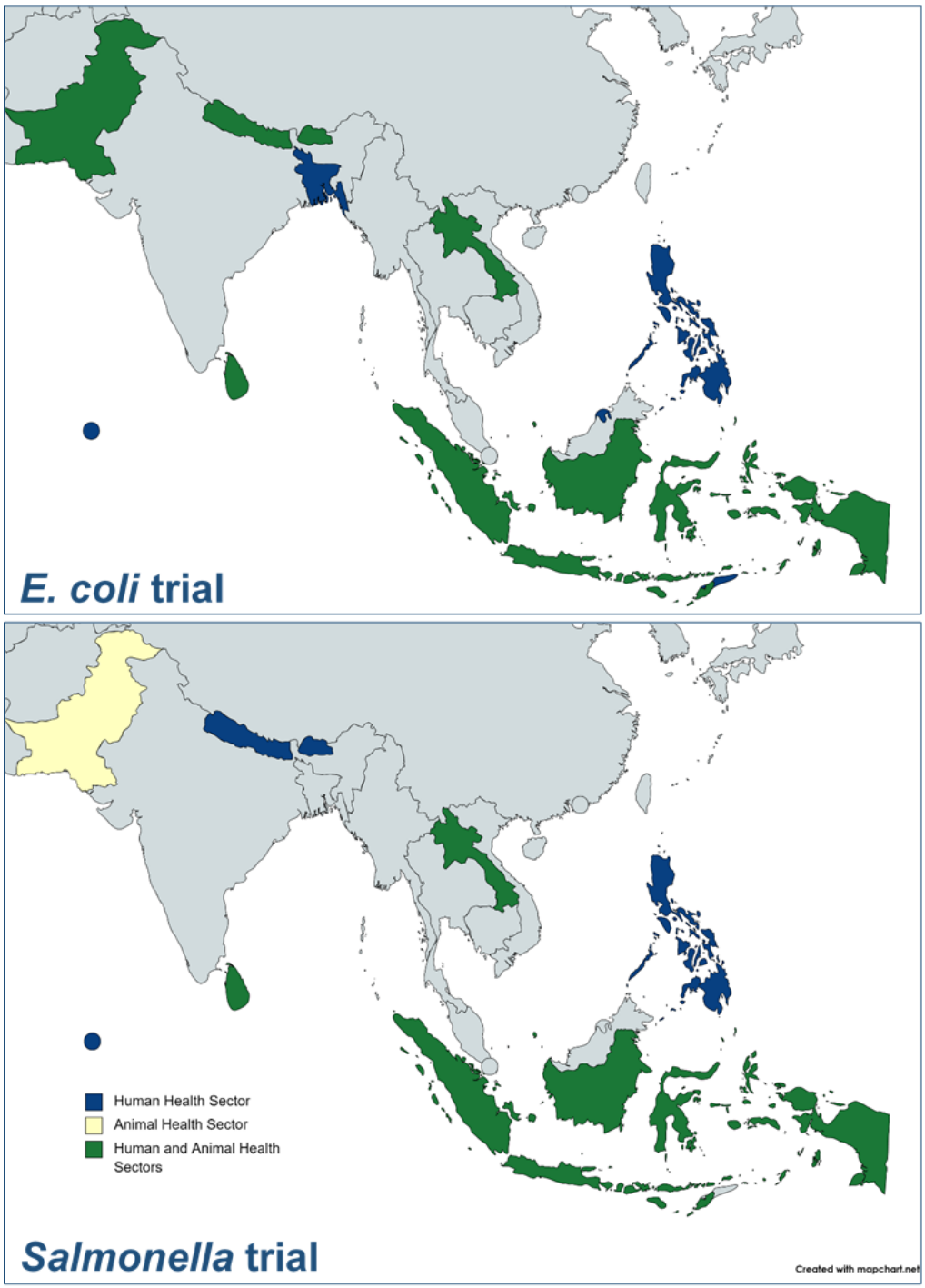

Figure 1: Countries participating in the first EQA of the EQAsia 2021 project on antimicrobial susceptibility testing of *E. coli* (top panel) and *Salmonella* (bottom panel). Color indicates sector affiliation of the participating laboratory as Human Health laboratory (blue), Animal Health laboratory (yellow) or both Human and Animal Health laboratories (green).

2.2 Strains

Participating laboratories could register for either *E. coli, Salmonella* spp. or both. For each registration, the laboratory received 11 bacterial strains of which only eight strains were the targeted species. Hence, the initial task was the identification of the bacterial species of interest using the laboratory's own routine method for bacterial identification.

The eight *E. coli* and eight *Salmonella* spp. strains were selected to represent a heterogeneous phenotypic profile. With the purpose to monitor and assess improvements and trends over time for each organism included in EQA1, one of the test strains is used as an internal control strain that will also be included in EQA3 (where *E. coli* and *Salmonella* are again offered) with varying strain code.

Expected MIC values (Appendix 2) for this EQA were obtained at DTU-FOOD and further verified by the external partner (FDA). Results could not be verified by the external partner for ertapenem, tigecycline and trimethoprim (*E. coli*); and azithromycin, cefepime, cefotaxime, cefotaxime and clavulanic acid, ceftazidime, ceftazidime and clavulanic acid, colistin, ertapenem and imipenem (*Salmonella*).

The reference strain *E. coli* ATCC 25922 was 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 were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-30th Ed. 2020 (Appendix 3).

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for both *E. coli* and *Salmonella* 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.

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA1 2021. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

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

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. 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. No interpretative criteria were available for tigecycline (*Salmonella*) and, therefore, the results were not scored. Results for presumptive beta-lactam resistance mechanisms were

interpreted according to the most recent EFSA (European Food Safety Authority) recommendations also included as an appendix in the EQA protocol (Appendix 1).

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

2.4 Distribution

The bacterial strains were dispatched as lyophilized strains in February 2021 by NIH and CU to the HH and AH laboratories, respectively. 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.

2.5 Procedure

Protocols and all relevant information were available at the EQAsia website, to allow access to all the necessary information at any time. The participants were recommended to store the lyophilized strains in a dark, cool place until performance of AST.

Participating laboratories were advised to perform identification and AST of the test strains according to the methods routinely applied in their laboratory. In addition, participants were asked to submit serotyping results for the *Salmonella* strains on a voluntary basis.

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. Participants were also encouraged to perform testing for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes.

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

Data analysis revealed several instances of misinterpretation of results. Participating laboratories were recommended to interpret the obtained results using the tables provided in the EQA protocol (Appendix 1). Due to misunderstanding or lack of clarification, several laboratories followed the guidelines routinely used in their work. This resulted in different categorisation as resistant or susceptible for each strain/antimicrobial combination, despite identical MIC/Inhibition Zone Diameter values. Such mistakes do not necessarily indicate a poor laboratory performance. Accordingly, the data retrieved from the informatics module was analysed in two different ways:

1) Reported data: all data submitted (categorisation as resistant or susceptible) was taken into account for the analysis;
2) Adjusted data: 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 taking into account these misinterpretations. Such adjusment may not be performed in future EQA reports as results' interpretation are an important factor to assure the quality of a given laboratory.

Further quality checks exposed a mistake in the S EQASIA 21.7 strain sent to the AH laboratories. The abovementioned strain, sent as a non-*Salmonella* strain, was identified by several AH laboratories as a *Salmonella*. This led to the conclusion that an error must have

occurred during the lyophilisation of this specific strain. For that reason, strain S EQASIA 21.7 was excluded from the analysis of results for the Animal Health laboratories.

3. Results – Human Health Laboratories

3.1 Overall participation

Among the Human Health laboratories, 13 laboratories submitted results for *E. coli*, and 7 of these laboratories additionally submitted results for *Salmonella*. Applied AST methods for the *E. coli* trial included disk diffusion for 7 out of 13 laboratories, as well as broth microdilution (n=4), broth macrodilution (n=1) and gradient test (n=1). For the *Salmonella* trial, 4 out of 7 laboratories applied the disk diffusion method, while the remaining laboratories reported MIC values obtained by broth microdilution (n=1), broth macrodilution (n=1) or gradient test (n=1). In addition, some laboratories performed AST using different methodologies and reported both Inhibition Zone Diameters and MIC depending on the antimicrobial drug tested, such as laboratories #01, #04, #07, #08, #10, #11 and #13.

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 EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested antimicrobials (**Table 1**). Therefore, the higher participation in the *E. coli* trial resulted in a larger number of total ASTs reported for this pathogen in comparison to *Salmonella* (**Table 2**). Among all tests and for both trials, sulfamethoxazole was tested by only a few laboratories, corresponding to only 1.8% and 1.2% of the total tests completed for *E. coli* and *Salmonella*, respectively. Similarly, colistin, tigecycline and trimethoprim were only tested by a few laboratories (**Table 2**). In contrast, ampicillin, cefepime, ciprofloxacin, gentamicin and meropenem were tested by most laboratories for the *E. coli* trial, whereas ampicillin, ceftazidime, imipenem and meropenem were tested by most laboratories for the *Salmonella* trial (**Table 2**).

Table 2. Total of Antimicrobial Susceptibility Tests performed for each antimicrobial and in total for each of the trials.

Antimicrobial	AST in total	
	E. coli	*Salmonella*
Ampicillin (AMP)	103 (7.6%)	56 (8.2%)
Azithromycin (AZI)	64 (4.7%)	40 (5.9%)
Cefepime (FEP)	101 (7.4%)	48 (7.0%)
Cefotaxime (FOT)	68 (5.0%)	40 (5.9%)
Cefoxitin (FOX)	66 (4.9%)	32 (4.7%)
Ceftazidime (TAZ)	94 (6.9%)	56 (8.2%)
Chloramphenicol (CHL)	80 (5.9%)	48 (7.0%)
Ciprofloxacin (CIP)	103 (7.6%)	50 (7.3%)
Colistin (COL)	49 (3.6%)	24 (3.5%)
Ertapenem (ETP)	78 (5.7%)	32 (4.7%)
Gentamicin (GEN)	104 (7.7%)	43 (6.3%)
Imipenem (IMI)	88 (6.5%)	56 (8.2%)
Meropenem (MERO)	104 (7.7%)	55 (8.1%)
Nalidixic Acid (NAL)	64 (4.7%)	23 (3.4%)
Sulfamethoxazole (SMX)	24 (1.8%)	8 (1.2%)
Tetracycline (TET)	80 (5.9%)	46 (6.8%)
Tigecycline (TGC)	48 (3.5%)	--
Trimethoprim (TMP)	39 (2.9%)	24 (3.5%)
Total	**1357 (100%)**	**681 (100%)**

3.2 *Escherichia coli* trial

Thirteen laboratories from 11 countries uploaded results for the *E. coli* trial.

