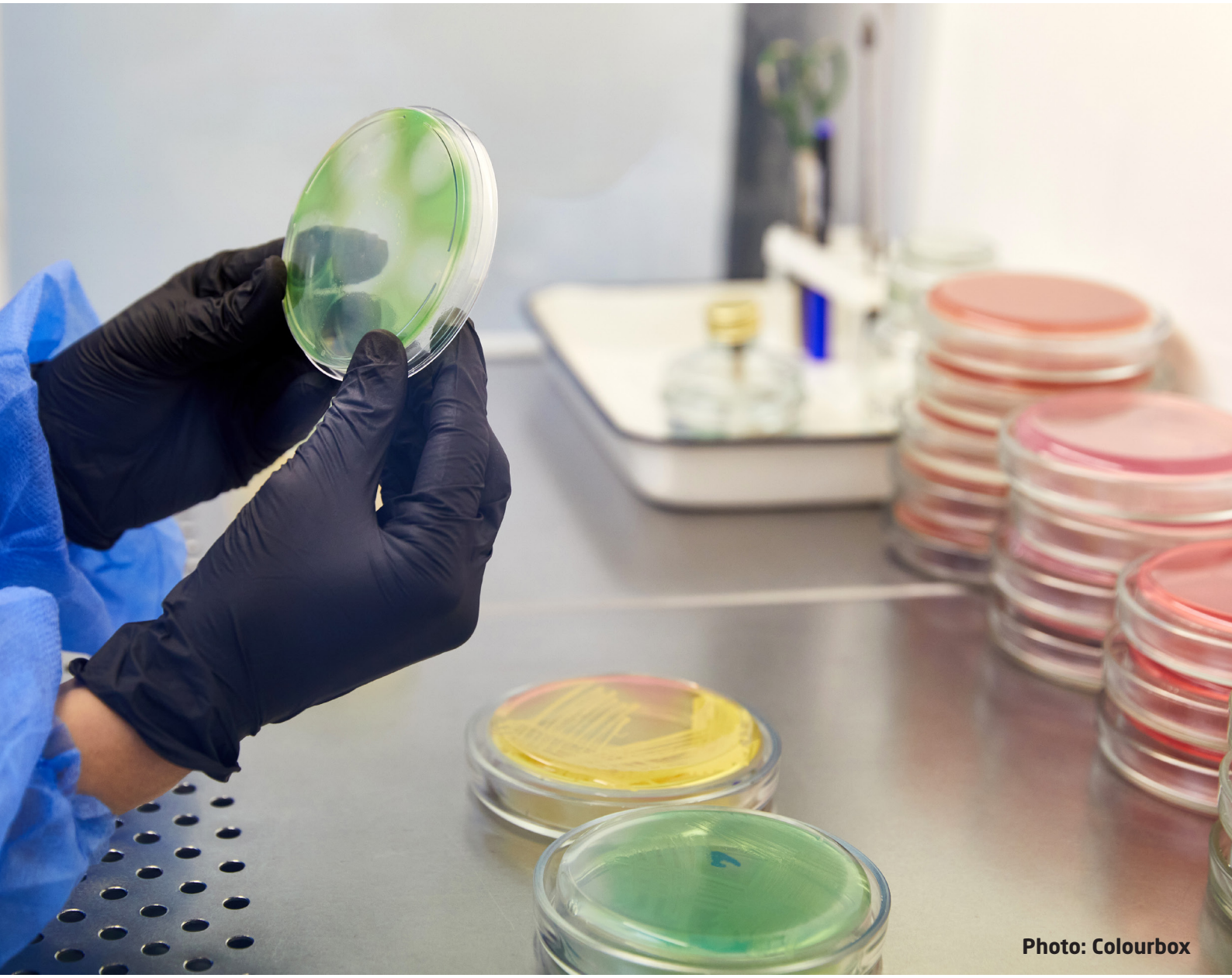
The 3rd EQAsia External Quality Assessment trial: Salmonella spp., Escherichia coli, Pseudomonas aeruginosa, Campylobacter jejuni / C. coli, Enterococcus faecium / E. faecalis and Streptococcus pneumoniae - 2021



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National Food Institute
Technical University of Denmark
Kemitorvet
Building 204
DK-2800 Kgs. Lyngby
Denmark

The 3rd EQAsia External Quality Assessment trial: *Salmonella* spp., *Escherichia coli, Pseudomonas aeruginosa, Campylobacter jejuni* / *C. coli, Enterococcus faecium* / *E. faecalis* and *Streptococcus pneumoniae* – 2021

Patrícia Teixeira dos Santos[1], Freshwork A. Abegaz[2], Ondari D. Mogeni[2], Pattrarat Chanchaithong[3], Paulina Tamez-Hidalgo[1], Pitak Santanirand[4], Rangsiya Prathan[3], Taradon Luangtongkum[3], Tobin Guarnacci[2], Watcharaporn Kamjumphol[4], Nimesh Poudyal[2], Rungtip Chuanchuen[3], Wantana Paveenkittiporn[4], Rene S. Hendriksen[1]

[1]National Food Institute, Technical University of Denmark, Denmark
[2]International Vaccine Institute, South Korea
[3]Faculty of Veterinary Science, Chulalongkorn University, Thailand
[4]National Institute of Health, Department of Medical Sciences, Thailand

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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 regional 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.

A total of five EQA trials are taking place during 2021-2022. The EQA trials focus on the WHO GLASS pathogens [1] and FAO priority pathogens [2]: *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Campylobacter* (*C. coli* and *C. jejuni*), Enterococci (*E. faecium* and *E. faecalis*) and *Streptococcus pneumoniae*. In addition, two Matrix EQAs are offered (one in each year), 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 food matrices.

For a given organism, candidate strains are assessed and validated by DTU Food and the external partner (The Peter Doherty Institute for Infection and Immunity, Australia). 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.

This report contains results from the third EQA trial of the EQAsia project (EQA3) carried out in November 2021-January 2022. The 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 are requested.

This third EQA trial includes serotyping of *Salmonella* spp., as well as identification and AST of *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter* (*C. jejuni* and *C. coli*), *Enterococci* (*E. faecium* and *E. faecalis*) and *Streptococcus pneumoniae*. 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 bacterial identification and 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 EQA3 protocol (Appendix 1) and allow for the obtained results to be interpreted into categories as resistant, intermediate or susceptible depending on the method used. Results in agreement with the expected interpretation are scored '4' (correct), while results deviating from the expected interpretation are scored as either '0' (incorrect: very major), '1' (incorrect: major) or '3' (incorrect: minor), as explained in the EQA3 protocol (Appendix 1). 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 to the respective participating laboratory. 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 (score of 0, 1 or 3) even though the actual MIC/Zone Diameter measured is only one-fold dilution/±3mm apart 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 Ben Howden (The Peter Doherty Institute for Infection and Immunity, Australia), Navin Karan (Pacific Pathology Training Centre, New Zealand) and Monica Lahra (WHO Collaborating Centre for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia).

2. Materials and Methods

2.1 Participants in EQAsia EQA3

A total of 25 laboratories participated in the third EQA survey of the EQAsia project: 14 laboratories belonging to the HH Sector and 11 belonging to the AH Sector, originating from: Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, Maldives, Nepal, Pakistan, Philippines, Sri Lanka and Timor-Leste (**Figure 1**).

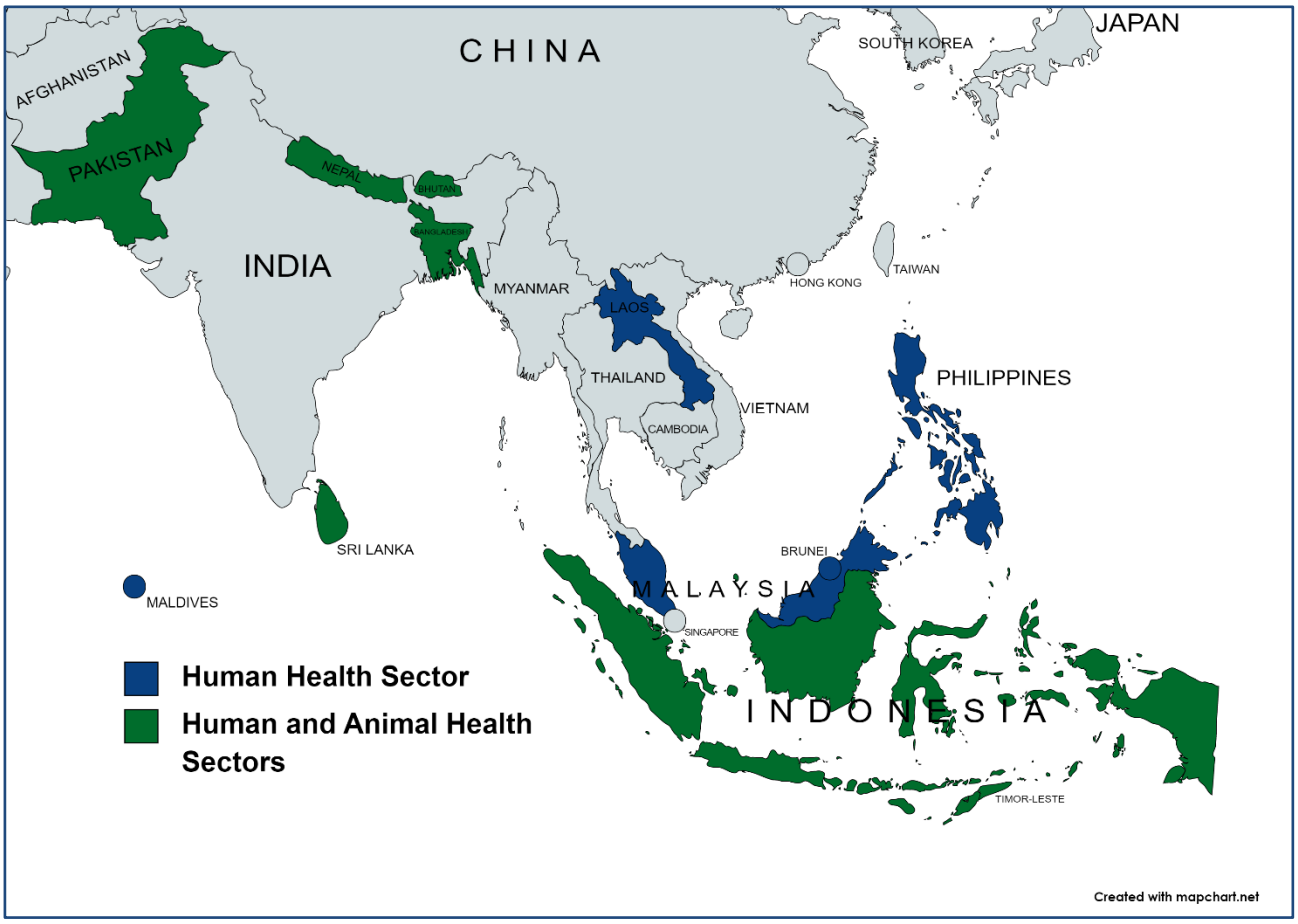

Figure 1: Countries participating in the 3rd EQA of the EQAsia 2021-22 project on antimicrobial susceptibility testing. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

2.2 Strains

Participating laboratories could register for any of the trials. 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 target species of each organism 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 EQA3, one of the test strains is used as an internal control strain that will also be included in future EQAs with varying strain code.

Candidate strains for this EQA were tested at DTU Food and additionally verified by the external partner (The Peter Doherty Institute for Infection and Immunity, Australia). Results could not be verified by the external partner for amikacin, cefepime, cefoxitin, ceftazidime, colistin, ertapenem and imipenem (*Salmonella*); azithromycin, cefotaxime, cefotaxime and clavulanic acid, cefoxitin, ceftazidime and clavulanic acid, chloramphenicol, ciprofloxacin, nalidixic acid, sulfamethoxazole and trimethoprim (*E. coli*); levofloxacin (*P. aeruginosa*); chloramphenicol and ertapenem (*Campylobacter*); ciprofloxacin, gentamicin, quinupristin/dalfopristin and teicoplanin (*Enterococci*). Expected MIC values (Appendix 2) of the selected strains for this EQA were further confirmed by NIH (*P. aeruginosa*, *Enterococci* and *S. pneumoniae*) and CUVET (*Salmonella*, *E. coli* and *Campylobacter*).

The reference strains *E. coli* ATCC 25922, *E. coli*

NCTC 13846, *P. aeruginosa* ATCC 27853, *C. jejuni* ATCC 33560, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619 were provided to all participants (in this trial or in previous trials) 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 strains (Appendix 3) were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-31st Ed., tables 4A-1 and 5A-1 [3], in document VET06-1st Ed., tables 21A and 21C [4], and from EUCAST in document "Routine and extended internal quality control for MIC determination and disk diffusion" [5].

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all six organisms are listed in the EQA3 protocol (Appendix 1) and summarized 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 zone diameter and MIC breakpoint values developed by CLSI (M100, 31st Ed. and VET06, 1st Ed.) [3, 4]. When not available, EUCAST clinical breakpoints (Tables v. 11.0, 2021) [5] or epidemiological cut off values [6] 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 EQA3 protocol (Appendix 1).

Participants were encouraged to test as many as possible of the antimicrobials listed, but always considering their relevance regarding the laboratory's routine work.

2.4 Distribution

The bacterial strains were dispatched as lyophilized strains in November 2021 by NIH and CUVET 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 [8], to allow access to all the necessary information at any time. The participants were recommended to store the lyophilized strains in a dark, dry and 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. Participants were encouraged to submit serotyping results for the *Salmonella* strains on a voluntary basis, as well as to perform testing for detection of ESBL-, AmpC-, and carbapenemase-producing *E. coli*.

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

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA3 2021. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

Salmonella	*E. coli*	*P. aeruginosa*	*Campylobacter*	*Enterococci*	*S. pneumoniae*
Amikacin (AMK)	Amikacin (AMK)	Amikacin (AMK)	Chloramphenicol (CHL)	Ampicillin (AMP)	Amoxicillin/
Ampicillin (AMP)	Ampicillin (AMP)	Aztreonam (AZT)	Ciprofloxacin (CIP)	Chloramphenicol (CHL)	clavulanic acid (AUG2)
Azithromycin (AZI)	Azithromycin (AZI)	Cefepime (FEP)	Ertapenem (ETP)	Ciprofloxacin (CIP)	Azithromycin (AZI)
Cefepime (FEP)	Cefepime (FEP)	Ceftazidime (TAZ)	Erythromycin (ERY)	Daptomycin (DAP)	Cefepime (FEP)
Cefotaxime (FOT)	Cefotaxime (FOT)	Ciprofloxacin (CIP)	Gentamicin (GEN)	Erythromycin (ERY)	Cefotaxime (FOT)
Cefoxitin (FOX)	Cefotaxime/	Colistin (COL)	Tetracycline (TET)	Gentamicin (GEN)	Ceftriaxone (AXO)
Ceftazidime (TAZ)	clavulanic acid (F/C)	Doripenem (DOR)		Linezolid (LZD)	Cefuroxime (FUR)
Chloramphenicol (CHL)	Cefoxitin (FOX)	Gentamicin (GEN)		Quinupristin/	Chloramphenicol (CHL)
Ciprofloxacin (CIP)	Ceftazidime (TAZ)	Imipenem (IMI)		dalfopristin (SYN)	Clindamycin (CLI)
Colistin (COL)	Ceftazidime/	Levofloxacin (LEVO)		Teicoplanin (TEI)	Ertapenem (ETP)
Ertapenem (ETP)	clavulanic acid (T/C)	Meropenem (MERO)		Tetracycline (TET)	Erythromycin (ERY)
Gentamicin (GEN)	Chloramphenicol (CHL)	Piperacillin/		Tigecycline (TGC)	Levofloxacin (LEVO)
Imipenem (IMI)	Ciprofloxacin (CIP)	tazobactam (P/T4)		Vancomycin (VAN)	Linezolid (LZD)
Meropenem (MERO)	Colistin (COL)	Tobramycin (TOB)			Meropenem (MERO)
Sulfamethoxazole (SMX)	Doripenem (DOR)				Penicillin (PEN)
Tetracycline (TET)	Ertapenem (ETP)				Tetracycline (TET)
Trimethoprim (TMP)	Gentamicin (GEN)				Trimethoprim/
	Imipenem (IMI)				sulfamethoxazole (SXT)
	Levofloxacin (LEVO)				Vancomycin (VAN)
	Meropenem (MERO)				
	Nalidixic Acid (NAL)				
	Piperacillin/				
	tazobactam (P/T4)				
	Sulfamethoxazole (SMX)				
	Tetracycline (TET)				
	Tigecycline (TGC)				
	Tobramycin (TOB)				
	Trimethoprim (TMP)				
	Trimethoprim/ sulfamethoxazole (SXT)				

2.6 Data management

Antimicrobial susceptibility testing of some of the reference strains revealed a number of incorrect results. These deviations (results outside the acceptance interval) were caused by the method used for MIC determination. This issue was also verified on EQA1 and EQA2 and reported. Briefly, MIC determination by broth microdilution 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.