3.2.1 Bacterial identification

All 13 participating laboratories submitted results for bacterial identification (**Table 3**). All of them

correctly identified the eight *E. coli* strains among the 11 test strains provided. Only one mistake occurred in the identification of strain E EQASIA 21.6, which was misidentified as *E. coli* by laboratory #02.

Table 3. Bacterial identification of each of the 11 test strains provided related to the *E. coli* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
E EQASIA 21.1	*E. coli*	13/13
E EQASIA 21.2	Non-*E. coli* (*Klebsiella pneumoniae*)	13/13
E EQASIA 21.3	*E. coli*	13/13
E EQASIA 21.4	*E. coli*	13/13
E EQASIA 21.5	*E. coli*	13/13
E EQASIA 21.6	Non-*E. coli* (*Cronobacter sakazakii*)	12/13
E EQASIA 21.7	*E. coli*	13/13
E EQASIA 21.8	*E. coli*	13/13
E EQASIA 21.9	*E. coli*	13/13
E EQASIA 21.10	Non-*E. coli* (*Salmonella*)	13/13
E EQASIA 21.11	*E. coli*	13/13

*E, *Escherichia coli*

3.2.2 AST performance

As explained in Section 2.6, the results are presented as 'reported data' and 'adjusted data', and both can be observed in **Table 4** and **Figures 2-3**. Only the 'adjusted data' results are, however, explained and discussed in the text, as these results truly reflect the laboratories' analytical performance.

In this subsection, the AST performance is analysed from a strain-, antimicrobial-, and laboratory-based perspective for a comprehensive overview of the trial.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/S) ranged from 87.7% (strain E EQASIA 21.5) to 97.6% (strain E EQASIA 21.4) for each strain (**Table 4**). The results from 2 out of 8 strains revealed more than 10% deviation (E EQASIA 21.5, E EQASIA 21.11).

Table 4. Total number of antimicrobial susceptibility tests performed and percentage of correct reported and correct adjusted results in agreement with expected interpretive results (R/S). Results are from 13 HH laboratories for the *E. coli* trial.

Strain	AST in total	% correct reported	% correct adjusted
E EQASIA 21.1	170	84.7	92.4
E EQASIA 21.3	170	92.9	92.9
E EQASIA 21.4	169	95.3	97.6
E EQASIA 21.5	171	80.7	87.7
E EQASIA 21.7	170	82.9	90.6
E EQASIA 21.8	168	97.0	97.0
E EQASIA 21.9	170	87.1	94.7
E EQASIA 21.11	169	84.6	88.8

*E, *Escherichia coli*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were ceftazidime (21.3%), sulfamethoxazole (20.8%) and trimethoprim (20.5%), whereas ampicillin, colistin, nalidixic acid and tetracycline revealed no deviation from the expected results (**Figure 2**). Of the 18 tested and scored antimicrobial agents, nine revealed to exceed a 10% deviation.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for only 5 participants (**Figure 3**). In average, the deviation was 6.9% (ranging from 0.0 to 14.3%). As the acceptance level was set to 5% deviation, 8 laboratories (#02, #12, #17, #13, #04, #10, #05 and #08) did not perform within the expected range for the *E. coli* trial.

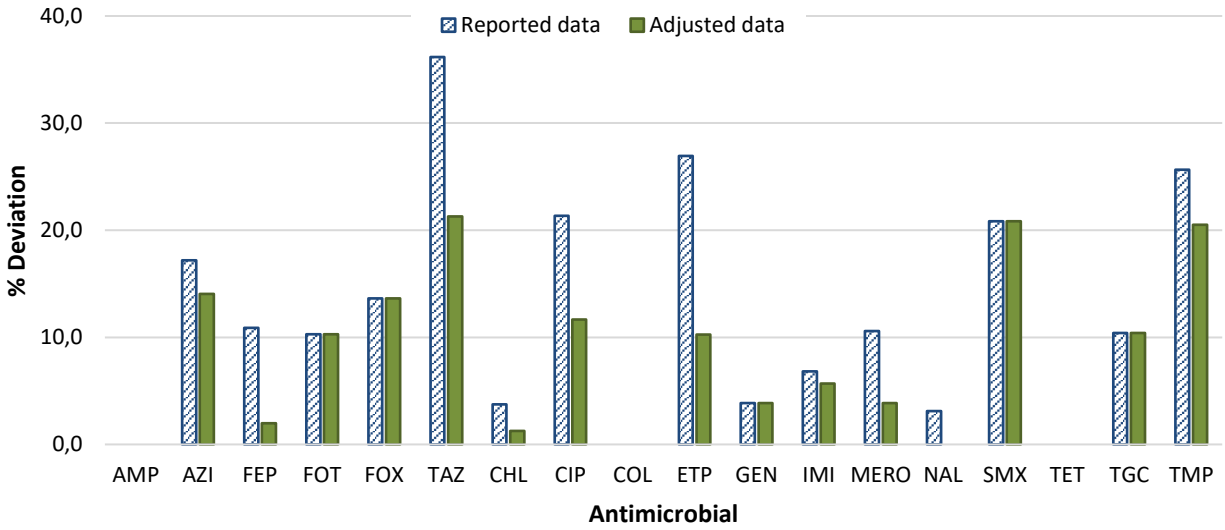

Figure 2. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=13) participating in the 1st EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

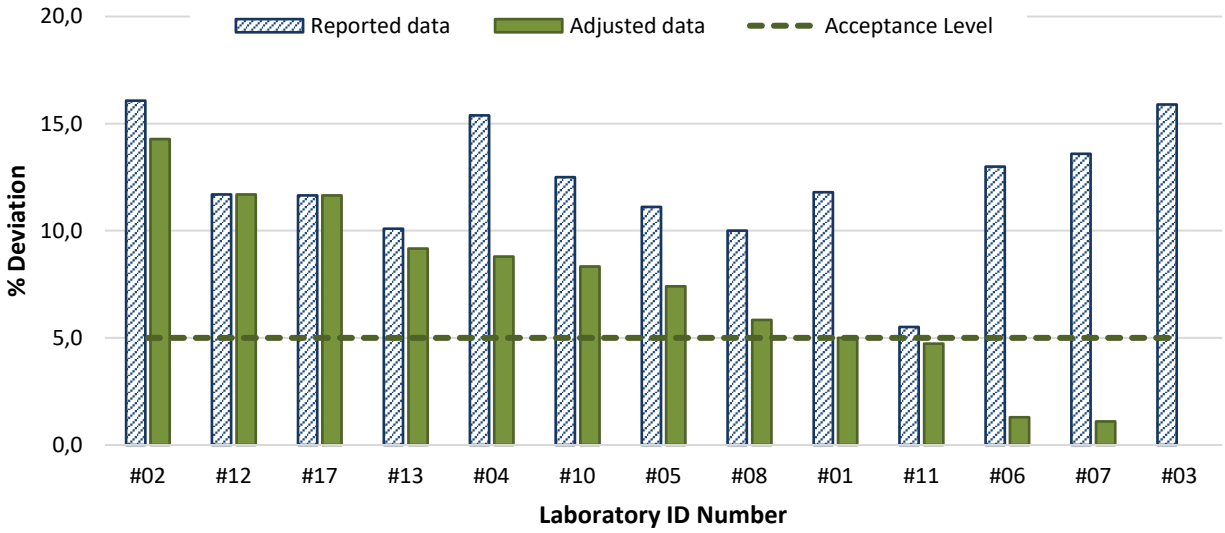

Figure 3. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=13) participating in the 1st EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.1.3 Beta-lactamase producing *E. coli*

Ten out of the 13 participating laboratories uploaded results for this component of the *E. coli* trial. Yet, for strains E EQASIA 21.5 and E EQASIA 21.11 only 9 laboratories tested for ESBL-production, and for strain E EQASIA 21.8 only 8 laboratories (**Table 5**). Of those, only laboratory #12 correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the eight *E. coli* strains. The highest deviation from the expected results was obtained for strain E EQASIA 21.1. The majority of the laboratories wrongly identified this carbapenemase-producing *E. coli* strain as an ESBL- or ESBL + AmpC-producer. This discrepancy can be explained by the wrong meropenem classification (which should be categorized as resistant) due to the use of

different Inhibition Zone Diameter/MIC values for the interpretation (as explained in Section 2.6). The remaining strains were correctly classified by at least 60% of the laboratories.

It is important to emphasize that the results were evaluated based on phenotypes, as genotypic characterization was optional.

Table 5. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *E. coli* test strains. 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. Results are from a total of 10 Human Health laboratories.

Strain code		E EQASIA-21.1	E EQASIA - 21.3	E EQASIA - 21.4	E EQASIA - 21.5	E EQASIA - 21.7	E EQASIA - 21.8	E EQASIA - 21.9	E EQASIA - 21.11
Expected results		Carbapene-mase	Susceptible	ESBL	AmpC	ESBL	Susceptible	ESBL+ AmpC	AmpC
Obtained results (n/N)	ESBL	5/10 (50.0%)	1/10 (10.0%)	**8/10 (80.0%)**	1/9 (11.1%)	**8/10 (80.0%)**	--	2/10 (20.0%)	1/9 (11.1%)
	AmpC	--	--	--	**7/9 (77.8%)**	--	--	1/10 (10.0%)	**6/9 (66.7%)**
	ESBL + AmpC	3/10 (30.0%)	--	1/10 (10.0%)	--	1/10 (10.0%)	--	**6/10 (60.0%)**	1/9 (11.1%)
	Carbapenemase	**1/10 (10.0%)**	--	--	--	--	--	--	--
	Other	--	--	--	--	--	--	--	--
	Susceptible*	1/10 (10.0%)	**9/10 (90.0%)**	1/10 (10.0%)	1/9 (11.1%)	1/10 (10.0%)	**8/8 (100.0%)**	1/10 (10.0%)	1/9 (11.1%)

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

3.3 *Salmonella* trial

Seven laboratories from seven different countries uploaded results for the *Salmonella* trial.

3.3.1 Bacterial Identification

The seven laboratories participating in the *Salmonella* trial submitted results for bacterial identification. All of the laboratories correctly identified the eight *Salmonella* strains and the three non-*Salmonella* (**Table 6**).

Table 6. Bacterial identification of each of the 11 test strains provided related to the *Salmonella* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
S EQASIA 21.1	*Salmonella*	7/7
S EQASIA 21.2	*Salmonella*	7/7
S EQASIA 21.3	Non-*Salmonella* (*Citrobacter freundii*)	7/7
S EQASIA 21.4	*Salmonella*	7/7
S EQASIA 21.5	*Salmonella*	7/7
S EQASIA 21.6	*Salmonella*	7/7
S EQASIA 21.7	Non-*Salmonella* (*Shigella flexneri*)	7/7
S EQASIA 21.8	*Salmonella*	7/7
S EQASIA 21.9	Non-*Salmonella* (*Escherichia coli*)	7/7
S EQASIA 21.10	*Salmonella*	7/7
S EQASIA 21.11	*Salmonella*	7/7

*S, *Salmonella*

3.3.2 Serotyping

Serotyping of *Salmonella* spp. was offered to the participants as a voluntary component. Of the seven participating laboratories in the *Salmonella* trial, only laboratory #02 did not submit results for *Salmonella* serotyping. In this component, the eight strains identified as *Salmonella* should be serotyped using the method routinely used by the laboratory. In case of lacking the necessary antisera for serotyping, serogroup could still be reported and further evaluated, meaning that serotype and serogroup were separately assessed in this trial. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (see Section 7. References).

Based on the results, serogroup O:4 (B) was the most accessible to identify as a higher participation is observed (**Table 7**). Among the submitted results, only one mistake occurred in the serogroup identification and three more mistakes in the reported serotypes. Regarding laboratory performance, laboratories #05, #08 and #11 submitted serogroup and serotype results for all eight strains and identified them correctly, except one serogroup for lab #11 (**Figure 4**). Laboratories #06, #12 and #17 were unable to report results for all serogroups/serotypes included in the trial.

Table 7. Serogroup, serotype and antigen of each of the 8 *Salmonella* strains. Number of correct serogroup/serotype out of the total submitted serogroup/serotype results are presented. Results are from a total of 6 Human Health laboratories.

Strain	Serogroup	No. correct Serogroup	Serotype	No. correct Serotype	Antigen
S EQASIA 21.1	O:4 (B)	6/6	Derby	3/4	4,12:f,g:-
S EQASIA 21.2	O:11 (F)	3/4	Rubislaw	3/4	11:r:e,n,x
S EQASIA 21.4	O:4 (B)	5/5	Typhimurium	3/3	I 4,12:i:1,2
S EQASIA 21.5	O:4 (B)	5/5	Heidelberg	3/3	I 4,12:r:1,2
S EQASIA 21.6	O:7 (C1)	3/3	Infantis	2/3	I 6,7:r:1,5
S EQASIA 21.8	O:9 (D1)	4/4	Enteritidis	3/3	I 9,12:g,m;-
S EQASIA 21.10	O:3.10 (E1)	4/4	Weltevreden	3/3	I 3,10:r;z6
S EQASIA 21.11	O:4 (B)	5/5	Schwarzengrund	3/3	I 4,12:d:1,7

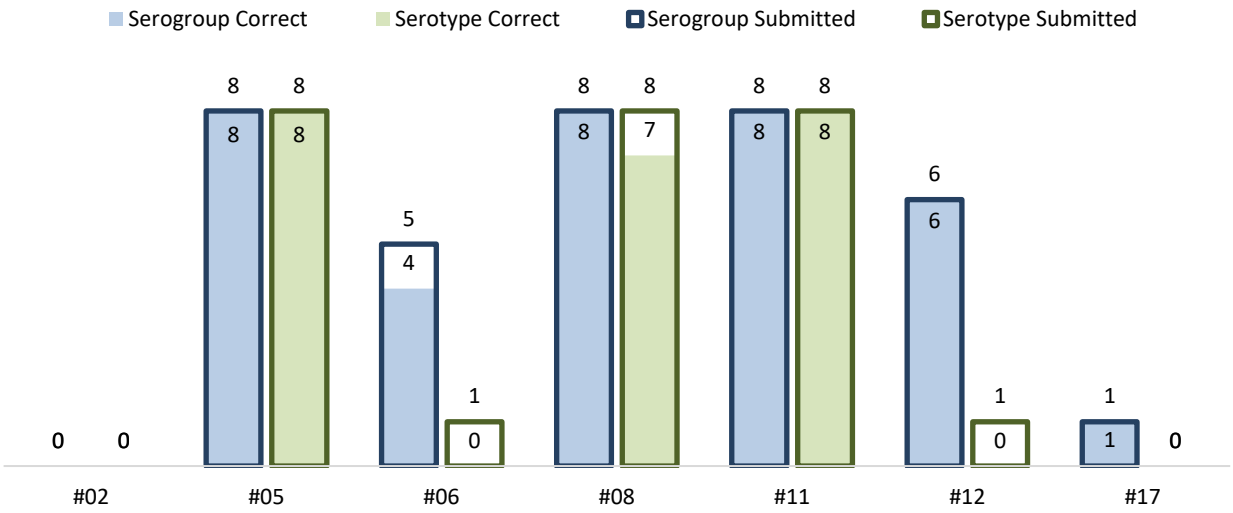

Figure 4. Number of correct serogroup/serotype out of the total of submitted serogroup/serotype results for each of the participating HH laboratories in the *Salmonella* trial.

3.3.3 AST performance

The AST performance in the *Salmonella* trial is analysed from a strain-, antimicrobial-, and laboratory-based perspective to allow for a broader interpretation of the results. **Table 8** and **Figures 5-6** contain 'reported data' and 'adjusted data' (see explanation in Section 2.6), whereas explanation and discussion of the observations described in the text are based on the 'adjusted data' only.

Strain-based analysis

Deviations among the *Salmonella* strains were all below 5% except for S EQASIA 21.6, where the deviation was as high as 9.4% (**Table 8**).

Antimicrobial-based analysis

The antimicrobials that resulted in highest percentage of deviations were sulfamethoxazole (25.0%), followed by ciprofloxacin (10.0%), nalidixic acid (8.7%) and trimethoprim (8.3%) (**Figure 5**). The results of eight antimicrobial agents (ampicillin, azithromycin, cefotaxime, chloramphenicol, ertapenem, imipenem, meropenem and tetracycline) revealed no deviation from the expected results.

Table 8. Total number of antimicrobial susceptibility tests performed and percentage of correct reported and correct adjusted results in agreement with expected interpretive results (R/S). Results are from seven HH laboratories for the *Salmonella* trial.

Strain	AST in total	% correct reported	% correct adjusted
S EQASIA 21.1	86	93.0	96.5
S EQASIA 21.2	85	97.6	100.0
S EQASIA 21.4	87	97.7	100.0
S EQASIA 21.5	85	95.3	96.5
S EQASIA 21.6	85	87.1	90.6
S EQASIA 21.8	85	94.1	96.5
S EQASIA 21.10	85	98.8	100.0
S EQASIA 21.11	83	95.2	97.6

*S, *Salmonella*

Laboratory-based analysis

For the *Salmonella* trial, two out of the seven HH laboratories presented a deviation above the acceptance level of 5% (#02 and #17). The average deviation was 2.8 % (ranging from 0.8 to 5.4%).

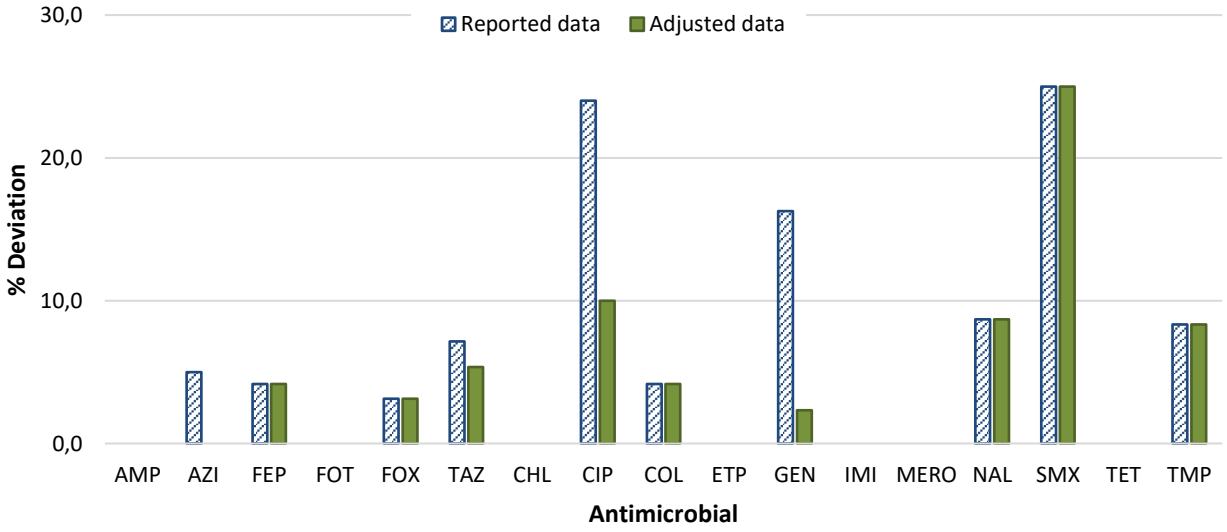

Figure 5. Percentage of deviation in the AST interpretation (R/S) among *Salmonella* strains by HH laboratories (n=7) participating in the 1st EQA of the EQAsia project. Results are categorized by antimicrobial agent.

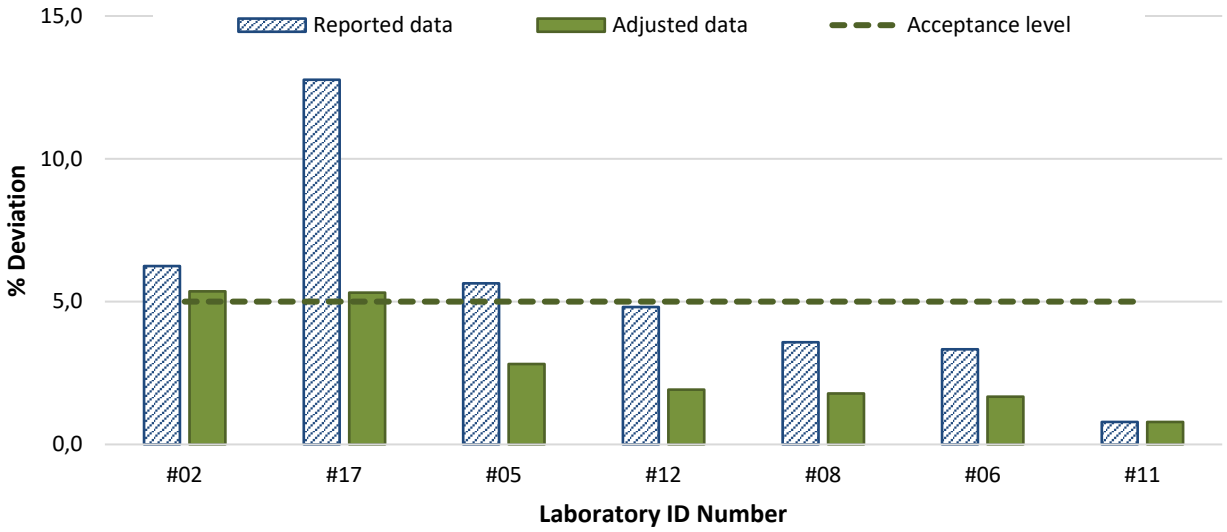

Figure 6. Percentage of deviation in the AST interpretation (R/S) among *Salmonella* spp. strains by HH laboratories (n=7) participating in the 1st proficiency testing of the EQAsia project in the *Salmonella* trial. Results are categorized by laboratory ID Number.