Table 2. Adjusted scores for reported MIC values for *E. coli* ATCC 25922 and *S. pneumoniae* ATCC 49619 reference strains. 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'
Doripenem	0.016-0.06	≤ 0.12	Changed to '1'
Ertapenem	0.004-0.016	≤ 0.5	Changed to '1'
Levofloxacin	0.008-0.06	≤ 0.12	Changed to '1'
Meropenem	0.008-0.06	≤ 0.25	Changed to '1'
Tigecycline	0.03-0.25	≤ 0.5	Changed to '1'
***S. pneumoniae* ATCC 49619**			
Antimicrobial	**MIC Quality Control Range**	**MIC reported by the labs**	**Score**
Clindamycin	0.03-0.12	≤ 0.25	Changed to '1'

3. Results – Human Health Laboratories

3.1 Overall participation

Among the 14 Human Health laboratories participating in the 3rd EQA of the EQAsia Programme, 12 laboratories submitted results for the *E. coli* and *P. aeruginosa* trials, 11 for the *E. faecium* / *E. faecalis* trial, 10 for the *S. pneumoniae* trial, eight for the *Salmonella* trial, and only one laboratory submitted results for the *C. jejuni* / *C. coli* trial. The methodologies applied varied amongst the participating laboratories and are summarized in **Figure 2**. Most of the laboratories opted for only one method (mainly disk diffusion or broth microdilution), whereas others performed AST using different methodologies and reported both Inhibition Zone Diameters and MIC depending on the antimicrobial drug tested.

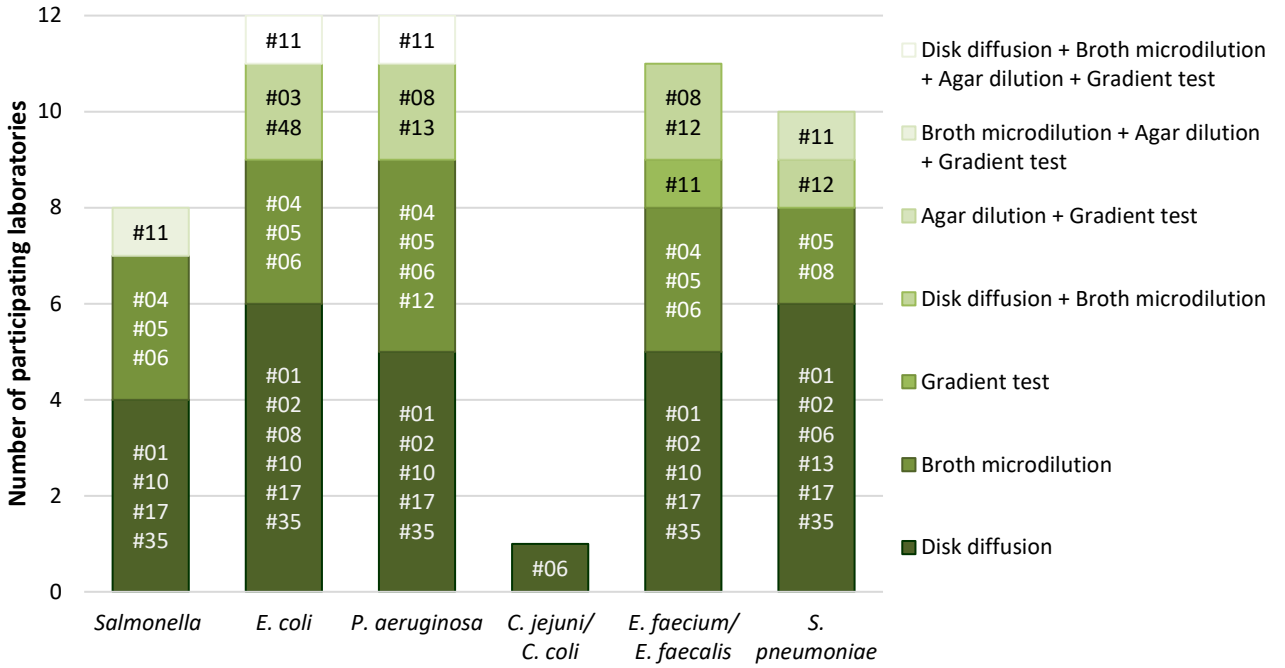

Figure 2. Methodologies applied by the HH laboratories participating in each of the trials.

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') 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**). For Gram negative bacteria *Salmonella*, *E. coli* and *P. aeruginosa* trials (**Table 3**), cefepime, ceftazidime, ciprofloxacin and meropenem were tested by 90-100% of the laboratories participating in each trial. In contrast, the last resort antibiotics such as colistin and tigecycline (*E. coli* trial only) were tested by less than half of the participating laboratories (**Table 3**). Antibiotics such as sulfamethoxazole and trimethoprim were not tested by the HH laboratories. Instead, the combination drug trimethoprim/ sulfamethoxazole was tested for the *E. coli* trial (**Table 3**). Regarding the *C. jejuni/ C. coli* trial, the only laboratory submitting results reported data for three out of the six recommended antimicrobials (**Table 3**).

For Gram positive bacteria, ampicillin, chloramphenicol, ciprofloxacin and vancomycin were tested by at least 10 out of the 11 laboratories participating in the *E. faecium/ E. faecalis* trial, as well as chloramphenicol, erythromycin, tetracycline and vancomycin were widely tested (all 10 laboratories) for the *S. pneumoniae* trial (**Table 3**). In opposition, daptomycin and quinupristin/dalfopristin (*E. faecium/ E. faecalis* trial), as well as amoxicillin/ clavulanic acid, cefuroxime and ertapenem (*S. pneumoniae* trial) were chosen for testing by less than 30% of the laboratories (**Table 3**).

Scattering of missing data or incomplete AST results entries were observed for all trials, except *C. jejuni/C. coli* trial (**Tables 4-8**). There was only one laboratory (#06) testing antibiotics against *C. jejuni*/*C. coli.* Three antibiotics were selected to test two *Campylobacter* strains, and there was no incomplete data.

Three of the eight laboratories selecting *Salmonella* did not submit complete results of their own available antimicrobial agents (**Table 4**). The highest number of incomplete results in the *Salmonella* trial was seen for laboratories #05 and #06. A closer look to laboratory #06 missing data suggests that this laboratory may

have wrongly selected amikacin instead of ampicillin for strain Salm EQASIA 21.3 when submitting results (**Table 4**). Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance.

Similarly, more than half of the laboratories selecting *E. coli* (n=8) submitted incomplete results of their own available antimicrobials (**Table 5**). The highest number of incomplete results in the *E. coli* trial was seen for laboratories #06 and #48 (**Table 5**).

Regarding the *P. aeruginosa* trial, four out of the 12 participating laboratories presented incomplete results of their own available antimicrobial agents (**Table 6**).

Only one out of the 11 laboratories selecting *E. faecium/ E. faecalis* did not submit complete results: daptomycin was not reported for some of the strains (**Table 8**).

Six out of the 10 laboratories selecting *S. pneumoniae* submitted incomplete results (**Table 9**). The highest number of incomplete results in the *S. pneumoniae* trial was seen for laboratories #05 and #06. Once again, it appears that laboratory #06 may have selected azithromycin for some strains and amoxicillin/ clavulanic acid for others (**Table 9**).

Table 3. Antimicrobial susceptibility tests performed by the HH laboratories for each trial per antimicrobial, and in total (shown in bold). For a given trial (Salm, Ec, Pa, Campy, Ef or Sp), the number of tests performed by all participating laboratories per antimicrobial is shown (n), as well as the percentage (%) of tests per antimicrobial out of the total number of tests performed (N) for the trial (% of n/N). The antimicrobials not included in a given trial are represented as --.

Antimicrobial	ASTs in total: n (% of n/N)					
	Salm	Ec	Pa	Campy	Ef	Sp
AMK	30 (4.4)	93 (6.4)	95 (10.1)	--	--	--
AMP	60 (8.9)	87 (5.9)	--	--	87 (12.7)	--
AUG2	--	--	--	--	--	10 (1.4)
AZI	23 (3.4)	48 (3.3)	--	--	--	50 (6.9)
AZT	--	--	47 (5.0)	--	--	--
FEP	59 (8.7)	86 (5.9)	94 (10.0)	--	--	15 (2.1)
FOT	39 (5.8)	67 (4.6)	--	--	--	31 (4.3)
FOX	28 (4.1)	71 (4.9)	--	--	--	--
AXO	--	--	--	--	--	24 (3.3)
FUR	--	--	--	--	--	8 (1.1)
TAZ	61 (9.0)	95 (6.5)	95 (10.1)	--	--	--
CHL	39 (5.8)	56 (3.8)	--	0	79 (11.5)	75 (10.4)
CIP	62 (9.2)	94 (6.4)	95 (10.1)	2 (33.3)	79 (11.5)	--
CLI	--	--	--	--	--	59 (8.1)
COL	24 (3.5)	27 (1.8)	39 (4.1)	--	--	--
DAP	--	--	--	--	21 (3.1)	--
DOR	--	24 (1.6)	32 (3.4)	--	--	--
ETP	52 (7.7)	74 (5.1)	--	0	--	9 (1.2)
ERY	--	--	--	2 (33.3)	71 (10.3)	73 (10.1)
GEN	29 (4.3)	95 (6.5)	95 10.1)	0	32 (4.7)	--
IMI	55 (8.1)	79 (5.4)	85 (9.0)	--	--	--
LEVO	--	55 (3.8)	71 (7.5)	--	--	59 (8.1)
LZD	--	--	--	--	72 (10.5)	58 (8.0)
MERO	62 (9.2)	93 (6.4)	95 (10.1)	--	--	15 (2.1)
NAL	--	64 (4.4)	--	--	--	--
PEN	--	--	--	--	--	23 (3.2)
P/T4	--	83 (5.7)	77 (8.2)	--	--	--
SYN	--	--	--	--	16 (2.3)	--
SMX	0	0	--	--	--	--
TEI	--	--	--	--	48 (7.0)	--
TET	46 (6.8)	55 (3.8)	--	2 (33.3)	71 (10.3)	74 (10.2)
TGC	--	16 (1.1)	--	--	24 (3.5)	--
TOB	--	16 (1.1)	24 (2.5)	--	--	--
TMP	8 (1.2)	0	--	--	--	--
SXT	--	85 (5.8)	--	--	--	68 (9.4)
VAN	--	--	--	--	87 (12.7)	73 (10.1)
Total	**677**	**1463**	**944**	**6**	**687**	**724**

Salm, *Salmonella*; Ec, *E. coli*; Pa, *P. aeruginosa*; Campy, *C.jejuni/ C. coli*; Ef, *E. faecium/ E. faecalis*; Sp, *S. pneumoniae*
(n/N) number of tests performed per antimicrobial (n) out of the total tests performed for the trial (N)

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *Salmonella* strains reported by HH laboratories (n=8) participating in the 3rd EQA of the EQAsia project.

Lab ID No.	Salm EQASIA 21.2	Salm EQASIA 21.3	Salm EQASIA 21.4	Salm EQASIA 21.5	Salm EQASIA 21.6	Salm EQASIA 21.7	Salm EQASIA 21.8	Salm EQASIA 21.11
#04	ETP	--	--	--	--	--	--	--
#05	--	FEP; FOX		--	--	FEP	AMK; AMP; FEP; FOX; TAZ; ETP; GEN	--
#06	AMK	AMP	AMK	AMK	AMK	AMK	AMK	AMK; ETP

Salm, *Salmonella*; blue shade, strain not tested

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *E. coli* strains reported by HH laboratories (n=12) participating in the 3rd EQA of the EQAsia project.

Lab ID No.	Ec EQASIA 21.1	Ec EQASIA 21.2	Ec EQASIA 21.3	Ec EQASIA 21.5	Ec EQASIA 21.7	Ec EQASIA 21.8	Ec EQASIA 21.9	Ec EQASIA 21.11
#02	FOX	CIP	--	--	--	--	--	--
#03	--	--	--	--	FEP	--	--	--
#04	--	--	--	SXT	--	--	--	
#05	MERO	--	--	--	--	--	AMK	AMK; ETP; MERO
#06	--	ETP	ETP	ETP	PT4	ETP; PT4	PT4	PT4
#17	--	--	ETP	--	--	--	--	--
#35	TET; SXT	--	--	--	--	--	--	--
#48	COL	--	FOT	FOT	COL	FOT	FOT; COL	FOT; COL

Ec, *E. coli*; blue shade, strain not tested

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *P. aeruginosa* strains reported by HH laboratories (n=12) participating in the 3rd EQA of the EQAsia.

Lab ID No.	Pa EQASIA 21.1	Pa EQASIA 21.2	Pa EQASIA 21.3	Pa EQASIA 21.4	Pa EQASIA 21.5	Pa EQASIA 21.6	Pa EQASIA 21.9	Pa EQASIA 21.10
#05	--	--	--	PT4	--	FEP	--	--
#06	--	--	--	PT4	--	--	--	--
#12	--	--	--	--	--	--	--	PT4
#13	--	IMI	--	--	--	--	IMI	--

Pa, *P. aeruginosa*

Table 7. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by HH laboratories (n=11) participating in the 3rd EQA of the EQAsia project.

Lab ID No.	Ef EQASIA 21.1	Ef EQASIA 21.2	Ef EQASIA 21.4	Ef EQASIA 21.5	Ef EQASIA 21.7	Ef EQASIA 21.8	Ef EQASIA 21.10	Ef EQASIA 21.11
#12	--	--	DAP	DAP	--	--	DAP	--

Ef, *E. faecium/ E. faecalis*

Table 8. Distribution of incomplete or missing data of antimicrobial agents among *S. pneumoniae* strains reported by HH laboratories (n=10) participating in the 3rd EQA of the EQAsia project.

Lab ID No.	Sp EQASIA 21.1	Sp EQASIA 21.2	Sp EQASIA 21.3	Sp EQASIA 21.6	Sp EQASIA 21.7	Sp EQASIA 21.8	Sp EQASIA 21.10	Sp EQASIA 21.11
#01		--	--	VAN	--	--	--	
#05	CLI; ERY; SXT	ETP; LZD	ETP; SXT	ETP; SXT	ETP; SXT	ETP; SXT	ETP; SXT	ETP; LZD; SXT
#06		AUG2	AZI	AUG2	AUG2	AZI	AUG2	AUG2
#12	--	--	VAN	--	--	--	--	--
#13	TET	LEVO	--	--	--	--	--	--
#35	--	--	--	--	ERY	--	--	--

Sp, *S. pneumoniae*; blue shade, strains not tested

3.2 *Salmonella* trial

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

3.2.1 Bacterial identification

All eight laboratories participating in the *Salmonella* trial submitted results for bacterial identification (**Table 9**). All laboratories, except one (laboratory #10), correctly identified the tested *Salmonella* and non-*Salmonella* strains. Laboratory #05 did not test strain Salm EQASIA 21.4 and laboratory #10 wrongly identified strain Salm EQASIA 21.2 as non-*Salmonella* (**Table 9**).