3.3.4 Beta-lactamase producing *Salmonella*

Six out of the seven participants uploaded results for this part of the *Salmonella* trial. However, for more than half of the strains, fewer than six laboratories reported data (**Table 9**). Of those, only laboratory #08 identified all the different ESBL / AmpC / carbapenemase phenotypes correctly among the eight *Salmonella* strains. Laboratory #12 also identified correctly but submitted results for only three strains.

The laboratories were able to identify the susceptible (no AmpC, ESBL and carbapenemase) isolates with almost no problems (80% to 100% of the laboratories). The remaining four non-susceptible strains generated more mistakes in correctly identifying the resistance phenotype. The highest deviation from the expected results was obtained for strain S EQASIA 21.8, for which no laboratories reported the correct phenotype. All three laboratories (#06, #08 and #12) reported the strain as being susceptible to cefotaxime and/or ceftazidime; these findings should exclude an ESBL or AmpC phenotype. In addition, the laboratories identified the strain as susceptible to meropenem, also excluding a carbapenemase phenotype. Laboratories #08 and #12 however found that S EQASIA 21.8 was resistant towards cefoxitin. This observation indicates that the strain should be classified as 'other phenotypes' as shown in **Table 9**.

The results reported were evaluated based on phenotypes, as genotypic characterization was optional.

Table 9. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *Salmonella* test strains. 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. Results are from a total of 7 HH laboratories.

Strain code		S EQASIA-21.1	S EQASIA - 21.2	S EQASIA - 21.4	S EQASIA - 21.5	S EQASIA - 21.6	S EQASIA - 21.8	S EQASIA - 21.10	S EQASIA - 21.11
Expected results		Susceptible	Susceptible	ESBL	AmpC	ESBL	Other	Susceptible	Susceptible
Obtained results (n/N)	ESBL	1/5 (20.0%)	1/5 (20.0%)	**5/5 (100.0%)**	1/6 (16.7%)	**6/6 (100.0%)**	1/3 (33.3%)	--	--
	AmpC	--	--	--	**3/6 (50.0%)**	--	2/3 (66.7%)	--	--
	ESBL + AmpC	--	--	--	1/6 (16.7%)	--	--	--	--
	Carbapenemase	--	--	--	--	--	--	--	--
	Other	--	--	--	1/6 (16.7%)	--	**--**	--	--
	Susceptible*	**4/5 (80.0%)**	**4/5 (80.0%)**	--	--	--	--	**5/5 (100.0%)**	**5/5 (100.0%)**

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

3.4 Quality control strain *E. coli* ATCC 25922

The quality control strain *E. coli* ATCC 25922 was sent to all participating laboratories to be used as a reference strain for both *E. coli* and *Salmonella* trials. Antimicrobial susceptibility test results for the quality control strain were evaluated separately for each of the trials.

3.4.1 Deviations in the *E. coli* trial

The 13 participating laboratories used different methodologies for testing the reference strain: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, macrobroth dilution or microbroth dilution. One laboratory tested colistin by disk diffusion, which is not the recommended standard method due to its large molecule. This result was therefore considered incorrect (**Table 10**, see *). The highest proportion of test results outside of the expected range were observed for cefepime, ciprofloxacin, ertapenem, meropenem and tigecycline (**Table 10**). Moreover, the majority of the inaccurate results seem to be caused by MIC determination methodologies (micro- and macrobroth dilution). A deeper look into the results that were outside of range revealed that the applied method tested for antimicrobial concentrations above the expected interval, making it impractical to determine the exact MIC. For example, the expected range for cefepime is 0.016 to 0.12, and most of the laboratories reported an MIC ≤1. The informatics module scores such results as '0' (incorrect) because either the exact MIC can be within (0.016-0.12) or outside (0.25-1) the expected range. This occurrence is not only seen for cefepime, but also for ceftazidime, ciprofloxacin, ertapenem, meropenem and tigecycline. On the other hand, the deviations originating from the disk diffusion method demonstrate that the reported value is 1-2mm above or below the expected range. Lastly, no deviations were seen when gradient test was applied.

Taking into consideration all the above-mentioned observations, the laboratories performance is also highly dependent on the methodology applied for AST of the quality control strain (**Figure 7).** Laboratory #11 presented no deviation, as the preferred method applied was gradient test. Inversely, laboratories' #03, #04, #06, #07 and #08 deviations were

solely caused by the applied MIC determination method, which tested antimicrobial concentrations above the expected range. Therefore, these deviations do not necessarily imply a poor performance of the laboratories, but rather an inappropriate method for testing the quality control strain.

Table 10. AST of the reference strain *E. coli* ATCC 25922 in the *E. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	**Total**
AMP	0/6	0/1	0/6	**0/13**
FEP	2/5	0/1	7/7	**9/13**
FOT	1/7	0/1	--	**1/8**
FOX	1/6	0/1	0/1	**1/8**
TAZ	1/7	0/1	3/4	**4/12**
CHL	1/8	0/1	0/1	**1/10**
CIP	1/7	0/1	5/5	**6/13**
COL	1/1*	0/1	0/4	**1/6**
ETP	1/5	--	5/5	**6/10**
GEN	1/6	0/1	1/6	**2/13**
IMI	1/7	--	2/4	**3/11**
MERO	1/6	--	7/7	**8/13**
NAL	1/6	0/1	0/1	**1/8**
SMX	1/1	--	0/2	**1/3**
TET	0/8	0/1	0/1	**0/10**
TGC	0/1	--	5/5	**5/6**
TMP	1/3	--	0/1	**1/4**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by micro- and macrobroth dilution
*Disk diffusion is not recommended for testing colistin

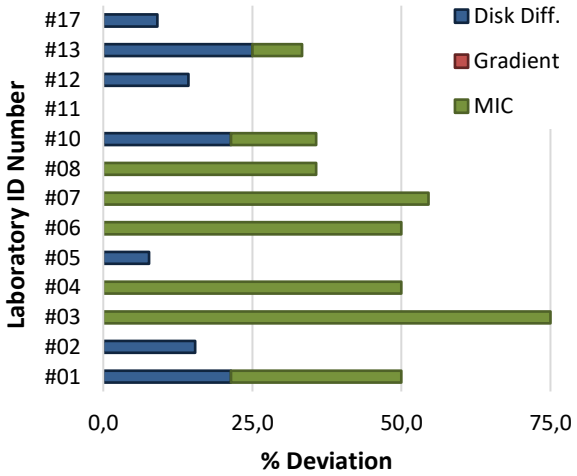

Figure 7. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *E. coli* trial by the HH laboratories.

3.4.2 Deviations in the *Salmonella* trial

Five laboratories submitted results regarding AST of *E. coli* ATCC 25922 reference strain in the *Salmonella* trial. Aligned with the *E. coli* trial, different methodologies were applied for testing the quality control strain (disk diffusion, gradient test, macrobroth dilution and microbroth dilution). However, fewer test results outside the expected range were observed (**Table 11**), and the majority of those were reported by laboratory #06 due to limitations of the applied MIC determination method.

Table 11. AST of the reference strain *E. coli* ATCC 25922 in the *Salmonella* trial. Proportion of test results outside of expected range is presented by methodology used.

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

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by micro- and macrobroth dilution

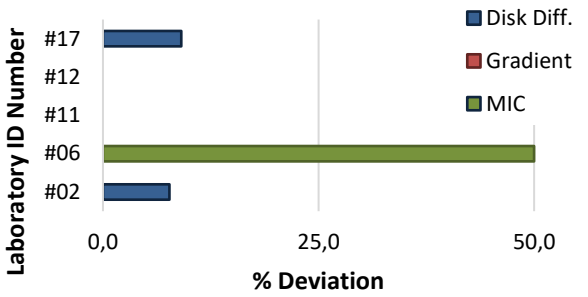

Figure 8. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *Salmonella* trial by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

For the Animal Health laboratories, 10 laboratories submitted results for *E. coli* and 8 of these laboratories additionally submitted results for *Salmonella*. The participants were invited to report MIC values or Inhibition Zone Diameters and interpretation as resistant or susceptible (R/S) for each strain/antimicrobial combination. Only the categorisation was evaluated, whereas the Inhibition Zone Diameters/MIC values were used as supplementary information.

The AST methods for the *E. coli* trial varied from disk diffusion (n=6) to broth microdilution (n=3), and agar dilution (n=1). Similarly, the participants in the *Salmonella* trial mostly applied the disk diffusion method (n=5), whereas only two laboratories chose broth microdilution and one laboratory agar dilution. Laboratory #20 performed AST using different methodologies and reported both Inhibition Zone Diameters and MIC, depending on the antimicrobial drug tested.

The EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested antimicrobials (**Table 1**).

As seen in **Table 12**, a larger number of total ASTs was reported for *E. coli*, which can be explained by the number of laboratories that submitted results to each of the trials. Regarding the number of tests performed for each individual antimicrobial, cefoxitin, ertapenem and imipenem were the least tested drugs amongst

the AH laboratories on both trials, as well as tigecycline in the *E. coli* trial (**Table 12**). On contrary, ampicillin, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin and tetracycline were tested by several laboratories on both *E. coli* and *Salmonella* trials (**Table 12**).

Table 12. Total of Antimicrobial Susceptibility Tests performed for each antimicrobial and in total for each of the trials.

Antimicrobial	AST in total	
	E. coli	*Salmonella*
Ampicillin (AMP)	70 (8.8%)	54 (8.5%)
Azithromycin (AZI)	39 (4.9%)	31 (4.9%)
Cefepime (FEP)	28 (3.5%)	30 (4.7%)
Cefotaxime (FOT)	49 (6.2%)	40 (6.3%)
Cefoxitin (FOX)	23 (2.9%)	24 (3.8%)
Ceftazidime (TAZ)	63 (7.9%)	46 (7.2%)
Chloramphenicol (CHL)	57 (7.2%)	53 (8.3%)
Ciprofloxacin (CIP)	59 (7.4%)	46 (7.2%)
Colistin (COL)	38 (4.8%)	30 (4.7%)
Ertapenem (ETP)	20 (2.5%)	16 (2.5%)
Gentamicin (GEN)	63 (7.9%)	47 (7.4%)
Imipenem (IMI)	18 (2.3%)	24 (3.8%)
Meropenem (MERO)	43 (5.4%)	39 (6.1%)
Nalidixic Acid (NAL)	54 (6.8%)	41 (6.4%)
Sulfamethoxazole (SMX)	39 (4.9%)	31 (4.9%)
Tetracycline (TET)	60 (7.6%)	54 (8.5%)
Tigecycline (TGC)	24 (3.0%)	--
Trimethoprim (TMP)	47 (5.9%)	31 (4.9%)
Total	**794 (100%)**	**637 (100%)**

4.2 *Escherichia coli* trial

Ten laboratories (five from South Asia and five from Southeast Asia) uploaded the results for the *E. coli* trial.

4.2.1 Bacterial identification

All 10 participating laboratories submitted results for bacterial identification (**Table 13**). All of them, except for one (laboratory #22, which misidentified E EQASIA 21.1 as non-*E. coli*), correctly identified the eight *E. coli* strains among the 11 test strains provided. However, fewer laboratories (n=5 or 6, **Table 13**) properly identified the three non-*E. coli* strains. This observation suggests that some laboratories may not have performed bacterial identification and simply reported all 11 strains as *E. coli*. This seems to be the case for laboratories #18, #21, #26 and #27.

Table 13. Bacterial identification of the 11 test strains provided related to the *E. coli* trial. Number of correct results out of the total of participating laboratories.

Strain	Bacterial ID	No. correct
E EQASIA 21.1	*E. coli*	9/10
E EQASIA 21.2	Non-*E. coli* (*Klebsiella pneumoniae*)	6/10
E EQASIA 21.3	*E. coli*	10/10
E EQASIA 21.4	*E. coli*	10/10
E EQASIA 21.5	*E. coli*	10/10
E EQASIA 21.6	Non-*E. coli* (*Cronobacter sakazakii*)	6/10
E EQASIA 21.7	*E. coli*	10/10
E EQASIA 21.8	*E. coli*	10/10
E EQASIA 21.9	*E. coli*	10/10
E EQASIA 21.10	Non-*E. coli* (*Salmonella*)	5/10
E EQASIA 21.11	*E. coli*	10/10

*E, *Escherichia coli*

4.2.2 AST performance

As explained in Section 2.6, the results are presented as 'reported data' and 'adjusted data', and can be found in **Table 14** and **Figures 9-10**. However, only the 'adjusted data' results are explained and discussed in the text, as these results truly reflect the laboratories analytical performance.

In this subsection, the AST performance is analysed from a strain-, antimicrobial-, and laboratory-based perspective for a comprehensive overview of the trial.

Strain-based analysis

The percentage of the results in agreement with expected interpretative results (R/S) among each strain ranged from 83.8% (strain E EQASIA 21.4) to 95.6% (E EQASIA 21.3) (**Table 14**). The result from 4 out of 8 strains revealed larger than

10% deviation.

Antimicrobial-based analysis

No deviations from expected results were obtained when testing susceptibility to imipenem (**Figure 9**). The antimicrobials with the largest deviations were ertapenem (45%), followed by cefoxitin (30.4%), azithromycin (28.2%) and ceftazidime (19.0%). Of the 18 tested and scored antimicrobial agents, six of them revealed over 10% deviation.

Laboratory-based analysis

Most of the AH laboratories participating in the *E. coli* trial had deviations above the acceptance level (5%), and therefore did not perform within the expected range in the *E. coli* trial (**Figure 10**). The average deviation was 11.2%, well above the acceptance level. Only two laboratories (#23 and #30) had results deviating less than 5%.

Table 14. Total number of antimicrobial susceptibility tests performed and percentage of correct reported and correct adjusted results in agreement with expected interpretive results (R/S). Results are from 10 Animal Health laboratories for the *E. coli* trial.

Strain	AST in total	% correct reported	% correct adjusted
E EQASIA 21.1	87	83.9	87.4
E EQASIA 21.3	91	95.6	95.6
E EQASIA 21.4	105	81.0	83.8
E EQASIA 21.5	103	83.5	88.3
E EQASIA 21.7	102	83.3	90.2
E EQASIA 21.8	100	88.0	89.0
E EQASIA 21.9	102	93.1	93.1
E EQASIA 21.11	104	90.4	92.3

*E, *Escherichia coli*

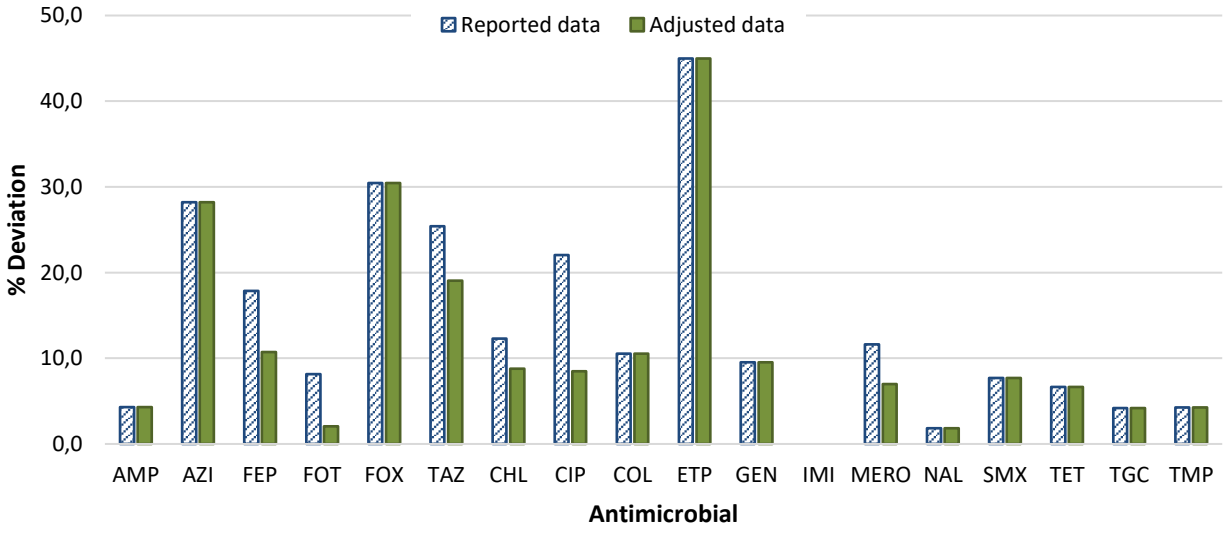

Figure 9. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=10) participating in the 1st EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

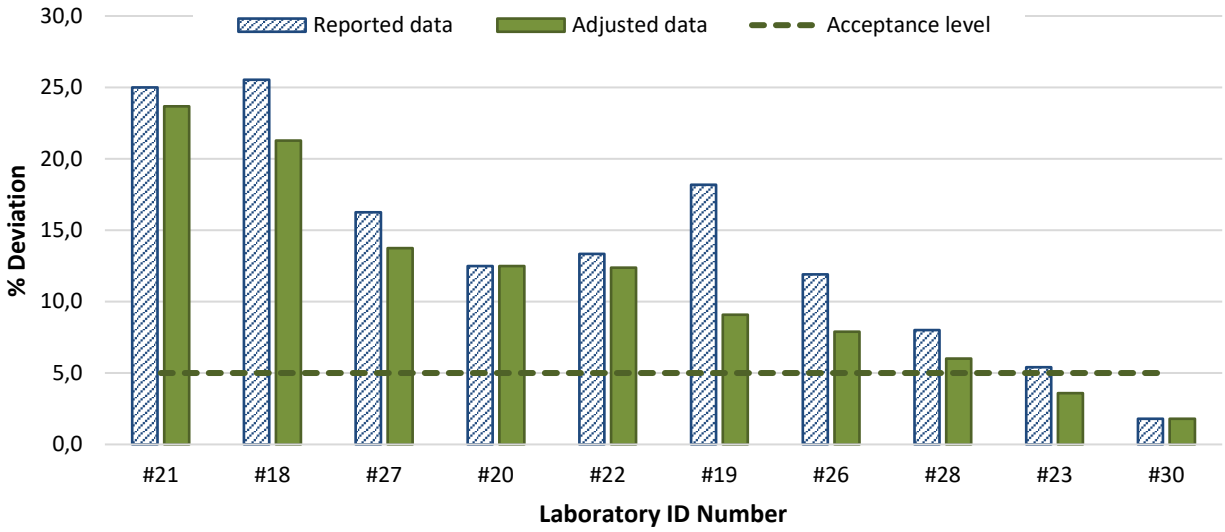

Figure 10. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=10) participating in the 1st EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 Beta-lactamase-producing *E. coli*

Only three (#18, #22 and #28) out of the 10 participating laboratories submitted results for this component of the *E. coli* trial, but not for all of the strains. Discrepancies from the expected results are summarized in **Table 15**.

Firstly, laboratories identified the strains that produced ESBL/AmpC/carbapenemase, and then reported the specific phenotype. Only strain E EQASIA 21.7 was correctly identified by all the laboratories submitting results. E EQASIA 21.1 was expected to be a carbapenemase-producer; however, laboratories #18 and #28 did not perform susceptibility testing towards meropenem and reported the strain as an ESBL-producer. Strain E EQASIA 21.4 was expected to be an ESBL-producer, but laboratory #18 classified it as susceptible despite reporting the strain as resistant to ceftazidime; in turn, laboratory #22 reported the strain as resistant to cefoxitin (E EQASIA 21.4 is susceptible to cefoxitin) and thus classified the strain as ESBL+ AmpC-producer. E EQASIA 21.5 and E EQASIA 21.11 were expected to be AmpC-producers, but two laboratories (#18 and #28) categorized it as not producing any beta-lactamase mediating ESBL/AmpC/carbapenemase phenotype (susceptible) despite reporting the strains as resistant to cefotaxime and/or ceftazidime. Strain E EQASIA 21.9 was classified as susceptible by laboratory #18 despite being found resistant to ceftazidime; laboratory #22 classified it correctly as ESBL+AmpC-producer; laboratory #28 classified as ESBL-producer as resistance to cefoxitin was not tested.

It is important to emphasize that the results were evaluated based on phenotypes, as genotypic characterization was optional.

Table 15. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *E. coli* test strains. 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. Results are from a total of 3 AH laboratories.

Strain code		E EQASIA-21.1	E EQASIA - 21.3	E EQASIA - 21.4	E EQASIA - 21.5	E EQASIA - 21.7	E EQASIA - 21.8	E EQASIA - 21.9	E EQASIA - 21.11
Expected results		Carbapene -mase	Susceptible	ESBL	AmpC	ESBL	Susceptible	ESBL+ AmpC	AmpC
Obtained results (n/N)	ESBL	2/2 (100.0%)	1/2 (50.0%)	**--**	--	**2/2 (100.0%)**	1/2 (50.0%)	1/3 (33.3%)	--
	AmpC	--	--	--	**1/3 (33.3%)**	--	--	--	**1/3 (33.3%)**
	ESBL + AmpC	--	--	1/2 (50.0%)	--	--	--	**1/3 (33.3%)**	--
	Carbapenemase	**--**	--	--	--	--	--	--	--
	Other	--	--	--	--	--	--	--	--
	Susceptible*	--	**1/2 (50.0%)**	1/2 (50.0%)	2/3 (66.7%)	--	**1/2 (50.0%)**	1/3 (33.3%)	2/3 (66.7%)

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

4.3 *Salmonella* trial

Eight laboratories from five different countries uploaded results for the *Salmonella* trial.