Table 9. 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
Salm EQASIA 21.1	Non-*Salmonella* (*Klebsiella pneumoniae*)	8/8
Salm EQASIA 21.2	*Salmonella*	7/8
Salm EQASIA 21.3	*Salmonella*	8/8
Salm EQASIA 21.4	*Salmonella*	7/7
Salm EQASIA 21.5	*Salmonella*	8/8
Salm EQASIA 21.6	*Salmonella*	8/8
Salm EQASIA 21.7	*Salmonella*	8/8
Salm EQASIA 21.8	*Salmonella*	8/8
Salm EQASIA 21.9	Non-*Salmonella* (*Shigella sonnei*)	8/8
Salm EQASIA 21.10	Non-*Salmonella* (*Escherichia coli*)	8/8
Salm EQASIA 21.11	*Salmonella*	8/8

Salm, *Salmonella*

3.2.2 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.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 86.3% (strain Salm EQASIA 21.2) to 97.2% (strain Salm EQASIA 21.5) for each strain (**Table 10**). Only strain Salm EQASIA 21.2 revealed a deviation above 10% (**Table 10**). This high deviation was mostly caused by the results submitted by laboratory #04, which reported a quite susceptible strain (Appendix 2a) as resistant to several antimicrobials.

Table 10. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/I/S). Results are from 8 HH laboratories for the *Salmonella* trial.

Strain	AST in total	% Correct
Salm EQASIA 21.2	75	86.3
Salm EQASIA 21.3	87	93.7
Salm EQASIA 21.4	79	90.2
Salm EQASIA 21.5	89	97.2
Salm EQASIA 21.6	89	96.9
Salm EQASIA 21.7	88	95.5
Salm EQASIA 21.8	82	94.8
Salm EQASIA 21.11	88	96.3

Salm, *Salmonella*

Antimicrobial-based analysis

The antimicrobials that resulted in highest

percentage of deviation were colistin (24.0%), amikacin (12.5%), cefoxitin (12.5%), ciprofloxacin (10.5%) and ceftazidime (10.2%), whereas trimethoprim revealed no deviation from the expected results (**Figure 3**). The deviation seen for colistin and amikacin can be in part explained by the fact that the AST results of these antimicrobials (and also gentamicin) should only be interpreted as intermediate or resistant, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1). Instead, several laboratories (2 out 3 for colistin and 2 out of 5 for amikacin) reported the *Salmonella* isolates as being susceptible towards these antimicrobials, which resulted in a score penalty (score of 3 instead of the full score 4). In addition, colistin testing requires the use of broth microdilution, which some laboratories may be lacking the required knowledge and experience for applying this methodology.

Regarding cefoxitin, the deviation observed seems to have been mostly due to the results of laboratory #01, which found the *Salmonella* isolates as more resistant to the drug than expected (smaller Inhibition Zone Diameters reported). For ciprofloxacin and ceftazidime, the deviation can be partially explained by the incorrect interpretation of obtained results.

Laboratory-based analysis

For the *Salmonella* trial, five out of the eight HH laboratories presented a deviation above the acceptance level of 5% (laboratories #04, #06, #05, #01 and #35). The average deviation was 6.3% (ranging from 1.9 to 11.9%) (**Figure 4**). The highest deviation was observed for laboratory #04 and can be explained by the abovementioned issues with misinterpreting the obtained results and the incorrect results reported for strain Salm EQASIA 21.2.

Laboratory #06 (second highest deviation) reported the isolates as susceptible to colistin instead of intermediate, whereas laboratory #05 made the same mistake but for amikacin and gentamicin. In addition, both laboratories (#05 and #06) used a ciprofloxacin concentration range (broth microdilution method was applied) that did not allow for discriminating the isolate as susceptible (≤ 0.06) or intermediate (0.12-0.5), since the lowest concentration tested was ≤ 0.25 µg/mL.

Laboratory #01 presented some issues related to cefoxitin testing as mentioned in the previous sub-section, and laboratory #35 deviation seems to have been caused by occasional performance errors.

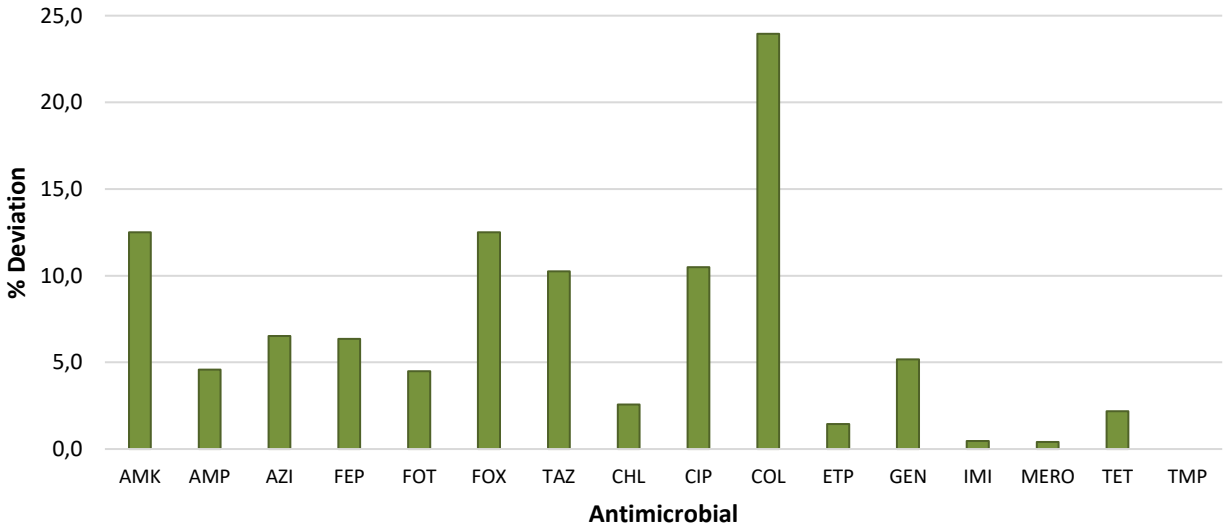

Figure 3. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by HH laboratories (n=8) participating in the 3rd EQA in the EQAsia project. Results are categorized by antimicrobial agent. Bars represent the average distribution of the deviation.

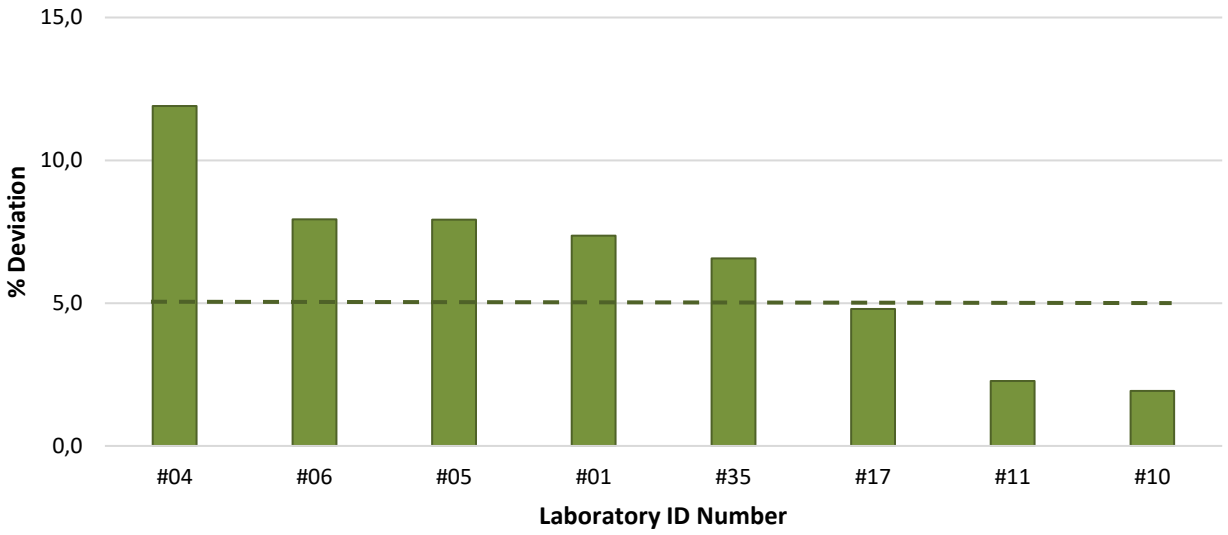

Figure 4. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by HH laboratories (n=8) participating in the 3rd EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 Serotyping

Serotyping of *Salmonella* was offered to the participants as a voluntary component. 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 [9].

Of the eight participating laboratories in the trial, four (#04, #05, #10 and #11) submitted results for *Salmonella* serogrouping, but only laboratory #11 provided serotyping results (**Table 11, Figure 5**). Laboratory #4 only submitted serogroup results for strain Salm EQASIA 21.2, which was correctly assigned; laboratory #05 uploaded serogroup results for seven strains (strain Salm EQASIA 21.4 was not tested as mentioned in section '3.2.1 Bacterial identification'), and identified the correct serogroup for six of them; laboratory #10 reported results for five strains and correctly identified the serogroup for three of them; lastly, laboratory #11 not only was the sole participant correctly identifying the serogroup of all eight *Salmonella* strains, as it was also the only one submitting serotyping data, and completely accurate as well (**Table 11**).

Table 11. 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 4 HH laboratories.

Strain	Serogroup	No. correct Serogroup	Serotype	No. correct Serotype	Antigen
Salm EQASIA 21.2	O:9 (D1)	3/3	Enteritidis	1/1	9,12:g,m:-
Salm EQASIA 21.3	O:3,10 (E1)	2/2	Anatum	1/1	3,10:e,h:1,6
Salm EQASIA 21.4	O:7 (C1)	1/2	Infantis	1/1	6,7:r:1,5
Salm EQASIA 21.5	O:8 (C2-C3)	2/2	Kentucky	1/1	8,20:i:z6
Salm EQASIA 21.6	O:4 (B)	3/3	Schwarzengrund	1/1	4,12:d:1,7
Salm EQASIA 21.7	O:4 (B)	3/3	Agona	1/1	4,12:f,g,s:-
Salm EQASIA 21.8	O:4 (B)	3/3	Derby	1/1	4,12:f,g:-
Salm EQASIA 21.11	O:8 (C2-C3)	1/3	Corvallis	1/1	8,20:z4,z23:-

Salm, *Salmonella*

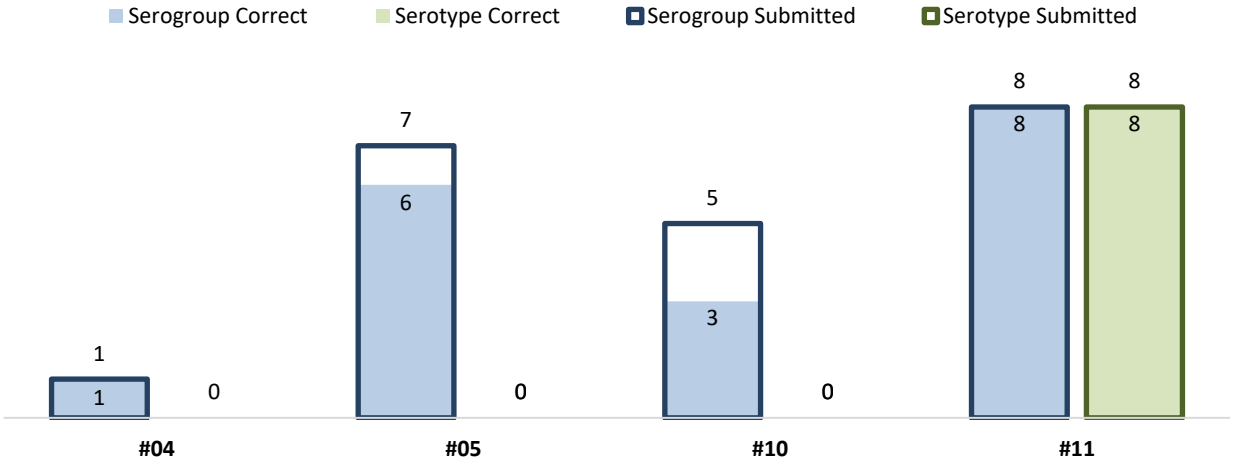

Figure 5. Number of correct serogroup/serotype out of the total of submitted serogroup/serotype results for each of the participating HH laboratories in the Serotyping component of the *Salmonella* trial.

3.2.4 Quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for both *Salmonella* and *E. coli* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the eight participating laboratories in the trial, six submitted results for the reference strain *E. coli* ATCC 25922 and only two performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, broth microdilution or agar dilution (**Table 12**). For testing *E. coli* NCTC 13846, MIC was determined by the standard broth microdilution method.

The highest proportion of test results outside of the expected range was observed for sulfamethoxazole (1 out of 1), colistin (1 out of 2), cefoxitin (1 out of 4), and gentamicin (1 out of 4) (**Table 12**). Except for colistin, the inaccurate results seemed to be caused by disk diffusion. Strikingly, sulfamethoxazole was included in the testing of *E. coli* ATCC 25922 by laboratory #35, even though this antimicrobial was not selected for the test strains susceptibility testing. For quality control purposes, the participating laboratories should test the same antimicrobials for both the reference strains and the test strains, as well as apply the same methodology in both situations.

Table 12. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) 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.	Gradient	MIC	**Total**
AMK	1/4	--	0/1	**1/5**
AMP	0/4	0/1	0/1	**0/6**
FEP	1/4	--	0/2	**1/6**
FOT	1/4	--	0/1	**1/5**
FOX	1/4	--	--	**1/4**
TAZ	0/4	0/1	0/1	**0/6**
CHL	1/4	0/1	--	**1/5**
CIP	1/4	0/1	0/1	**1/6**
COL	--	--	1/2	**1/2**
ETP	0/2	0/1	0/1	**0/4**
GEN	1/4	--	--	**1/4**
IMI	0/4	0/1	0/1	**0/6**
MERO	1/4	--	0/2	**1/6**
SMX	1/1	--	--	**1/1**
TET	0/4	0/1	--	**0/5**
TMP	0/1	--	--	**0/1**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth microdilution or agar dilution.

Regarding the laboratories' performance (**Figure 6**), laboratories #01, #11 and #17 had no deviations. While laboratories #01 and #17 applied disk diffusion, laboratory #11 used a mixture of gradient test and agar dilution, as well as broth microdilution for colistin testing.

In reverse, the other three laboratories had deviations ranging from 7.1 to 66.7% (**Figure 6**). Laboratories #06 and #35 had only one deviation each, whereas laboratory #10 presented eight deviations. The deviation observed for laboratory #06 was for colistin testing, which may suggest that *E. coli* ATCC 25922 was used instead of the recommended *E. coli* NCTC 13846; laboratory #35 only deviated from the accepted range for sulfamethoxazole, which was actually not included for assessing the test strains as mentioned above; finally, laboratory #10 inaccurate results consisted in Inhibition Zone Diameter values below the expected range (except for chloramphenicol), which could indicate, for example, contamination or a non-viable strain.

These overall deviations may imply a poor performance of individual laboratories, as well as poor handling of strains, which needs to be strengthened particularly on disk diffusion, a well-known and routinely used method.

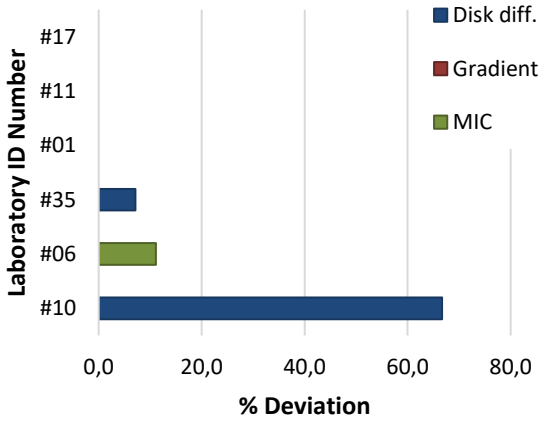

Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Salmonella* trial by the HH laboratories.

3.3 *Escherichia coli* trial

Twelve laboratories from 10 countries uploaded results for the *E. coli* trial.

3.3.1 Bacterial identification

All 12 participating laboratories submitted results for bacterial identification (**Table 13**). Ten out of twelve laboratories correctly identified the eight *E. coli* and three non-*E.coli* strains. The non-*E. coli* strain Ec EQASIA 21.6 (*Shigella flexneri*) was reported by laboratories #04 and #10 as *E. coli*. Laboratory #04 additionally misidentified the Ec EQASIA 21.11 strain as non-*E. coli*.