4.3.1 Bacterial identification

The eight laboratories participating in the *Salmonella* trial submitted results for bacterial identification. Laboratory #30 misidentified S EQASIA 21.2 as non-*Salmonella*, but all remaining laboratories correctly identified the eight *Salmonella* strains (**Table 16**).

As observed in the *E. coli* trial, fewer laboratories (n=3 to 5, **Table 16**) properly identified the non-*Salmonella* strains S EQASIA 21.3 and S EQASIA 21.9. Again, it suggests that laboratories #21, #26 and #27 may not have performed bacterial identification and simply reported all strains as *Salmonella*.

4.3.2 Serotyping

No Animal Health laboratories participating in the *Salmonella* trial performed serotyping.

Table 16. Bacterial identification of the test strains provided related to the *Salmonella* trial. Number of correct results out of the total of participating Animal Health laboratories is presented. S EQASIA 21.7 is excluded from data analysis (see section 2.6 for details).

Strain	Bacterial ID	No. correct
S EQASIA 21.1	*Salmonella*	8/8
S EQASIA 21.2	*Salmonella*	7/8
S EQASIA 21.3	Non-*Salmonella* (*Citrobacter freundii*)	3/8
S EQASIA 21.4	*Salmonella*	8/8
S EQASIA 21.5	*Salmonella*	8/8
S EQASIA 21.6	*Salmonella*	8/8
S EQASIA 21.8	*Salmonella*	8/8
S EQASIA 21.9	Non-*Salmonella* (*Escherichia coli*)	5/8
S EQASIA 21.10	*Salmonella*	8/8
S EQASIA 21.11	*Salmonella*	8/8

*S, *Salmonella*

4.3.3 AST performance

The AST performance in the *Salmonella* trial is analysed from a strain-, antimicrobial-, and laboratory-based perspective to allow for a broader interpretation of the results. **Table 17**

and **Figures 11-12** contain 'reported data' and 'adjusted data' (see explanation in Section 2.6), whereas explanation and discussion of the observations described in the text are base in the 'adjusted data' only.

Strain-based analysis

The percentage of the results in agreement with expected interpretative results (R/S) among each strain ranged from 83.1% (strain S EQASIA 21.6) to 92.8% (S EQASIA 21.4) (**Table 17**). The result from 5 out of 8 strains revealed larger than 10% deviation and among these, 3 strains (S EQASIA 21.1, S EQASIA 21.2, S EQASIA 21.6), exceeded 15% deviation.

Antimicrobial-based analysis

All 17 antimicrobials showed deviations from the expected results. The antimicrobials that resulted in the highest percentage of deviations were azithromycin (45.2%), followed by cefoxitin (37.5%), ceftazidime (19.6%) and ertapenem (25.0%) (**Figure 11**).

Table 17. Total number of antimicrobial susceptibility tests performed and percentage of correct reported and correct adjusted results in agreement with expected interpretive results (R/S). Results are from eight AH laboratories for the *Salmonella* trial.

Strain	AST in total	% correct reported	% correct adjusted
S EQASIA 21.1	80	82.5	83.8
S EQASIA 21.2	68	83.8	83.8
S EQASIA 21.4	83	89.2	92.8
S EQASIA 21.5	80	87.5	88.8
S EQASIA 21.6	83	81.9	83.1
S EQASIA 21.8	80	86.3	88.8
S EQASIA 21.10	82	91.5	92.7
S EQASIA 21.11	81	91.4	91.4

*S, *Salmonella*

Laboratory-based analysis

Two laboratories (#28 and #30) reported all results in agreement with those expected, presenting no deviation (**Figure 12**). However, the remaining six laboratories had deviations above the acceptance level of 5%, where the average deviation was 11.0%.

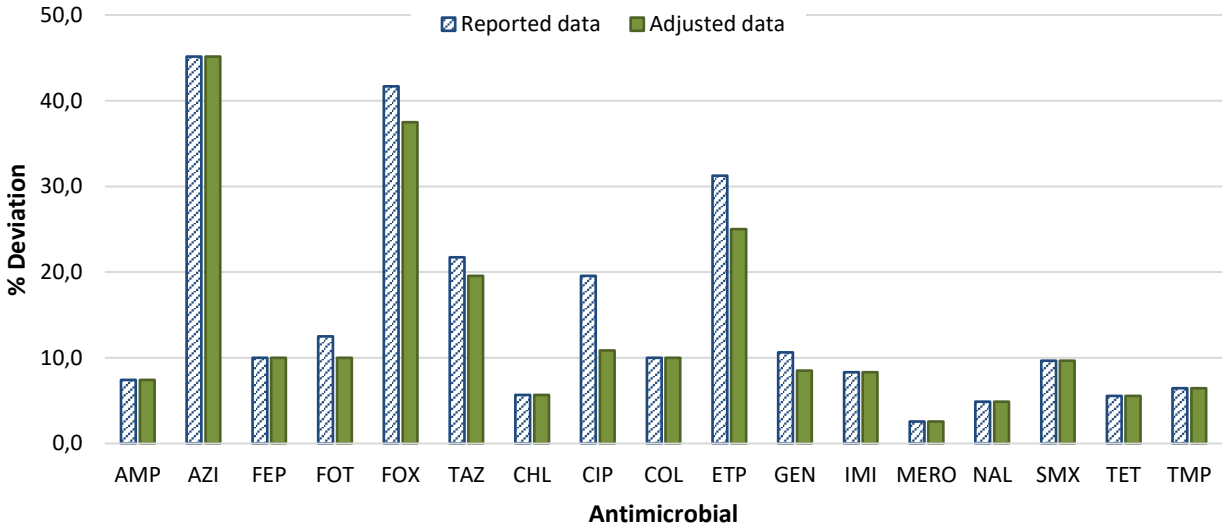

Figure 11. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=8) participating in the 1st EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

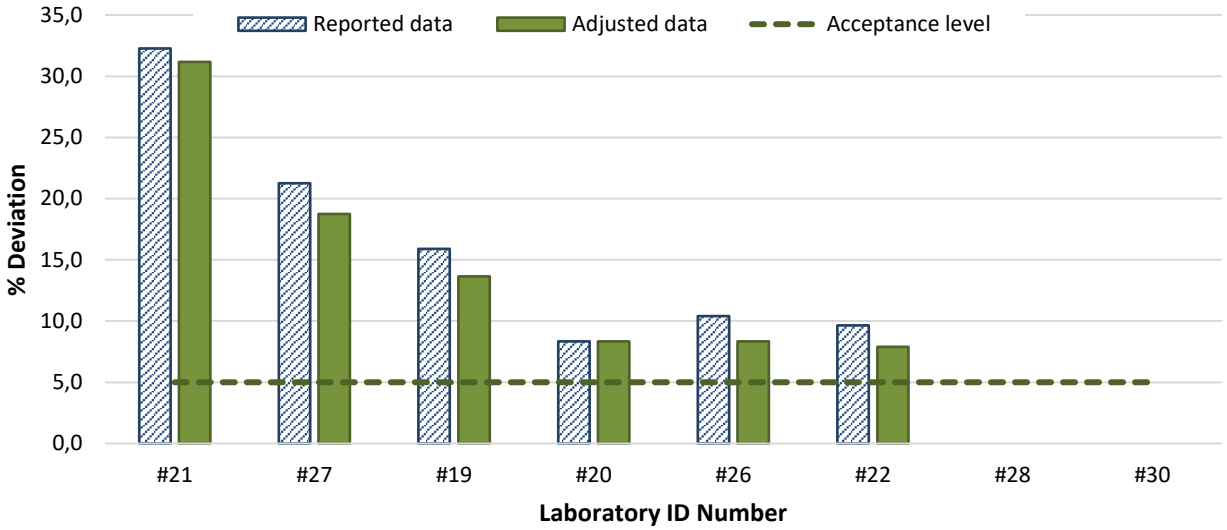

Figure 12. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=8) participating in the 1st EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 Beta-lactamase-producing *Salmonella*

Laboratory #22 was the only one reporting results for all eight *Salmonella* strains, whereas laboratory #28 submitted data for 3 out of 8 strains (**Table 18**).

Strain S EQASIA 21.1 was classified as an ESBL-producer by laboratory #22, even though the strain was reported as susceptible towards cefotaxime and ceftazidime.

The same laboratory misclassified strain S EQASIA 21.5 as ESBL + AmpC-producer, as in their results they observed positive synergy of the drug combinations cefotaxime/ceftazidime and clavulanic acid. In its turn, laboratory #28 classified the strain as susceptible because despite being resistant to cefotaxime and ceftazidime, no synergy was observed. As the laboratory did not test for cefoxitin, they were unable to complete the detection of AmpC-type beta-lactamases.

Lastly, laboratory #22 classified the strain S EQASIA 21.8 as ESBL + AmpC-producer despite reporting the strain as susceptible to cefotaxime and ceftazidime, and only resistant to cefoxitin.

Table 18. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *E. coli* test strains. 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. Results are from a total of two AH laboratories.

Strain code		S EQASIA-21.1	S EQASIA - 21.2	S EQASIA - 21.4	S EQASIA - 21.5	S EQASIA - 21.6	S EQASIA - 21.8	S EQASIA - 21.10	S EQASIA - 21.11
Expected results		Susceptible	Susceptible	ESBL	AmpC	ESBL	Other	Susceptible	Susceptible
Obtained results (n/N)	ESBL	1/1 (100.0%)	--	**2/2 (100.0%)**	--	**2/2 (100.0%)**	--	--	--
	AmpC	--	--	--	**--**	--	--	--	--
	ESBL + AmpC	--	--	--	1/2 (50.0%)	--	1/1 (100.0%)	--	--
	Carbapenemase	--	--	--	--	--	--	--	--
	Other	--	--	--	--	--	**--**	--	--
	Susceptible*	**--**	**1/1 (100.0%)**	--	1/2 (50.0%)	--	--	**1/1 (100.0%)**	**1/1 (100.0%)**

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

4.4 Quality control strain *E. coli* ATCC 25922

The quality control strain *E. coli* ATCC 25922 was sent to all participating laboratories to be used as a reference strain for both *E. coli* and *Salmonella* trials. Antimicrobial susceptibility test results for the quality control strain were evaluated separately for each of the trials.

4.4.1 Deviations in the *E. coli* trial

Nine out of 10 participants in the *E. coli* trial tested the reference strain *E. coli* ATCC 25922, although some of these laboratories tested the strain against very few antimicrobials (laboratory #18 tested five antimicrobials, laboratory #28 tested six antimicrobials, and laboratory #20 tested only 3 drugs).