Table 13. 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
Ec EQASIA 21.1	*E. coli*	12/12
Ec EQASIA 21.2	*E. coli*	12/12
Ec EQASIA 21.3	*E. coli*	12/12
Ec EQASIA 21.4	Non- *E. coli* (*Klebsiella pneumoniae*)	12/12
Ec EQASIA 21.5	*E. coli*	12/12
Ec EQASIA 21.6	Non- *E. coli* (*Shigella flexneri*)	10/12
Ec EQASIA 21.7	*E. coli*	12/12
Ec EQASIA 21.8	*E. coli*	12/12
Ec EQASIA 21.9	*E. coli*	12/12
Ec EQASIA 21.10	Non- *E. coli* (*Salmonella*)	12/12
Ec EQASIA 21.11	*E. coli*	11/12

Ec, *E. coli*

3.3.2 AST performance

In this subsection, the AST performance was 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/I/S) ranged from 95.0% (strain Ec EQASIA 21.7) to 98.5% (strain Ec EQASIA 21.8) for each strain (**Table 14**). All strains revealed deviations below or

equal to 5%, which is considered a highly satisfactory result.

Table 14. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 12 HH laboratories for the *E. coli* trial.

Strain	AST in total	% Correct
Ec EQASIA 21.1	183	97.1
Ec EQASIA 21.2	186	97.0
Ec EQASIA 21.3	185	96.5
Ec EQASIA 21.5	185	96.4
Ec EQASIA 21.7	185	95.0
Ec EQASIA 21.8	185	98.5
Ec EQASIA 21.9	184	96.3
Ec EQASIA 21.11	170	97.4

Ec, *E. coli*

Antimicrobial-based analysis

Of the 22 tested and scored antimicrobial agents, three revealed to exceed a 10% deviation: doripenem (19.8%), colistin (15.7%) and azithromycin (11.5%). On the contrary, cefotaxime, ciprofloxacin, nalidixic acid, tetracycline and tigecycline revealed no deviation from the expected results (**Figure 7**).

Doripenem was tested by only three laboratories, meaning that even few incorrect results would result in a high percentage of deviation. In this case, most of the incorrect results were observed for laboratory #01, which reported smaller Inhibition Zone Diameters than what would be expected.

The deviation from colistin expected results could be explained once again (also observed in the *Salmonella* trial) by AST results being interpreted as susceptible, when intermediate or resistant are the only possible options, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1).

Regarding azithromycin, the incorrect reported results were close to the threshold for categorising the strain as susceptible or resistant (intermediate option is not available), which resulted in a heavy score penalty (score of 0 or 1 out of the possible full score 4).

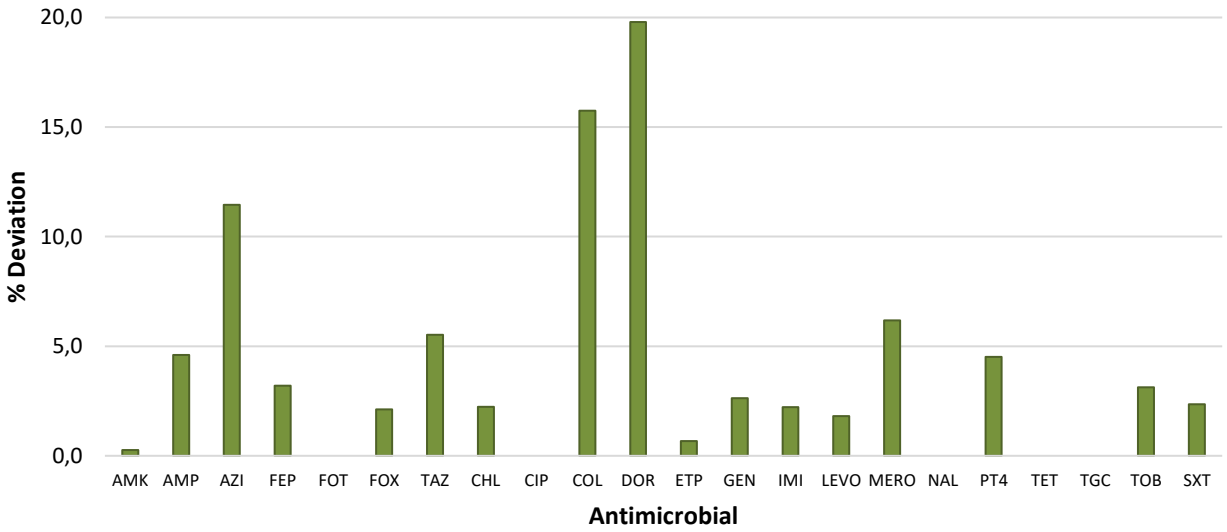

Figure 7. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by HH laboratories (n=12) participating in the 3rd EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

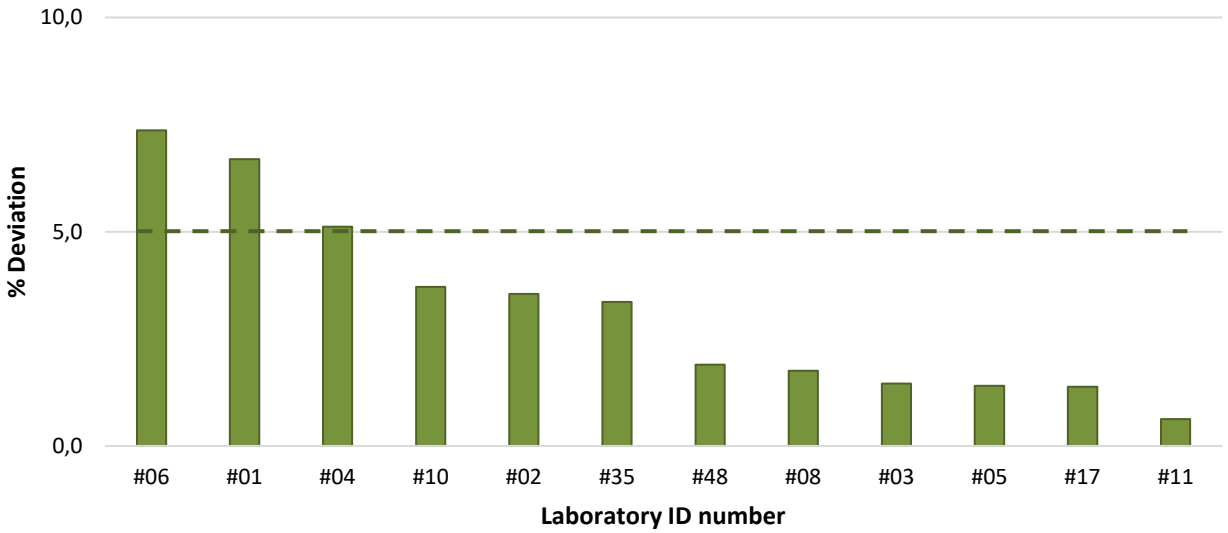

Figure 8. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by HH laboratories (n=12) participating in the 3rd EQA in the EQAsia project. Results are categorized by laboratory ID number.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for nine of the participants (**Figure 8**). In average, the deviation was 3.2% (ranging from 0.6 to 7.4%). As the acceptance level was set to 5% deviation, three laboratories (#06, #01 and #04) did not perform within the expected range for the *E. coli* trial.

Laboratory #04 deviation is only slightly above 5% and can be explained by misinterpretation of the obtained colistin results, which means that it is not an actual performance issue; laboratory #01 deviation seems to have originated mostly from the abovementioned incorrect doripenem results, as well as other occasional mistakes when testing, for example, azithromycin and piperacillin/tazobactam; lastly, laboratory #06 lower performance was caused by misinterpretation of colistin results and a few incorrect results reported for strain Ec EQASIA 21.2.

3.3.3 β-lactamase producing *E. coli*

Ten out of the 12 participating laboratories uploaded results for this component of the *E. coli* trial. Yet, for strains Ec EQASIA 21.9, and Ec EQASIA 21.11 only eight and seven laboratories, respectively, tested for ESBL-production (**Table 15**).

Of all 10 laboratories, only laboratory #10 correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the eight *E. coli* strains. The laboratories were able to identify the susceptible (no AmpC, ESBL and carbapenemase) isolates with no problems (strains Ec EQASIA 21.9, and Ec EQASIA 21.11, **Table 15**). The remaining six non-susceptible strains generated more mistakes in correctly identifying the resistance phenotype, with deviations from the expected results varying from 20.0 to 60.0% (**Table 15**).

Strains Ec EQASIA 21.1 and Ec EQASIA 21.7, ESBL-producing *E. coli* strains, were identified as susceptible by some laboratories. Laboratories #06 (both strains) and #5 (strain Ec EQASIA 21.1) obtained an MIC result for ceftazidime of ≤ 1 µg/mL, which led to the classification as susceptible. On the contrary, laboratory #17 reported strain Ec EQASIA 21.1 as resistant to cefotaxime (Inhibition Zone Diameter = 6 mm) and laboratory #04 reported MIC = 2 µg/mL for ceftazidime, and therefore should have consider it a non-susceptible strain and perform confirmatory testing.

The carbapenemase-producing *E. coli* strains Ec EQASIA 21.2, Ec EQASIA 21.3, Ec EQASIA 21.5 and Ec EQASIA 21.8, were wrongly classified by several laboratories. The most problematic strain was Ec EQASIA 21.3, with six laboratories reporting wrong results. Yet, five of them obtained values for meropenem > 0.12 µg/mL (laboratories #03, #04 and #11) or < 25 mm (laboratories #01 and #48), indicating that this strain should be classified as a carbapenemase-producing *E. coli*.

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 10 HH laboratories.

Strain code		Ec EQASIA 21.1	Ec EQASIA 21.2	Ec EQASIA 21.3	Ec EQASIA 21.5	Ec EQASIA 21.7	Ec EQASIA 21.8	Ec EQASIA 21.9	Ec EQASIA 21.11
Expected results		ESBL	Carbapene-mase	Carbapene-mase	Carbapene-mase	ESBL	Carbapene-mase	Susceptible	Susceptible
Obtained results (n/N)	ESBL	**6/10 (60.0%)**	1/10 (10.0%)	1/10 (10.0%)	--	**8/10 (80.0%)**	--	--	--
	AmpC	1/10 (10.0%)	1/10 (10.0%)	1/10 (10.0%)	1/10 (10.0%)	--	1/10 (10.0%)	--	--
	ESBL + AmpC	--	--	4/10 (40.0%)	4/10 (40.0%)	--	1/10 (10.0%)	--	--
	Carbapenemase	--	**8/10 (80.0%)**	**4/10 (40.0%)**	**5/10 (50.0%)**	--	**8/10 (80.0%)**	--	--
	Other	--	--	--	--	--	--	--	--
	Susceptible*	3/10 (30.0%)	--	--	--	2/10 (20.0%)	--	**8/8 (100.0%)**	**7/7 (100.0%)**

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

3.3.4 Quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for both *Salmonella* and *E. coli* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the 12 participating laboratories in the trial, eight submitted results for the reference strain *E. coli* ATCC 25922 and only two performed colistin testing and reported results for *E. coli* NCTC 13846. Different methodologies were applied for testing the quality control strain *E. coli* ATCC 25922 (disk diffusion, gradient test, broth microdilution and agar dilution) (**Table 16**). For testing *E. coli* NCTC 13846, MIC was determined by the standard broth microdilution method.

The highest proportion of test results outside of the expected range was observed for colistin (1 out of 2) and trimethoprim/sulfamethoxazole (2 out of 7) (**Table 16**). Moreover, the majority of the inaccurate results seemed to be caused by disk diffusion.

Regarding the laboratories' performance, laboratories #01, #03, #08, #11, #17 and #35 presented no deviation. While laboratories #01, #08, #17 and #35 applied disk diffusion, laboratory #03 used a mixture of disk diffusion and broth microdilution and laboratory #11 used a mixture of disk diffusion, gradient test, broth microdilution for colistin testing and agar dilution. The remaining two laboratories (#06 and #10) presented deviations that ranged from 14.3% to 66.7% (**Figure 9**), corresponding to two (laboratory #06) and 10 (laboratory #10) deviations.

The deviations observed for laboratory #06 resulted from colistin testing (suggesting that *E. coli* ATCC 25922 was used instead of the recommended *E. coli* NCTC 13846, as seen in the *Salmonella* trial) and from trimethoprim/ sulfamethoxazole testing (reported MIC of ≤ 20, which may suggest a typing error). As seen in the *Salmonella* trial, laboratory #10 reported Inhibition Zone Diameter values below the expected range (except for chloramphenicol), which could indicate, for example, contamination or a non-viable strain. Again, handling of reference strains needs to be strengthened to assure the laboratories' performance.

Table 16. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) 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
AMK	0/5	--	0/3	**0/8**
AMP	1/5	0/1	0/2	**1/8**
FEP	1/4	--	0/2	**1/6**
FOT	1/6	--	0/1	**1/7**
FOX	1/6	0/1	--	**1/7**
TAZ	1/5	0/1	0/1	**1/7**
CHL	1/5	0/1	--	**1/6**
CIP	1/5	0/1	0/1	**1/7**
COL	--	--	1/2	**1/2**
DOR	0/2	--	0/1	**0/3**
ETP	0/3	0/1	0/1	**0/5**
GEN	1/5	0/1	0/1	**1/7**
IMI	0/5	0/1	0/1	**0/7**
LEVO	0/3	0/1	0/1	**0/5**
MERO	0/5	--	0/2	**0/7**
NAL	1/4	0/1	0/1	**1/6**
PT4	0/2	--	0/2	**0/4**
SMX	--	--	--	**--**
TET	0/4	0/1	--	**0/5**
TGC	0/1	--	0/2	**0/3**
TOB	0/1	--	--	**0/1**
TMP	--	--	--	**--**
SXT	1/5	0/1	1/1	**2/7**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth microdilution or agar dilution.

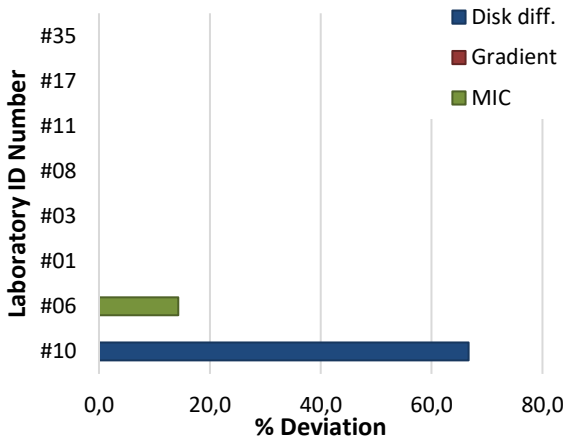

Figure 9. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *E. coli* trial by the HH laboratories.

3.4 *Pseudomonas aeruginosa* trial

A total of 12 laboratories from 10 countries uploaded results for the *P. aeruginosa* trial.

3.4.1 Bacterial identification

All 12 laboratories participating in the *P. aeruginosa* trial submitted results for bacterial identification. Among these, ten laboratories correctly identified the eight *P. aeruginosa* strains and the three non-*P. aeruginosa* (**Table 17**). The non-*P. aeruginosa* strain Pa EQASIA 21.7 (*Acinetobacter baumannii*) was reported by laboratory #13 as *P. aeruginosa*. Inversely, the *P. aeruginosa* strain Pa EQASIA 21.1 was apparently misidentified as non-*P. aeruginosa* by laboratory #04, but, in fact, the strain was not tested by the laboratory since the strain could not be revived.