Antimicrobial susceptibility testing of the quality control strain was performed by disk diffusion (Inhibition Zone Diameter determination) or by either agar dilution, macrobroth dilution or microbroth dilution for MIC determination. One laboratory tested colistin by disk diffusion, which is not the recommended standard method due to its large molecule. This result was therefore considered incorrect (**Table 19**, see *). The highest proportion of test results outside of the expected range were observed for cefotaxime, ciprofloxacin, ertapenem and trimethoprim (**Table 19**). The inaccurate results seem not to be methodology-dependent, as the results outside of range arise from both disk diffusion and MIC. However, when microbroth dilution was the method applied, the tested antimicrobial ranges were mostly above the expected ranges, making it impractical to determine the exact MIC, and generating incorrect results (scored as “0” by the informatics module). This occurrence was seen for cefotaxime (laboratories #23, #26 and #30), ciprofloxacin (laboratories #19 and #23) and two other isolated cases (cefepime and trimethoprim for laboratory #26). The deviations from the disk diffusion method were in either direction from the expected range.

Considering these observations, the laboratories’ performance is at some extent dependent on the methodology applied for AST of the quality control strain (**Figure 13**). Laboratories’ #23 and #26 deviations were

mostly caused by the applied MIC determination method, which tested antimicrobial concentrations above the expected range. Laboratory #18 presented 100% deviation, as the Inhibition Zone Diameters determined for the five tested antimicrobials were below the expected range. Laboratories #19, #20 and #30 had only one deviating result, whereas laboratories #22 and #28 presented no deviation from the expected range.

Table 19. Antimicrobial susceptibility testing of the reference strain *E. coli* ATCC 25922 in the *E. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

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

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; MIC – MIC determination by agar dilution or microbroth dilution

*Disk diffusion is not recommended for testing colistin

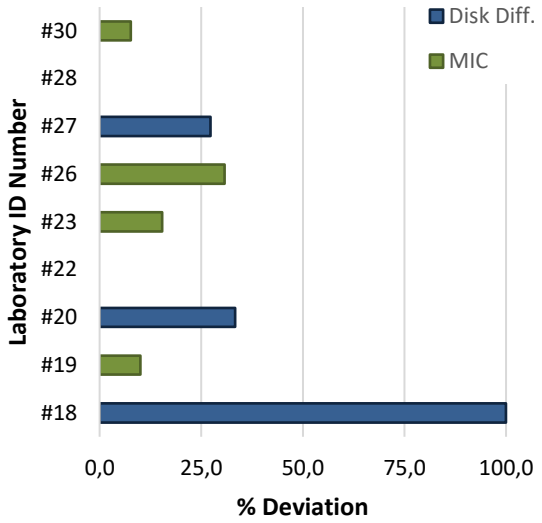

Figure 13. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *E. coli* trial by the AH laboratories.

4.4.2 Deviations in the *Salmonella* trial

Six laboratories submitted results regarding AST of *E. coli* ATCC 25922 reference strain in the *Salmonella* trial. Also here, different methodologies were applied for testing the quality control strain (disk diffusion, agar dilution and microbroth dilution). A few test results outside the expected range were observed across the list of antimicrobials (**Table 20**). Most of the deviations were observed for laboratory #21 (**Figure 14**), which used disk diffusion method and reported Zone Inhibition Diameters below the expected range.

Table 20. Antimicrobial susceptibility testing of the reference strain *E. coli* ATCC 25922 in the *Salmonella* trial. Proportion of test results outside of expected range is presented by methodology used.

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

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by micro- and macrobroth dilution

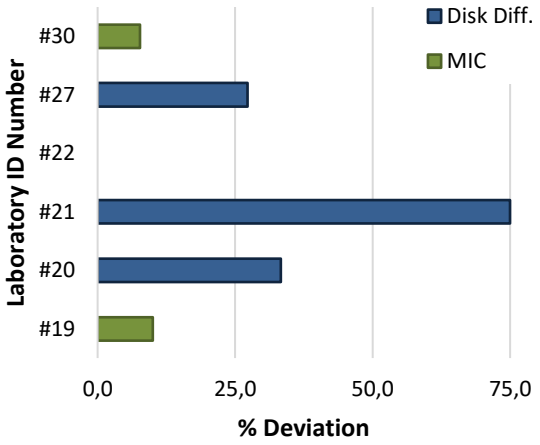

Figure 14. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *Salmonella* trial by the AH laboratories.

5. Discussion

5.1 Human Health Laboratories

A total of 13 Human Health laboratories participated in EQAsia 1st EQA, either on the *E. coli* trial alone or in both *E. coli* and *Salmonella* trials. In the bacterial identification component, the participants showed high proficiency in correctly identifying the target species (*E. coli* or *Salmonella*) among the provided test strains.

The antimicrobial susceptibility testing performance was assessed from different perspectives to better identify deviations from the expected results. Hence, the strain-based analysis revealed a higher difficulty in testing E EQASIA 21.5, for which a notable proportion of deviations was caused by expected MIC/Inhibition Zone Diameter values being close to the cut-off epidemiological values. In this situation, a one-fold dilution/±3mm difference from an expected value resulted in a different interpretation and was scored as incorrect. For the *Salmonella* strains, the deviations observed for each of them did not exceed 10%.

On both trials, sulfamethoxazole was the least tested antimicrobial. In fact, sulfamethoxazole and trimethoprim are rarely tested alone by HH laboratories, but rather in combination, which explains the few testing results submitted for these two drugs. Sulfamethoxazole was also one of the antimicrobials causing the highest deviations from expected results. Sulfamethoxazole MIC/Inhibition Zone Diameter

reading is challenging due to the bacteriostatic effect, and thus 1) MIC has to be read at the concentration in which there is ≥ 80% reduction in growth compared to the positive control, and 2) slight growth (≤ 20% of the lawn of growth) should be disregarded, and the more obvious margin measured to determine the zone diameter. This situation may lead to subjective interpretations.

In addition to sulfamethoxazole, ceftazidime resulted in several deviations in the *E. coli* trial, possibly because the expected MIC/Inhibition Zone Diameter values for strain E EQASIA 21.7 were close to the cut-off epidemiological value for this antimicrobial, resulting in the strain being reported as susceptible when it should be resistant.

Regarding the HH laboratories' AST performance, on average, the deviation was 6.9% in the *E. coli* trial (above the acceptance level) and 2.8 % in the *Salmonella* trial (below the acceptance level). Eight laboratories (#02, #12, #17, #13, #04, #10, #05 and #08) did not perform within the expected range in the *E. coli* trial. Two of those laboratories (#02 and #17) also obtained a result deviation larger than 5% in the *Salmonella* trial.

Detection and confirmation of presumptive beta-lactamase producing phenotypes was an optional component of this EQA, but highly encouraged due to its importance. A big part of the participating laboratories submitted results and in most of the cases were able to differentiate the susceptible (no ESBL, AmpC or carbapenemase) from the ESBL/AmpC/ carbapenemase-producers. However, some mistakes were observed in the correct classification of the phenotypes, suggesting that the laboratories still need support on capacity building.

Serotyping of *Salmonella* was also a component with voluntary participation, but six out of the seven participating laboratories reported results, which is a positive outcome. Based on the results, it was noticeable that some laboratories only have the capacity to identify certain serogroups and consequently serotyping. Within their capacity, the reported results were satisfactory.

Lastly, AST of the quality control strain demonstrated that the HH laboratories' performance was highly methodology-dependent. The results outside of range revealed that the MIC method applied presented limitations for an exact MIC determination (antimicrobial ranges tested above the expected interval). Therefore, these deviations do not necessarily imply a poor performance of the laboratories, but rather an inappropriate method for testing the quality control strain.

5.2 Animal Health Laboratories

For the Animal Health sector, 10 laboratories submitted results for *E. coli* and 8 of these laboratories additionally submitted results for *Salmonella*. Four of the participating laboratories (#18, #21, # 26 and #27) demonstrated limited capacity for performing bacterial identification; the remaining laboratories were able to correctly identify the strains, with only a couple of misidentifications reported.

Ertapenem, cefoxitin, azithromycin and ceftazidime were the antimicrobials with highest percentage deviations amongst the AH laboratories on both trials. Actually, these antimicrobials are not commonly used in food animals in Asia.

Regarding laboratories performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. Several AH laboratories did not perform within the expected range (deviations above the acceptance level of 5%). In fact, the average deviation was 11.2% and 11.0%, in the *E. coli* and *Salmonella* trials, respectively. Eight AH laboratories (#18, #19, #20, #21, #22, #26, #27 and #28) underperformed in one or both of the trials. Even

though some of the deviations can be explained by expected MIC/Inhibition Zone Diameter values close to the cut-off epidemiological value resulting in different interpretations, technical performance issues seem to have occurred in testing the susceptibility of several antimicrobials.

Relatively few AH laboratories (only #18, #22 and #28) had the capacity to identify the beta-lactam resistance phenotypes and further classifying them into Extended-Spectrum Beta-Lactamase (ESBL)/AmpC/carbapenemase production. Some of the laboratories tested the strains towards cefotaxime and/or ceftazidime, allowing the identification of beta-lactam resistance phenotypes, but the absence of test results from these drugs in combination with the beta-lactam inhibitor clavulanic acid prevented them from further confirming a presumptive ESBL-phenotype. The same occurred with cefoxitin and subsequent detection of an AmpC-phenotype, which was not a commonly tested drug and with many deviations from expected results. Meropenem susceptibility tests were not performed by the laboratories submitting results for this component, which was also reflected in the misinterpretation of the carbapenemase-producers.

No AH laboratories performed *Salmonella* serotyping. This part of the trial was voluntary, but it is evident that there is a need for capacity building within this area among the AH sector laboratories in South and Southeast Asia.

Finally, laboratories performed antimicrobial susceptibility testing of *E. coli* ATCC 25922. Deviations were defined as AST results of the reference strain that were outside the quality control acceptance intervals. The deviations that originated from MIC determination methodologies were mostly due to acceptance intervals being outside the antimicrobial test range, whereas Inhibition Zone Diameters determined by disk diffusion were either above or below the expected range, often very different from the expected interval of values, demonstrating technical problems in performing AST.

6. Conclusions

This report presented the results of the first EQAsia EQA trial 2021, which included *E. coli* and *Salmonella*. This EQA assessed the performance in 1) bacterial identification, 2) AST determination and interpretation, 3) detection of beta-lactam resistance phenotypes mediated by ESBL/AmpC/carbapenemase and 4) serotyping of *Salmonella*.

The goal of EQAsia EQAs is to have all participating Human and Animal Health laboratories performing antimicrobial susceptibility testing of the offered pathogens (here *E. coli* and *Salmonella*) with a result deviation level below 5%, and to address underperformance by supporting the laboratories with technical guidance and capacity building.

For both Human and Animal Health laboratories, a higher participation rate was found for *E. coli* than for *Salmonella*, with 23 and 15 laboratories participating, respectively. This may indicate that laboratories in South and Southeast Asia may be more used to routine testing of *E. coli* compared to *Salmonella*.