Table 17. Bacterial identification of each of the 11 test strains provided related to the *P. aeruginosa* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Pa EQASIA 21.1	*P. aeruginosa*	11/12
Pa EQASIA 21.2	*P. aeruginosa*	12/12
Pa EQASIA 21.3	*P. aeruginosa*	12/12
Pa EQASIA 21.4	*P. aeruginosa*	12/12
Pa EQASIA 21.5	*P. aeruginosa*	12/12
Pa EQASIA 21.6	*P. aeruginosa*	12/12

Pa EQASIA 21.7	Non- *P. aeruginosa* (*Acinetobacter baumannii*)	11/12
Pa EQASIA 21.8	Non- *P. aeruginosa* (*Acinetobacter lowffii*)	12/12
Pa EQASIA 21.9	*P. aeruginosa*	12/12
Pa EQASIA 21.10	*P. aeruginosa*	12/12
Pa EQASIA 21.11	Non- *P. aeruginosa* (*Acinetobacter pittii*)	12/12

Pa, *P. aeruginosa*

3.4.2 AST 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/I/S) ranged from 88.9% (strain Pa EQASIA 21.10) to 99.4% (strain Pa EQASIA 21.5) for each strain (**Table 18**). The high deviation for strain Pa EQASIA 21.10 (above 10%) seems to have been caused by several instances of results' misinterpretation, as well as varying results for levofloxacin, which had an expected MIC of 2 and interpretation as intermediate, but the reported MIC values varied from 0.25 to 1, resulting in different interpretations and score penalties.

Table 18. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 12 HH laboratories for the *P. aeruginosa* trial.

Strain	AST in total	% Correct
Pa EQASIA 21.1	110	93.9
Pa EQASIA 21.2	119	92.4
Pa EQASIA 21.3	120	90.4
Pa EQASIA 21.4	118	94.5
Pa EQASIA 21.5	120	99.4
Pa EQASIA 21.6	119	94.5
Pa EQASIA 21.9	119	98.1
Pa EQASIA 21.10	119	88.9

Pa, *P. aeruginosa*

Antimicrobial-based analysis

All 13 antimicrobials presented deviations from the expected results, where doripenem (13.3%) and colistin (12.2%) presented the highest deviation (**Figure 10**). As seen in the *E. coli* trial, testing of these two antimicrobials seems to cause several incorrect results. And again, a part of doripenem's incorrect results were observed for laboratory #01, which reported smaller Inhibition Zone Diameters than what would be expected. Similarly, laboratory #12 also reported higher MIC values than expected for some of the isolates. The deviation from colistin was again caused by misinterpretation of the results.

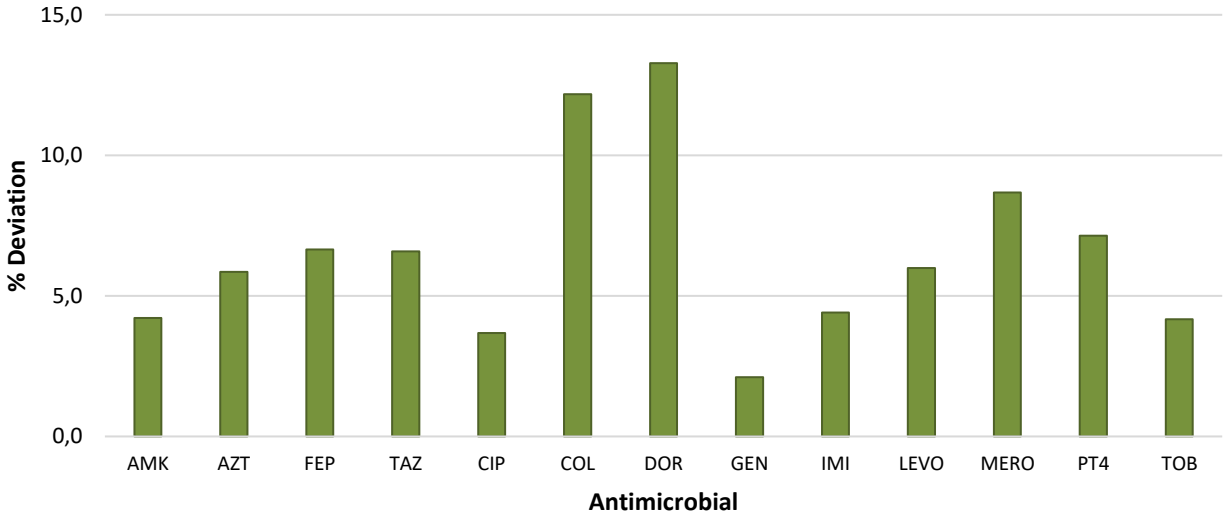

Figure 10. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by HH laboratories (n=12) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

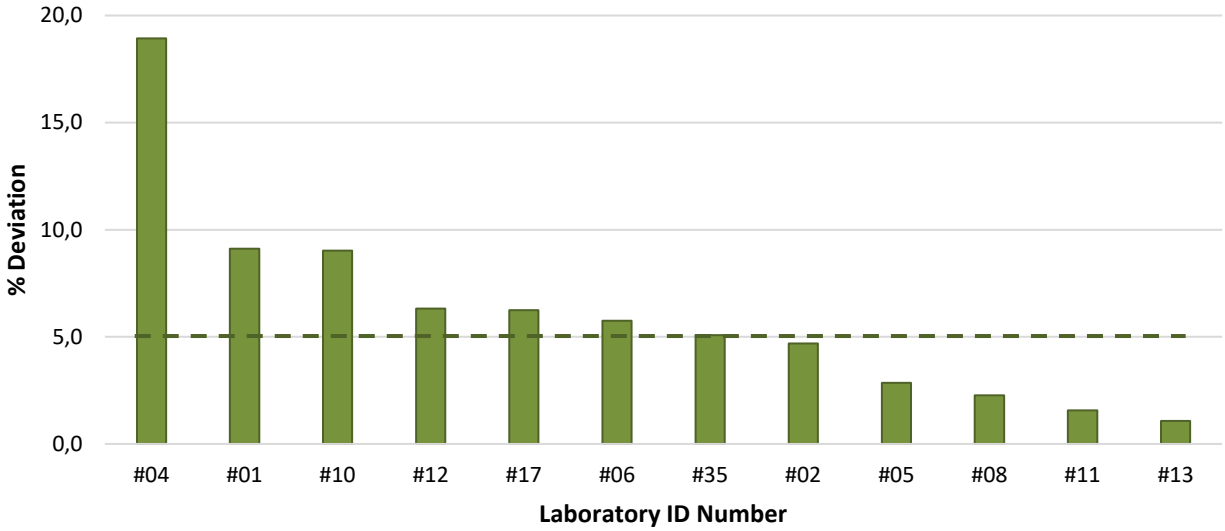

Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by HH laboratories (n=12) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

Laboratory-based analysis

For the *P. aeruginosa* trial, seven out of the 12 HH laboratories presented a deviation above the acceptance level of 5% (laboratories #04, #01, #10, #12, #17, #06 and #35). The average deviation was 6.1% (ranging from 1.1 to 18.9%) (**Figure 10**).

Laboratory #04 presented a deviation quite high compared to the remaining laboratories, and can once again be explained by misinterpretation of the obtained colistin results, as well as incorrect interpretation of several other antimicrobial susceptibility results. Even though this deviation may not reflect an actual performance issue, correct interpretation of the obtained results is as important as testing the antimicrobials, because it can lead to inappropriate clinical treatment.

3.4.3 Quality control strain *P. aeruginosa* ATCC 27853

The quality control strain *P. aeruginosa* ATCC 27853 was sent to all participating laboratories free of charge (in this trial or in previous trials) to be used as a reference strain for the *P. aeruginosa* trial.

Table 19. AST of the reference strain *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	**Total**
AMK	1/5	--	0/4	**1/9**
AZT	0/3	--	0/2	**0/5**
FEP	1/5	--	0/5	**1/10**
TAZ	1/5	0/1	0/4	**1/10**
CIP	0/5	0/1	0/4	**0/10**
COL	--	--	0/4	**0/4**
DOR	0/2	--	0/2	**0/4**
GEN	0/5	0/1	0/4	**0/10**
IMI	0/5	0/1	1/2	**1/8**
LEVO	1/3	0/1	0/3	**1/7**
MERO	2/5	--	0/5	**2/10**
P/T4	0/6	--	2/4	**2/10**
TOB	0/1	--	0/1	**0/2**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth microdilution or agar dilution.

Among the 12 participating laboratories, 10 submitted results for the reference strain. The participants used different methodologies for testing the reference strain: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test,

broth microdilution or agar dilution (**Table 19**).

The highest proportion of test results outside of the expected range was observed for meropenem (2 out of 10) and piperacillin/ tazobactam (2 out of 10) (**Table 19**). While the inaccurate results for meropenem were caused by disk diffusion, incorrect results for piperacillin/ tazobactam were seen when MIC was determined by broth dilution.

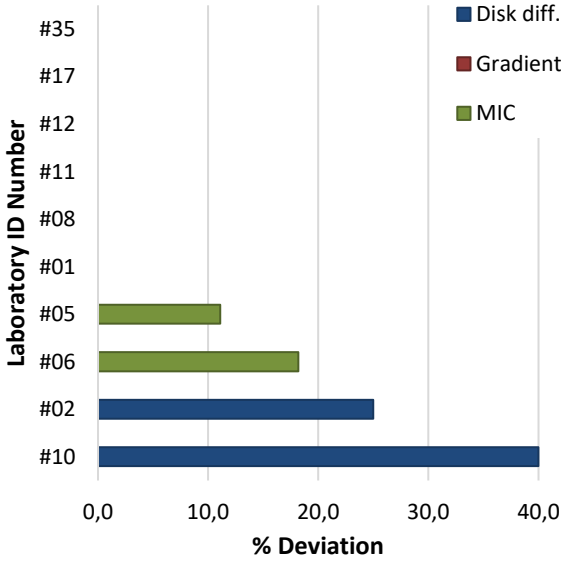

Figure 12. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial by the HH laboratories.

Regarding the laboratories' performance (**Figure 12**), six laboratories (#01, #08, #11, #12, #17 and #35) presented no deviation. Some of these laboratories tested the antimicrobials by using solely broth microdilution (#12, #17 and #35) or disk diffusion (#01); laboratory #08 opted for broth macrodilution, except for piperacillin/ tazobactam testing (disk diffusion was used), and laboratory #11 applied agar dilution, gradient test and broth microdilution (for colistin).

On the contrary, the other four laboratories had deviations ranging from 11.1 to 40.0% (**Figure 12**). Laboratories #05 and #06 presented one and two deviations, respectively, that occurred when broth microdilution was applied. Both laboratories reported MIC values for piperacillin/ tazobactam way above the acceptable range. Inversely, laboratories #10 and #02 presented deviations that were caused by the disk diffusion methodology. Laboratory #02 had two deviations below the acceptance interval, and laboratory #10 had four deviations both above and below the acceptance interval.

3.5 *Campylobacter jejuni* / *C. coli* trial

Only one laboratory (#06) participated and uploaded results for the *C. jejuni/ C. coli* trial.

3.5.1 Bacterial identification

The results for bacterial identification are shown in **Table 20**. Laboratory #06 submitted data for only two strains (Campy EQASIA 21.9 and Campy EQASIA 21.11) out of the eight *C. jejuni/ C. coli* provided strains. While Campy EQASIA 21.11 (*C. jejuni*) was correctly identified, Campy EQASIA 21.9 (*C. coli*) was incorrectly identified as *C. jejuni*.

Table 20. Bacterial identification of each of the 11 test strains provided related to the *C. jejuni/ C. coli* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Campy EQASIA 21.1	*C. coli*	--
Campy EQASIA 21.2	Non-*C. jejuni/ C. coli (C. lari)*	--
Campy EQASIA 21.3	*C. coli*	--
Campy EQASIA 21.4	Non-*C. jejuni/ C. coli (C. lari)*	--
Campy EQASIA 21.5	*C. coli*	--
Campy EQASIA 21.6	*C. jejuni*	--
Campy EQASIA 21.7	*C. jejuni*	--
Campy EQASIA 21.8	Non-*C. jejuni/ C. coli (C. lari)*	--
Campy EQASIA 21.9	*C. coli*	0/1
Campy EQASIA 21.10	*C. coli*	--
Campy EQASIA 21.11	*C. jejuni*	1/1

Campy, *C. jejuni/ C. coli*

3.5.2 AST 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/I/S) was 66.7% for both Campy EQASIA 21.9 and Campy EQASIA 21.11 strains (**Table 21**).

Table 21. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from one HH laboratory for the *C. jejuni/ C. coli* trial.

Strain	AST in total	% Correct
Campy EQASIA 21.1	--	--
Campy EQASIA 21.3	--	--
Campy EQASIA 21.5	--	--
Campy EQASIA 21.6	--	--
Campy EQASIA 21.7	--	--
Campy EQASIA 21.9	3	66.7
Campy EQASIA 21.10	--	--
Campy EQASIA 21.11	3	66.7

Campy, *C. jejuni/ C. coli*

Antimicrobial-based analysis

Laboratory #06 tested the three recommended antimicrobials for the HH sector: ciprofloxacin, erythromycin and tetracycline. Erythromycin results were correct for both strains tested; ciprofloxacin and tetracycline results were, however, incorrect for Campy EQASIA 21.9 and Campy EQASIA 21.11, respectively (**Figure 13**).

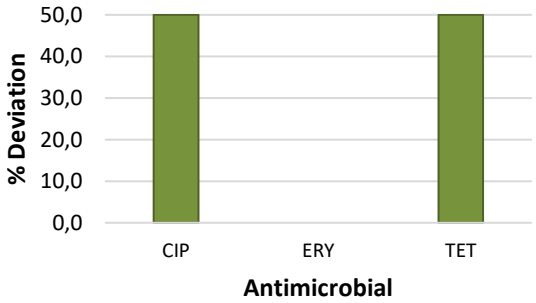

Figure 13. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by HH laboratories (n=1) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

Laboratory #06 presented a deviation from the expected results of 33.3% (**Figure 14**), due to the incorrect results mentioned in the previous section. In fact, the expected result for both of these antimicrobial/strain pairs was resistant; instead, the laboratory reported as susceptible. The remaining antimicrobial/strain pairs were indeed susceptible and were reported as such, meaning that laboratory #06 reported all results as susceptible. A closer look into the submitted data reveals very large Inhibition Zone Diameter values, way above the breakpoint. These observations could indicate, for example, that a light bacterial inoculum was used for plating or that inappropriate growth conditions were applied for the *Campylobacter* strains.

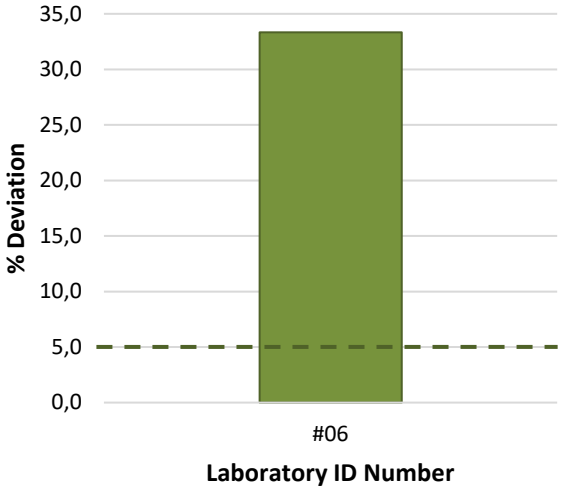

Figure 14. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by HH laboratories (n=1) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.5.3 Quality control strain *C. jejuni* ATCC 33560

The quality control strain *C. jejuni* ATCC 33560 was sent to all participating laboratories free of charge to be used as a reference strain for the *C. jejuni/ C. coli* trial. Laboratory #06 tested the reference strain and submitted disk diffusion results (**Table 22**).

Disk diffusion acceptance intervals are only available for ciprofloxacin and erythromycin, when *C. jejuni* ATCC 33560 is grown at 42°C for

24h [4], meaning that even though the laboratory submitted results for all three antimicrobials, only ciprofloxacin and erythromycin were evaluated. The obtained Zone Diameter for ciprofloxacin was within the acceptance interval, whereas the Zone Diameter for erythromycin was slightly below the acceptance interval (**Table 22**). Therefore, the laboratory's performance was of 50.0% (**Figure 15**).