Performance issues were detected for both sectors, but larger deviations were observed among the AH laboratories demonstrating the need for supporting with training and capacity building the reference laboratories in the South and Southeast Asian region, and the AH sector in particular.

In this report, the data was adjusted (see Section 2.6), due to erroneous interpretation of

MIC/Inhibition Zone Diameter values, which were otherwise obtained within the acceptable range. This was caused by laboratories using guidelines different from those indicated in the EQA protocol. In the future, such adjustments will not be conducted. Rather, it is recommended and will be emphasized to solely use the interpretative criteria available in the EQA protocol as well as to implement quality control procedures such as having two different persons reading the results and the respective interpretations. It is a requirement that all participating laboratories follow the same interpretation criteria to allow for comparison of results.

7. References

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.

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.

EQAsia Website: https://antimicrobialresistance.dk/eqasia.aspx

EUCAST Website: https://www.eucast.org/

Clinical and Laboratory Standards Institute; M100. Performance standards for Antimicrobial Susceptibility Testing 30th edit, January 2020.

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.

Grimont P. A., Weill F.-X. *Antigenic Formulae of the Salmonella Serovars.* 9th. Paris, France: WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur; 2007.

8. Appendices

Appendix 1: EQA1 Protocol

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

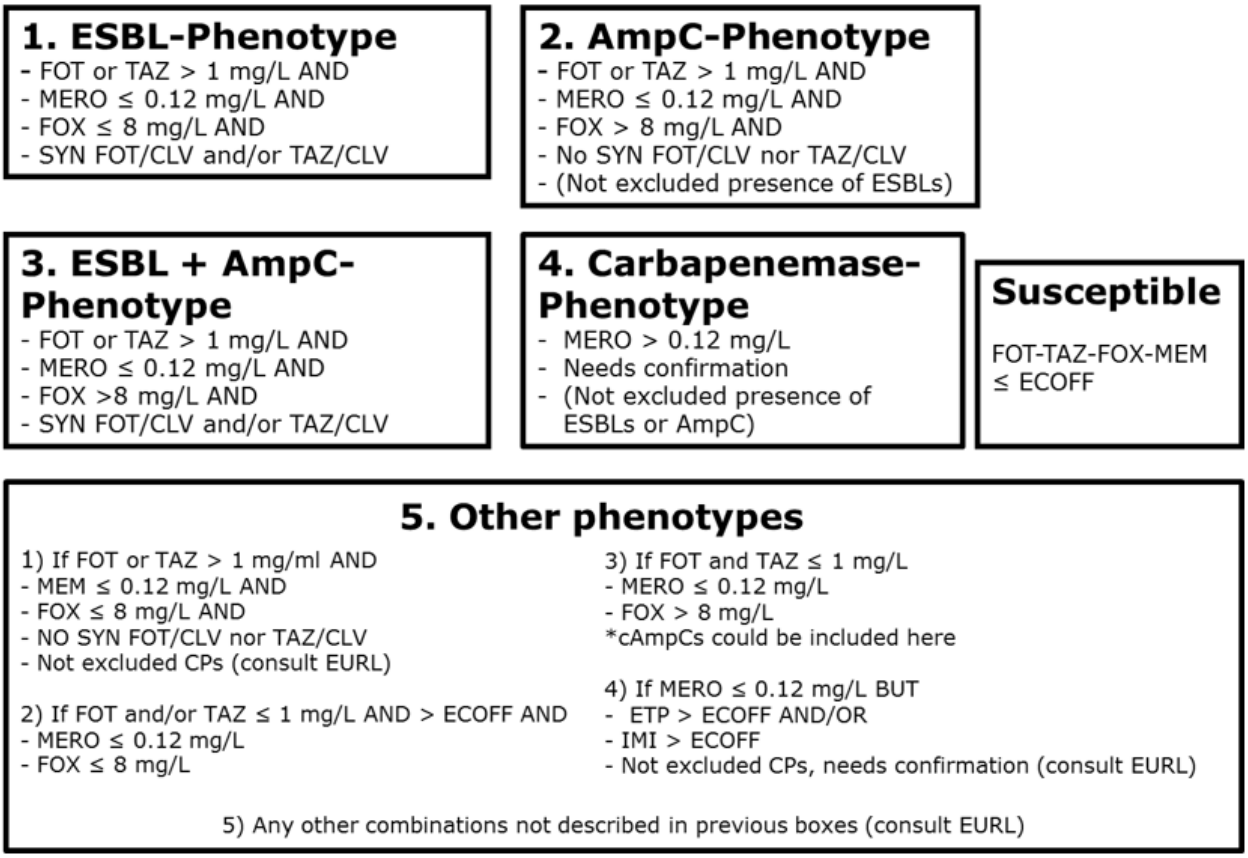

Figure 1: EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018.

Appendix 2a: 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	
E EQASIA 21.1	>1024	R	64	R	>32	R	>64	R	0,25/4	64	R	4	R	0,5/4	128	R	>8	R
E EQASIA 21.3	>64	R	64	R	0,25	S	≤0,25	S	0,12/4	16	R	≤0,50	S	0,25/4	>128	R	>8	R
E EQASIA 21.4	>64	R	8	S	>32	R	>64	R	0,12/4	4	S	4	R	0,25/4	≤8	S	≤0,015	S
E EQASIA 21.5	>64	R	64	R	≤0,06	S	2	R	2/4	>64	R	8	R	8/4	≤8	S	0,12	R
E EQASIA 21.7	>64	R	64	R	8	R	32	R	≤0,06/4	4	S	1	R	≤0,12/4	64	R	0,12	R
E EQASIA 21.8	4	S	8	S	≤0,06	S	≤0,25	S	≤0,06/4	4	S	≤0,25	S	≤0,12/4	≤8	S	≤0,015	S
E EQASIA 21.9	>64	R	8	S	32	R	>64	R	2/4	64	R	16	R	4/4	>128	R	>8	R
E EQASIA 21.11	>64	R	16	S	0,25	S	16	R	8/4	64	R	32	R	16/4	>128	R	2	R

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	
E EQASIA 21.1	≤1	S	2	R	>32	R	0,5	S	0,5	R	>128	R	>1024	R	>64	R	≤0,25	S	>32	R
E EQASIA 21.3	≤1	S	≤0,015	S	1	S	0,25	S	≤0,03	S	>128	R	>1024	R	>64	R	≤0,25	S	>32	R
E EQASIA 21.4	≤1	S	≤0,015	S	≤0,5	S	0,25	S	≤0,03	S	≤4	S	≤8	S	≤2	S	≤0,25	S	≤0,25	S
E EQASIA 21.5	2	S	0,03	S	2	S	0,5	S	≤0,03	S	≤4	S	≤8	S	≤2	S	≤0,25	S	>32	R
E EQASIA 21.7	≤1	S	≤0,015	S	≤0,5	S	0,25	S	≤0,03	S	≤4	S	>1024	R	>64	R	≤0,25	S	>32	R
E EQASIA 21.8	≤1	S	≤0,015	S	≤0,5	S	≤0,12	S	≤0,03	S	≤4	S	≤8	S	≤2	S	≤0,25	S	≤0,25	S
E EQASIA 21.9	≤1	S	0,06	R	1	S	0,25	S	≤0,03	S	>128	R	≤8	S	>64	R	0,5	S	>32	R
E EQASIA 21.11	≤1	S	0,06	R	32	R	0,25	S	≤0,03	S	>128	R	>1024	R	>64	R	0,5	S	≤0,25	S

R, Resistant; S, Susceptible

Appendix 2b: Reference values (MIC) – *Salmonella*

Reference values (MIC values and interpretation) – *Salmonella*

	Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		FOT+clav F/C	Cefoxitin FOX		Ceftazidime TAZ		TAZ+clav T/C	Chloramphenicol CHL		Ciprofloxacin CIP	
S EQASIA 21.1	>64	R	8	S	0,12	S	≤0,25	S	0,12/4	4	S	≤0,5	S	0,5/4	≤8	S	0,5	R
S EQASIA 21.2	2	S	8	S	≤0,06	S	≤0,25	S	0,12/4	2	S	≤0,5	S	0,25/4	≤8	S	≤0,015	S
S EQASIA 21.4	>64	R	>64	R	>32	R	>64	R	0,25/4	4	S	32	R	1/4	>128	R	1	R
S EQASIA 21.5	>64	R	8	S	0,5	S	32	R	32/4	64	R	32	R	32/4	>128	R	≤0,015	S
S EQASIA 21.6	>64	R	4	S	>32	R	>64	R	0,25/4	8	S	16	R	0,5/4	≤8	S	0,25	R
S EQASIA 21.8	2	S	8	S	0,25	S	0,5	S	0,5/4	16	R	1	S	0,5/4	≤8	S	0,03	S
S EQASIA 21.10	≤1	S	8	S	≤0,06	S	≤0,25	S	0,12/4	2	S	≤0,5	S	0,25/4	128	R	≤0,015	S
S EQASIA 21.11	≤1	S	8	S	≤0,06	S	≤0,25	S	≤0,06/4	4	S	≤0,5	S	0,25/4	≤8	S	≤0,015	S

R, Resistant; S, Susceptible

	Colistin COL		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Meropenem MERO		Nalidixic acid NAL		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP	
S EQASIA 21.1	2	S	≤0,015	S	≤0,5	S	0,5	S	0,06	S	8	S	16	S	>64	R	≤0,25	S
S EQASIA 21.2	2	S	≤0,015	S	≤0,5	S	0,25	S	0,06	S	≤4	S	16	S	≤2	S	≤0,25	S
S EQASIA 21.4	2	S	0,06	S	>32	R	0,5	S	0,06	S	>128	R	>1024	R	>64	R	>32	R
S EQASIA 21.5	2	S	0,03	S	≤0,5	S	0,5	S	0,06	S	≤4	S	>1024	R	>64	R	≤0,25	S
S EQASIA 21.6	2	S	0,03	S	≤0,5	S	0,5	S	0,06	S	>128	R	512	R	64	R	>32	R
S EQASIA 21.8	4	R	0,03	S	32	R	0,5	S	0,06	S	≤4	S	>1024	R	≤2	S	≤0,25	S
S EQASIA 21.10	2	S	≤0,015	S	≤0,5	S	0,25	S	≤0,03	S	≤4	S	>1024	R	>64	R	>32	R
S EQASIA 21.11	2	S	≤0,015	S	≤0,5	S	0,25	S	0,06	S	≤4	S	1024	R	>64	R	>32	R

R, Resistant; S, Susceptible

Appendix 3: Quality control ranges for ATCC reference strain

Quality Control ranges for ATCC reference strain

E. coli ATCC 25922		
Antimicrobial	**MIC**	**Disk Difusion**
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
Colistin, COL	0.25-2	--
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 30th edition

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