Table 22. AST of the reference strain *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diff.	Total
CIP	0/1	**0/1**
ERY	1/1	**1/1**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

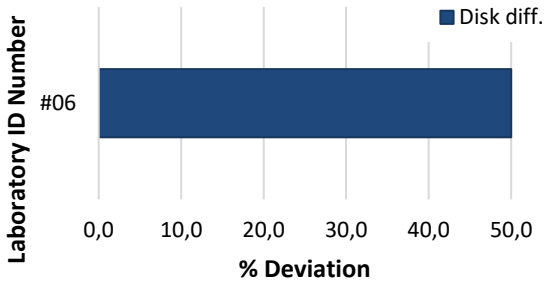

Figure 15. Percentage of deviation in the AST of *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial by the HH laboratory.

3.6 *Enterococcus faecium / E. faecalis* trial

Eleven laboratories from nine countries uploaded results for the *E. faecium/ E. faecalis* trial.

3.6.1 Bacterial identification

All 11 participating laboratories submitted results for bacterial identification (**Table 23**). Only six laboratories correctly identified the eight *E. faecium/ E. faecalis* strains and the three non-*E. faecium/ E. faecalis*. Among the eight *E. faecium/ E. faecalis* strains, four were *E. faecium* and the other four were *E. faecalis* (**Table 23**).

The *E. faecium* strains generated few instances of misidentification: strains Ef EQASIA 21.4 and Ef EQASIA 21.11 were both incorrectly identified as *E. faecalis* by laboratories #35 and #10, respectively.

The *E. faecalis* strains caused a few more incorrect results: strains Ef EQASIA 21.1 and Ef EQASIA 21.2 were misidentified as *E. faecium* by three (#02, #04 and #10) and one (#04) laboratories, respectively; Ef EQASIA 21.8 was considered as a non-*E. faecium/ E. faecalis* by laboratory #02.

Regarding the non-*E. faecium/ E. faecalis* strains, Ef EQASIA 21.3 and Ef EQASIA 21.6 (both *E. mundtii*) were identified as *E. faecium* by laboratory #35, and as *E. faecium* and *E. faecalis*, respectively, by laboratory #05; strain Ef EQASIA 21.9 (*E. gallinarum*) was incorrectly identified as *E. faecium* by laboratories #05 and #35, and as *E. faecalis* by laboratory #02.

Table 23. Bacterial identification of each of the 11 test strains provided related to the *E. faecium/ E. faecalis* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ef EQASIA 21.1	*E. faecalis*	8/11
Ef EQASIA 21.2	*E. faecalis*	10/11
Ef EQASIA 21.3	Non-*E. faecium/ E. faecalis* (*E. mundtii*)	9/11
Ef EQASIA 21.4	*E. faecium*	10/11
Ef EQASIA 21.5	*E. faecium*	11/11
Ef EQASIA 21.6	Non-*E. faecium/ E. faecalis* (*E. mundtii*)	9/11
Ef EQASIA 21.7	*E. faecalis*	11/11
Ef EQASIA 21.8	*E. faecalis*	10/11
Ef EQASIA 21.9	Non-*E. faecium/ E. faecalis* (*E. gallinarum*)	8/11
Ef EQASIA 21.10	*E. faecium*	11/11
Ef EQASIA 21.11	*E. faecium*	10/11

Ef, *E. faecium/ E. faecalis*

3.6.2 AST 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/I/S) ranged from 92.6% (strain Ef EQASIA 21.8) to 99.7% (strain Ef EQASIA 21.11) for each strain (**Table 24**). The AST results submitted for the eight *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species.

Even though none of the strains revealed more than 10% deviation (**Table 24**), the highest deviation seen for Ef EQASIA 21.8 can be explained by the testing results for some of the antimicrobials, such as chloramphenicol, daptomycin and vancomycin, being around the threshold for categorising the strain as susceptible / intermediate or intermediate / resistant, which originated a reported interpretation different from what would be expected and, consequently, score penalties.

Table 24. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 11 HH laboratories for the *E. faecium /E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQASIA 21.1	87	94.0
Ef EQASIA 21.2	87	98.0
Ef EQASIA 21.4	86	94.5
Ef EQASIA 21.5	86	94.5
Ef EQASIA 21.7	87	98.3
Ef EQASIA 21.8	81	92.6
Ef EQASIA 21.10	86	98.5
Ef EQASIA 21.11	87	99.7

Ef, *E. faecium/ E. faecalis*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were quinupristin/dalfopristin (14.1%), daptomycin (11.9%) and teicoplanin (9.4%), whereas erythromycin and tigecycline revealed no deviation from the expected results (**Figure 16**).

Quinupristin/dalfopristin was tested by only two laboratories (#01 and #05), meaning that even few incorrect results would result in a high percentage of deviation. In this case, both laboratories reported strain Ef EQASIA 21.4 as susceptible to the drug, even though it was expected to be resistant, resulting in the highest score penalty possible (score of 0).

Susceptibility testing results for daptomycin should be interpreted differently for *E. faecium* and *E. faecalis*, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1). Consequently, some of the incorrect results reported were due to incorrect interpretation of the results.

In its turn, the deviation observed for teicoplanin was mostly due to values around the breakpoint for strains Ef EQASIA 21.1 and Ef EQASIA 21.5 (MIC = 8), which resulted in slight different results and interpretations.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for seven participants (**Figure 17**). In average, the deviation was 3.7% (ranging from 0.9 to 7.0%). As the acceptance level was set to 5% deviation, four laboratories (#04, #06, #12 and #35) did not perform within the expected range for the *E. faecium/ E. faecalis* trial. Still, the deviations were only slightly above the acceptance level (**Figure 17**).

Laboratory #04 presented the highest deviation observed for this trial, which was largely caused by misinterpretation of the resulted obtained for several of the antimicrobials (chloramphenicol, ciprofloxacin, daptomycin, linezolid, teicoplanin and vancomycin).

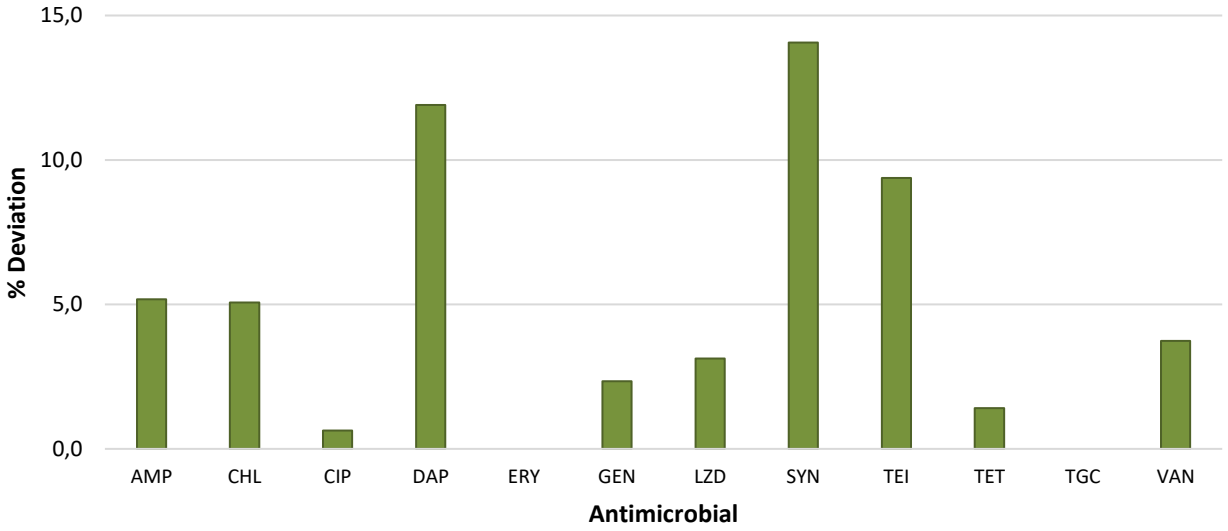

Figure 16. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by HH laboratories (n=11) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

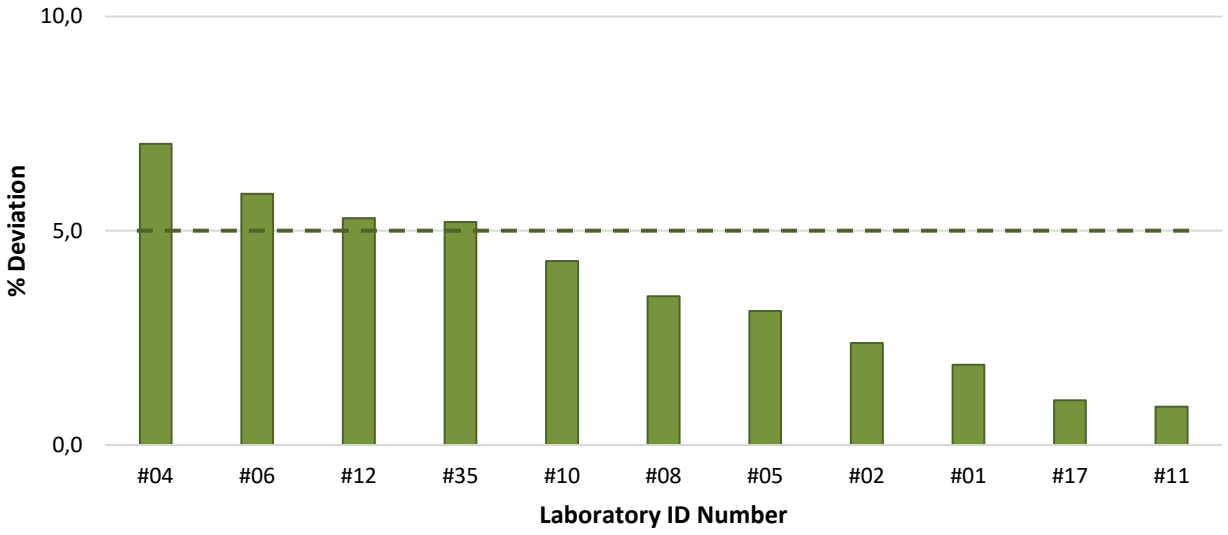

Figure 17. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by HH laboratories (n=11) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.6.3 Quality control strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212

The quality control strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 for testing when disk diffusion or MIC determination methodologies are applied, respectively, were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *E. faecium/ E. faecalis* trial.

Among the 11 participating laboratories, 10 submitted results for the reference strains, but only eight were assessed because laboratories #01 and #10 tested *E. faecalis* ATCC 29212 by disk diffusion, which is not recommended (Appendix 3d). Different methodologies for

testing the reference strain *E. faecalis* ATCC 29212 were applied: MIC was determined by either gradient test or broth microdilution (**Table 25**, **). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine Inhibition Zone Diameters by disk diffusion (**Table 25**, *).

The highest proportion of test results outside of the expected range were observed for gentamicin (2 out of 4) and tetracycline (1 out of 6) (**Table 26**). The inaccurate results seem to be caused equally by both disk diffusion and broth microdilution.

Table 25. AST of the reference strain *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* 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	0/4	0/1	1/3	**1/8**
CHL	0/4	0/1	1/2	**1/7**
CIP	0/3	0/1	0/3	**0/7**
DAP	--	--	0/2	**0/2**
ERY	0/3	0/1	0/3	**0/7**
GEN	1/3	--	1/1	**2/4**
LZD	0/1	0/1	0/4	**0/6**
SYN	--	--	0/2	**0/2**
TEI	0/1	--	0/3	**0/4**
TET	1/3	0/1	0/2	**1/6**
TGC	--	--	0/2	**0/2**
VAN	1/3	0/1	0/4	**1/8**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth microdilution
**S. aureus* ATCC 25923 for disk diffusion
***E. faecalis* ATCC 29212 for MIC

A closer look at the laboratories' performance (**Figure 18**) shows that four laboratories had no deviation. Of those, laboratory #05 opted for broth microdilution as the sole methodology, laboratory #11 opted for gradient test, and laboratory #35 for disk diffusion; in reverse, laboratory #12 applied both disk diffusion and broth microdilution.

The remaining four laboratories had deviations ranging from 10.0 to 28.6% (**Figure 18**). Laboratories #02 and #06 presented the same number of deviations (n=2), but laboratory #02 tested fewer antimicrobials and thus, the deviation observed was higher (**Figure 18**); laboratories #04 and #17 accounted for one deviation each. The Inhibition Zone Diameters reported by laboratories #02 and #17 were below the acceptance interval; similarly, the MIC reported by #04 was also below the expected range.

Laboratory #06 reported that broth microdilution was the methodology applied for testing the test strains and the reference strain; however, the values submitted for ampicillin and chloramphenicol when testing *E. faecalis* ATCC 29212 seemed to be Inhibition Zone Diameters and not MIC values, which led to the two already mentioned deviations. This observation can be problematic in two ways: first, applying disk diffusion for testing these antimicrobials cannot be used as quality control for the results obtained for the test strains, as different methodologies were applied; second, if disk diffusion was applied, then *S. aureus* ATCC 25923 should be tested instead of *E. faecalis* ATCC 29212.

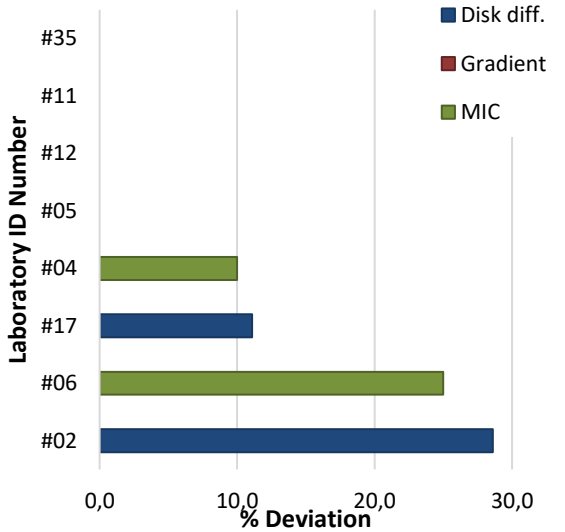

Figure 18. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* trial by the HH laboratories.

3.7 *Streptococcus pneumoniae* trial

Ten laboratories from eight countries uploaded results for the *S. pneumoniae* trial.

3.7.1 Bacterial identification

All 10 participating laboratories submitted results for bacterial identification (**Table 26**). All laboratories correctly identified the tested *S. pneumoniae* and non-*S. pneumoniae* strains. Strain Sp EQASIA 21.1 was not tested by laboratories #02 and #06.

Table 26. Bacterial identification of each of the 11 test strains provided related to the *S. pneumoniae* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Sp EQASIA 21.1	*S. pneumoniae*	8/8
Sp EQASIA 21.2	*S. pneumoniae*	10/10
Sp EQASIA 21.3	*S. pneumoniae*	10/10
Sp EQASIA 21.4	Non-*S. pneumoniae* (*E. faecalis*)	10/10
Sp EQASIA 21.5	Non-*S. pneumoniae* (*Listeria innocua*)	10/10
Sp EQASIA 21.6	*S. pneumoniae*	10/10
Sp EQASIA 21.7	*S. pneumoniae*	10/10
Sp EQASIA 21.8	*S. pneumoniae*	10/10
Sp EQASIA 21.9	Non-*S. pneumoniae* (*E. faecalis*)	10/10
Sp EQASIA 21.10	*S. pneumoniae*	10/10
Sp EQASIA 21.11	*S. pneumoniae*	10/10

Sp, *S. pneumoniae*

3.7.2 AST 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/I/S) ranged from 93.0% (strain Sp EQASIA 21.3) to 99.0% (strain Sp EQASIA 21.7) for each strain (**Table 27**). Only a couple of strains revealed a deviation higher than 5%. Strain Sp EQASIA 21.3 owes its deviation to some incorrect results mostly reported by laboratory #12, whereas strain Sp EQASIA 21.11 deviation comes from the testing of trimethoprim/sulfamethoxazole, which the strain was expected to be susceptible to, but some of the laboratories found it to be intermediate or resistant.

Table 27. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 10 HH laboratories for the *S. pneumoniae* trial.

Strain	AST in total	% Correct
Sp EQASIA 21.1	71	98.9
Sp EQASIA 21.2	96	96.9
Sp EQASIA 21.3	96	93.0
Sp EQASIA 21.6	84	95.5
Sp EQASIA 21.7	96	99.0
Sp EQASIA 21.8	97	96.9
Sp EQASIA 21.10	97	96.9
Sp EQASIA 21.11	87	93.7

Sp, *S. pneumoniae*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were ceftriaxone (12.5%), trimethoprim/sulfamethoxazole (8.8%), penicillin (8.7%) and cefotaxime (8.1%), whereas cefuroxime, ertapenem, linezolid and vancomycin revealed no deviation from the expected results (**Figure 19**).

Ceftriaxone was tested by only three laboratories (#05, #11 and #12), and all of them reported results different from what would be expected, but also different from each other. For example, strain Sp EQASIA 21.3 was expected to be interpreted as intermediate towards ceftriaxone; one of the laboratories interpreted the result incorrectly, another one reported as susceptible and the other as resistant. In summary, different reasons led to the observed deviation.

In the case of trimethoprim/sulfamethoxazole, as mentioned before, the deviation comes mostly from the testing of strain Sp EQASIA 21.11.

Penicillin and cefotaxime deviations come from only three (#08, #11 and #12) and four (#05, #08, #11 and #12) laboratories, respectively, and

were due to just slight variations in the obtained results that led to different interpretations.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for six of the participants (**Figure 20**). In average, the deviation was 3.9% (ranging from 1.1 to 7.9%). The remaining four laboratories (#06, #06, #12 and #02) presented deviations above the acceptance level of 5%, and therefore did not perform within the expected range for the *S. pneumoniae* trial.

Laboratory #06 had several azithromycin and erythromycin results around the breakpoint, resulting in interpretations different from what was expected. Most of these deviations were within the method variation and thus, do not reflect a poor performance.

Laboratory #05 deviation was mostly caused by misinterpretation of cefotaxime and ceftriaxone results; laboratories #12 and #02 deviations were mostly around the breakpoint and generated slight score penalties.

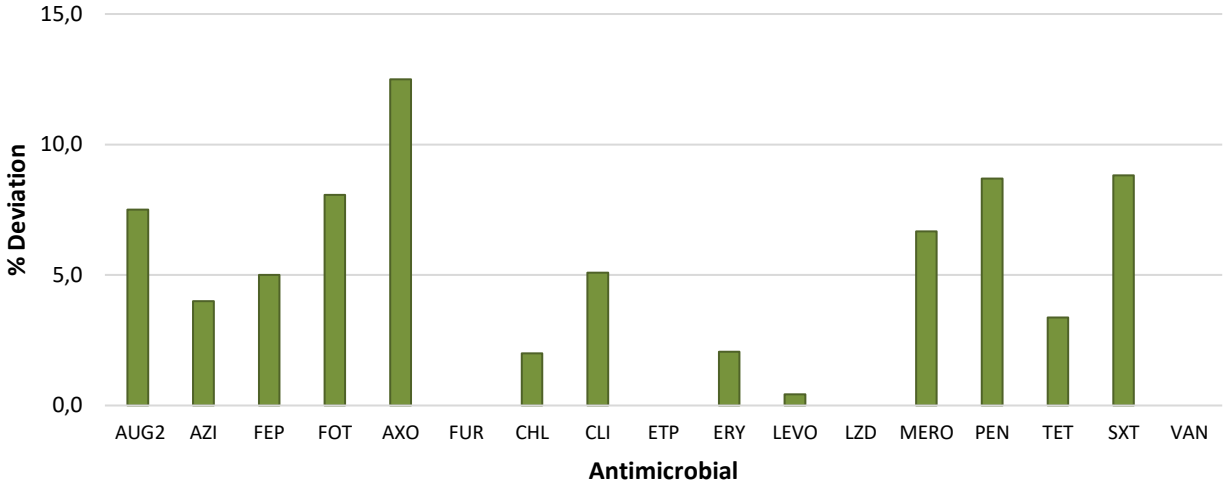

Figure 19. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by HH laboratories (n=10) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

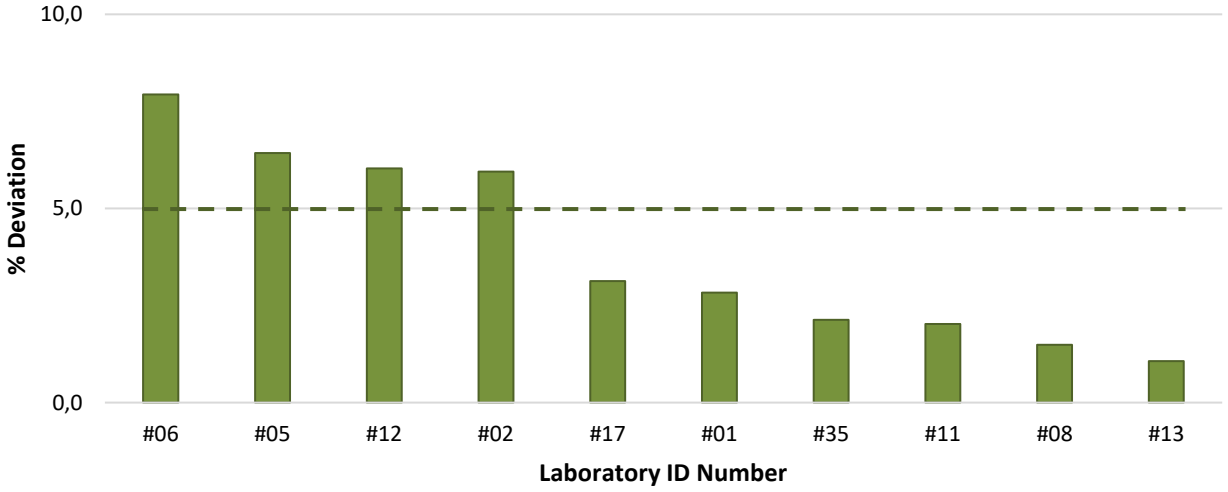

Figure 20. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by HH laboratories (n=10) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.7.3 Quality control strain *S. pneumoniae* ATCC 49619

The quality control strain *S. pneumoniae* ATCC 49619 was sent to all participating laboratories free of charge to be used as a reference strain for the *S. pneumoniae* trial.

Among the 10 participating laboratories, eight submitted results for the reference strain. Different methodologies for testing the reference strain *S. pneumoniae* ATCC 49619 were applied: disk diffusion, gradient test, agar dilution and broth microdilution (**Table 28**).

Table 28. AST of the reference strain *S. pneumoniae* ATCC 49619 in the *S. pneumoniae* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	**Total**
AUG	--	0/1	--	**0/1**
AZI	3/6	0/1	--	**3/7**
FEP	0/3	0/1	--	**0/4**
FOT	1/2	0/1	0/2	**1/5**
AXO	0/2	0/1	0/2	**0/5**
FUR	--	0/1	--	**0/1**
CHL	1/4	0/1	0/2	**1/7**
CLI	1/3	0/1	0/2	**1/6**
ETP	0/3	0/1	--	**0/4**
ERY	1/5	0/1	0/2	**1/8**
LEVO	1/3	0/1	0/2	**1/6**
LZD	0/3	0/1	0/2	**0/6**
MERO	0/3	--	0/1	**0/4**
PEN	0/2	0/1	0/1	**0/4**
TET	3/5	0/1	0/2	**3/8**
SXT	2/5	0/1	1/1	**3/7**
VAN	1/5	0/1	0/2	**1/8**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth microdilution or agar dilution

The highest proportion of test results outside of the expected range was observed for azithromycin (3 out of 7), trimethoprim/ sulfamethoxazole (3 out of 7) and tetracycline (3 out of 8) (**Table 28**). Most of the inaccurate results seem to be caused by disk diffusion.

A closer look at the laboratories' performance (**Figure 21**) shows that three laboratories had no deviation. Of those, laboratory #05 opted for broth microdilution as the sole methodology and laboratory #17 chose disk diffusion as the main methodology; in reverse, laboratory #11 applied both gradient test and agar dilution. The remaining five laboratories had deviations ranging from 9.1 to 55.6% (**Figure 21**).

Laboratory #12 had only one deviation, which resulted from the testing of trimethoprim/ sulfamethoxazole; however, the reported MIC value (≤ 10) was likely a typo and does not reflect the performance of the participant.

Laboratories #01, #02 and #35 presented three deviations each: while the Inhibition Zone Diameters outside the expected range reported by laboratory #02 were below the acceptance intervals, the deviations reported by laboratory #35 were above the acceptance interval; in its turn, laboratory #01 presented deviations both above and below the expected range.

Laboratory #06 presented the highest number of deviations (n=5), all above the acceptance interval.

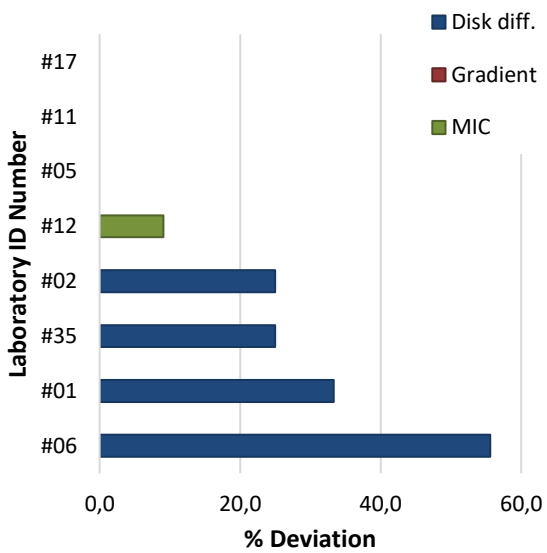

Figure 21. Percentage of deviation in the AST of *S. pneumoniae* ATCC 49619 in the *S. pneumoniae* trial by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 11 Animal Health laboratories participating in the 3rd EQA of the EQAsia Programme, nine laboratories submitted results for the *E. coli* trial, seven for the *Salmonella* and *E. faecium/ E. faecalis* trials, four for the *C. jejuni/ C. coli* trial, three for the *P. aeruginosa* trial, and only one laboratory submitted results for the *S. pneumoniae* trial (**Figure 22**). Applied AST methodologies for the six trials are presented in **Figure 22**. Disk diffusion as the sole method was the preferred choice for all the trials. Laboratory #37 was the only participant that used a mixture of disk diffusion and broth microdilution. Laboratories #20, #23 and #47 reported MIC values obtained solely by broth microdilution method. It is also worth noticing that laboratory #47 performed bacterial identification but did not submit AST results for the *C. jejuni / C. coli* trial (**Figure 22**).

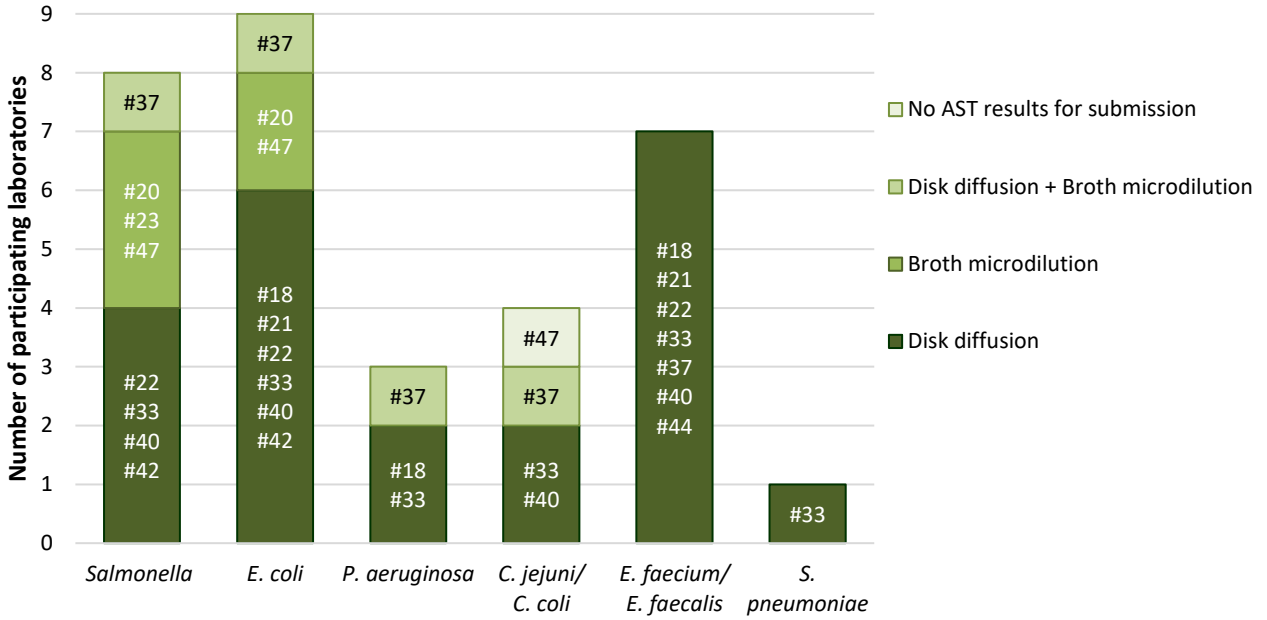

Figure 22. Methodologies applied by the AH laboratories participating in each of the trials.

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') 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**). Regarding the number of tests performed for each individual antimicrobial agent, tetracycline was tested by all laboratories participating in the *Salmonella* and *E. coli* trials (**Table 28**). In contrast, colistin and ertapenem were amongst the least tested drugs in both trials (less than half of the participating laboratories), as well as doripenem (2 laboratories) and tobramycin (1 laboratory) on the *E. coli* trial in particular (**Table 28**). For the *P. aeruginosa* trial, aztreonam, colistin, doripenem, piperacillin/ tazobactam and tobramycin were tested by only one of the three laboratories submitting results, which explains the fewer tests performed for these drugs (**Table 28**). Regarding the *C. jejuni/ C. coli* trial, no results were reported for ertapenem, and only one laboratory tested chloramphenicol (**Table 28**).

For the *E. faecium/ E. faecalis* trial, no results were reported for daptomycin, whereas ampicillin, chloramphenicol and tetracycline were tested by all seven participating laboratories (**Table 28**). Only one laboratory submitted results for the *S. pneumoniae* trial, and of the 17 recommended antimicrobials, the laboratory reported data for seven of them (azithromycin, chloramphenicol, erythromycin, levofloxacin, linezolid, tetracycline and vancomycin) (**Table 28**).

Scattering of missing data or incomplete AST results entries was observed for one of the trials, where four of the nine laboratories participating in the *E. coli* trial revealed incomplete results (**Table 29**). A closer look suggests that laboratory #20 may have wrongly selected tigecycline instead of tetracycline for the strain Ec EQASIA 21.11; similarly, laboratory #42 may have wrongly selected amikacin instead of ampicillin for strain Ec EQASIA 21.1 when submitting results. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance. Another example of missing data is the results submitted by laboratory #21, which reported colistin results only for strain Ec EQASIA 21.7. This situation does not allow for a proper assessment of the laboratory's capacity for testing this specific antimicrobial.

Table 28. Antimicrobial susceptibility tests performed by the AH laboratories for each trial per antimicrobial, and in total (shown in bold). For a given trial (Salm, Ec, Pa, Campy, Ef or Sp), the number of tests performed by all participating laboratories per antimicrobial is shown (n), as well as the percentage (%) of tests per antimicrobial out of the total number of tests performed (N) for the trial (% of n/N). The antimicrobials not included in a given trial are represented as --.

Antimicrobial	ASTs in total: : n (% of n/N)					
	Salm	Ec	Pa	Campy	Ef	Sp
AMK	32 (4.5)	47 (4.9)	24 (11.5)	--	--	--
AMP	56 (7.9)	58 (6.0)	--	--	49 (11.2)	--
AUG2	--	--	--	--	--	0
AZI	48 (6.7)	47 (4.9)	--	--	--	5 (14.3)
AZT	--	--	8 (3.8)	--	--	--
FEP	32 (4.5)	39 (4.0)	16 (7.7)	--	--	0
FOT	48 (6.7)	51 (5.3)	--	--	--	0
FOX	32 (4.5)	41 (4.2)	--	--	--	--
AXO	--	--	--	--	--	0
FUR	--	--	--	--	--	0
TAZ	48 (6.7)	54 (5.6)	24 (11.5)	--	--	--
CHL	48 (6.7)	53 (5.5)	--	5 (8.2)	49 (11.2)	5 (14.3)
CIP	48 (6.7)	54 (5.6)	24 (11.5)	14 (23.0)	41 (9.4)	--
CLI	--	--	--	--	--	0
COL	24 (3.4)	17 (1.8)	8 (3.8)	--	--	--
DAP	--	--	--	--	0	--
DOR	--	16 (1.7)	8 (3.8)	--	--	--
ETP	24 (3.4)	32 (3.3)	--	0	--	0
ERY	--	--	--	14 (23.0)	44 (10.0)	5 (14.3)
GEN	48 (6.7)	47 (4.9)	24 (11.5)	14 (23.0)	36 (8.2)	--
IMI	32 (4.5)	46 (4.8)	24 (11.5)	--	--	--
LEVO	--	31 (3.2)	16 (7.7)	--	--	5 (14.3)
LZD	--	--	--	--	36 (8.2)	5 (14.3)
MERO	48 (6.7)	47 (4.9)	16 (7.7)	--	--	0
NAL	--	47 (4.9)	--	--	--	--
PEN	--	--	--	--	--	0
P/T4	--	31 (3.2)	8 (3.8)	--	--	--
SYN	--	--	--	--	32 (7.3)	--
SMX	40 (5.6)	31 (3.2)	--	--	--	--
TEI	--	--	--	--	29 (6.6)	--
TET	64 (9.0)	68 (7.0)	--	14 (23.0)	49 (11.2)	5 (14.3)
TGC	--	25 (2.6)	--	--	32 (7.3)	--
TOB	--	8 (0.8)	8 (3.8)	--	--	--
TMP	40 (5.6)	32 (3.3)	--	--	--	--
SXT	--	46 (4.8)	--	--	--	0
VAN	--	--	--	--	41 (9.4)	5 (14.3)
Total	**712**	**968**	**208**	**61**	**438**	**35**

Salm, *Salmonella*; Ec, *E. coli*; Pa, *P. aeruginosa*; Campy, *C.jejuni/ C. coli*; Ef, *E. faecium/ E. faecalis*; Sp, *S. pneumoniae*
(n/N) number of tests performed per antimicrobial (n) out of the total tests performed for the trial (N)

Table 29. Distribution of incomplete or missing data of antimicrobial agents among *E. coli* strains reported by AH laboratories (n=9) participating in the 3rd EQA of the EQAsia project.

Lab ID No.	Ec EQASIA 21.1	Ec EQASIA 21.2	Ec EQASIA 21.3	Ec EQASIA 21.5	Ec EQASIA 21.7	Ec EQASIA 21.8	Ec EQASIA 21.9	Ec EQASIA 21.11
#18	FOX	FOX	FOX	FOX	AMP; FOT		FOT; FOX	AMP; FOT; CHL
#20	TGC	TGC	TGC	TGC	TGC	TGC	TGC	TET
#21	COL	COL	COL	COL	--	COL	COL	COL
#42	AMP	AMK	AMK		AMK	AMK	AMK	AMK

Ec, *E. coli*; blue shade, strains not tested

4.2 *Salmonella* trial

Eight laboratories from 5 countries uploaded results for the *Salmonella* trial.

4.2.1 Bacterial identification

All 8 laboratories participating in the *Salmonella* trial submitted results for bacterial identification. All of them correctly identified the eight *Salmonella* strains and the three non-*Salmonella* (**Table 30**).

Table 30. Bacterial identification of each of the 11 test strains provided related to the *Salmonella* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Salm EQASIA 21.1	Non-*Salmonella* (*Klebsiella pneumoniae*)	8/8
Salm EQASIA 21.2	*Salmonella*	8/8
Salm EQASIA 21.3	*Salmonella*	8/8
Salm EQASIA 21.4	*Salmonella*	8/8
Salm EQASIA 21.5	*Salmonella*	8/8
Salm EQASIA 21.6	*Salmonella*	8/8
Salm EQASIA 21.7	*Salmonella*	8/8
Salm EQASIA 21.8	*Salmonella*	8/8
Salm EQASIA 21.9	Non-*Salmonella* (*Shigella sonnei*)	8/8
Salm EQASIA 21.10	Non-*Salmonella* (*Escherichia coli*)	8/8
Salm EQASIA 21.11	*Salmonella*	8/8

Salm, *Salmonella*

4.2.2 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.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 85.1% (strain Salm EQASIA 21.6) to 96.9% (strain Salm EQASIA 21.2) for each strain (**Table 31**). The results from Salm EQASIA 21.6 revealed more than 10% deviation, which can be explained by the several incorrect results reported by laboratories #33 and #47.

Table 31. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/I/S). Results are from 8 AH laboratories for the *Salmonella* trial.

Strain	AST in total	% Correct
Salm EQASIA 21.2	89	96.9
Salm EQASIA 21.3	89	88.8
Salm EQASIA 21.4	89	92.4
Salm EQASIA 21.5	89	94.4
Salm EQASIA 21.6	89	85.1
Salm EQASIA 21.7	89	96.6
Salm EQASIA 21.8	89	90.4
Salm EQASIA 21.11	89	95.5

Salm, *Salmonella*

Antimicrobial-based analysis

The antimicrobials that resulted in highest percentage of deviations were ceftazidime (18.8%), amikacin (18.0%), colistin (14.6%) and azithromycin (13.5%), whereas ertapenem, imipenem and meropenem revealed no deviation from the expected results (**Figure 23**). The deviation seen for amikacin and colistin can be explained by the fact that the AST results of these antimicrobials (as well as gentamicin) should only be interpreted as intermediate or resistant, as recommended in the CLSI guidelines and stated in the EQA3 protocol

(Appendix 1). Instead, several laboratories reported the *Salmonella* isolates as being susceptible towards these antimicrobials, which resulted in a score penalty (score of 3 instead of the full score 4).

Regarding ceftazidime, the deviation observed seems to have been mostly due to the results of laboratory #23, which found most of the *Salmonella* isolates susceptible to the drug, which was not expected for some of them.

For azithromycin, the results could only be interpreted as susceptible or resistant (intermediate option is not available); in cases where the expected result was close to the threshold for categorising the strain as susceptible or resistant, a one-fold dilution/ ±3mm difference may result in different interpretations, which will cause a heavy score penalty (score of 0 or 1 out of the possible full score 4).

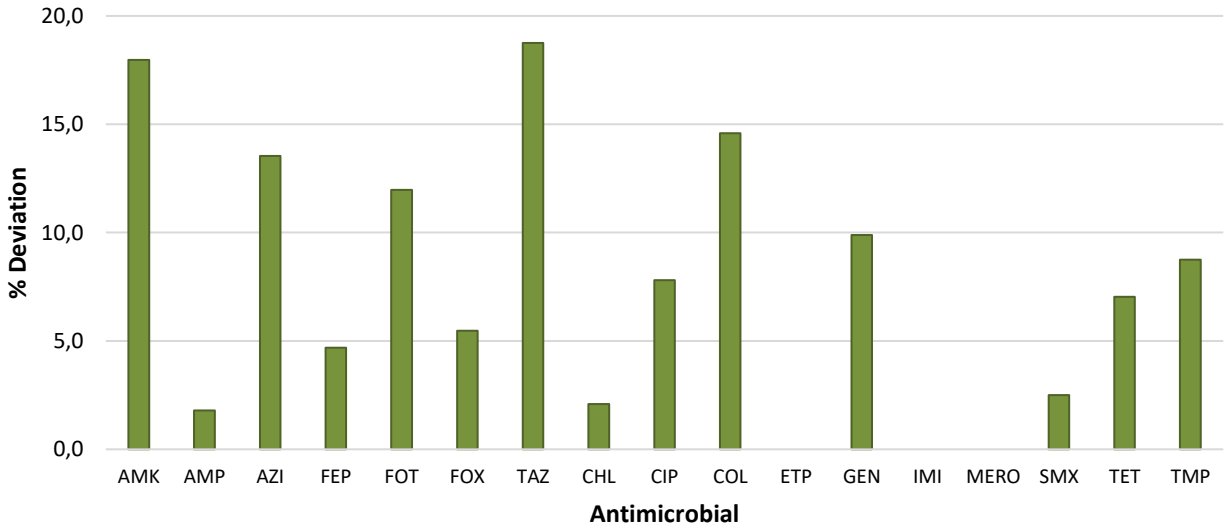

Figure 23. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=8) participating in the 3rd EQA in the EQAsia project. Results are categorized by antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

For the *Salmonella* trial, five out of the eight AH laboratories presented a deviation above the acceptance level of 5% (laboratories #23, #47, #22, #33 and #40). On the contrary, laboratories #20 and #42 presented no deviation. The average deviation was 6.1% (ranging from 0.0 to 12.8%) (**Figure 24**).

The highest deviation was observed for laboratory #23 and can be explained by the abovementioned issues with misinterpreting the obtained results for colistin, as well as for gentamicin. In addition, the laboratory presented several incorrect results for ceftazidime as stated before, and also for cefotaxime.

Laboratory #47 deviations come mostly from colistin misinterpretation and from strains Salm EQASIA 21.5 and Salm EQASIA 21.6. In fact, a deeper analysis suggests that the laboratory may have switched around these two strains, as the values reported for strain Salm EQASIA 21.5 resemble the results expected for Salm EQASIA 21.6 and vice-versa.

Laboratories #22 and #40 deviations seem to have originated mostly from reporting the isolates as susceptible to amikacin and gentamicin instead of intermediate, as well as other occasional mistakes when testing, for example, azithromycin and ciprofloxacin.

Laboratory #33 underperformance is greatly due to only one strain, Salm EQASIA 21.6, for which the reported results for several antimicrobials deviate from the expected outcome.

Regarding laboratories #20 and #42, which

presented no deviation from the expected results, it is worth mentioning that laboratory #20 only tested one antimicrobial (tetracycline) out of the 17 recommended, and laboratory #42 only tested for ampicillin and tetracycline.

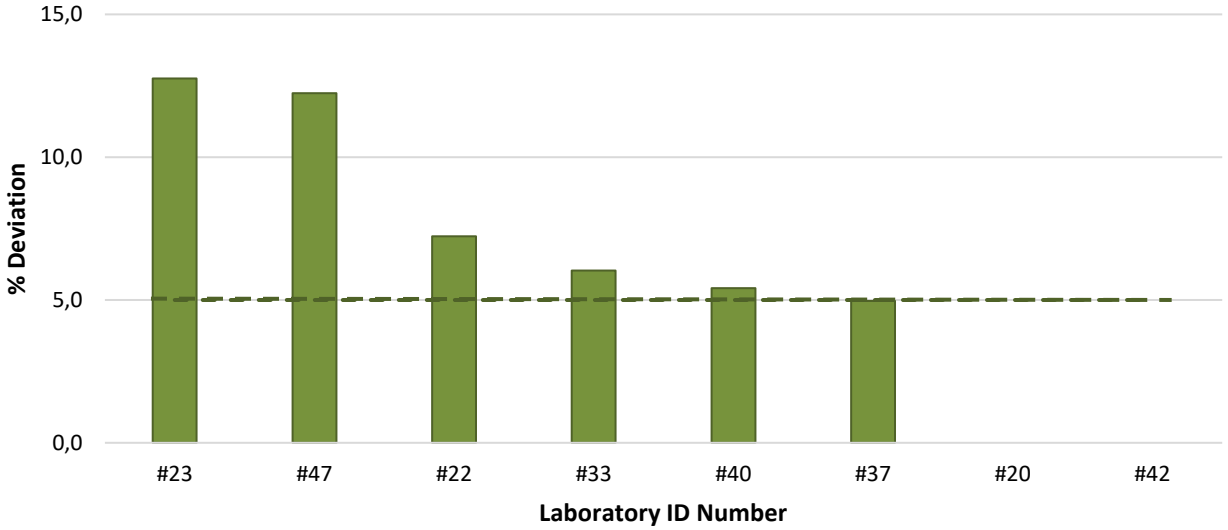

Figure 24. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=8) participating in the 3rd EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 Serotyping

Serotyping of *Salmonella* was offered to the participants as a voluntary component. 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 [9].

Of the eight participating laboratories in the *Salmonella* trial, only one laboratory submitted results for *Salmonella* serogrouping and serotyping (#40). Laboratory #40 submitted results for six of the eight strains and correctly assigned the serogroup to each of them (**Table 32**); in terms of serotyping, laboratory #40 reported accurate results for all strains, except for Salm EQASIA 21.5 (**Table 32**).

Table 32. 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 1 AH laboratories.

Strain	Serogroup	No. correct Serogroup	Serotype	No. correct Serotype	Antigen
Salm EQASIA 21.2	O:9 (D1)	1/1	Enteritidis	1/1	9,12:g,m:-
Salm EQASIA 21.3	O:3,10 (E1)	1/1	Anatum	1/1	3,10:e,h:1,6
Salm EQASIA 21.4	O:7 (C1)	1/1	Infantis	1/1	6,7:r:1,5
Salm EQASIA 21.5	O:8 (C2-C3)	1/1	Kentucky	0/1	8,20:i:z6
Salm EQASIA 21.6	O:4 (B)	--	Schwarzengrund	--	4,12:d:1,7
Salm EQASIA 21.7	O:4 (B)	1/1	Agona	1/1	4,12:f,g,s:-
Salm EQASIA 21.8	O:4 (B)	--	Derby	--	4,12:f,g:-
Salm EQASIA 21.11	O:8 (C2-C3)	1/1	Corvallis	1/1	8,20:z4,z23:-

Salm, *Salmonella*

4.2.4 Quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for both *Salmonella* and *E. coli* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the eight participating laboratories in the trial, six submitted results for the reference strain *E. coli* ATCC 25922 and only one (#37) performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used disk diffusion and broth microdilution for testing the reference strain *E. coli* ATCC 25922; for testing *E. coli* NCTC 13846, MIC was determined by broth microdilution (**Table 33**).

Table 33. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *Salmonella* trial. Proportion of test results outside of expected range is presented by methodology used.

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

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

The highest proportion of test results outside of the expected range was observed for sulfamethoxazole (2 out of 4), trimethoprim (2 out of 4) and chloramphenicol (2 out of 5) (**Table 33**). The inaccurate results seem to be caused by both disk diffusion and broth microdilution.

Considering the deviations, the laboratories' performance seems to be independent of the methodology applied for AST of the quality control strains (**Figure 25**). Laboratories #20, #33 and #47 presented no deviations. While laboratories #20 and #47 applied broth microdilution, laboratory #33 used disk diffusion.

In reverse, the other three laboratories had deviations ranging from 14.3 to 70.0% (**Figure 25)**. Laboratories #40 and #37 presented two and three deviations each, respectively, that occurred when disk diffusion was applied. Both participants had deviations both above and below the acceptance interval. Laboratory #23, which obtained a total of seven deviations, applied broth microdilution for testing *E. coli* ATCC 25922 and all the deviations, except for chloramphenicol, were due to MIC values way above the acceptance interval. These findings may suggest a possible contamination of the quality control strain, which indicates that handling of reference strains needs to be strengthened to assure the laboratories' performance.

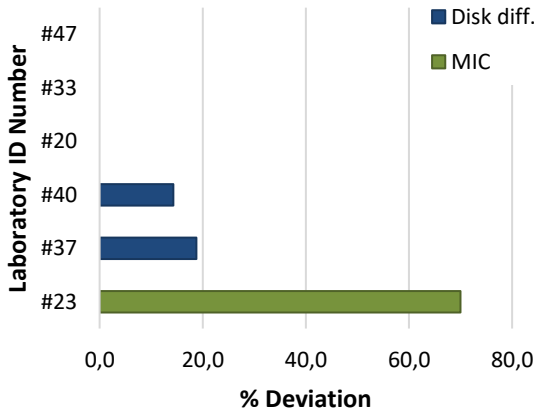

Figure 25. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Salmonella* trial by the AH laboratories.

4.3 *Escherichia coli* trial

Nine laboratories from five countries uploaded results for the *E. coli* trial.

4.3.1 Bacterial identification

All nine participating laboratories submitted results for bacterial identification (**Table 34**).Of those, six correctly identified the tested *E. coli* and non-*E.coli* strains, including laboratory #42, which did not test strain Ec EQASIA 21.5. The remaining three laboratories were unable to accurately identify all 11 test strains: the *E. coli* strain Ec EQASIA 21.8 was misidentified as non-*E. coli* by laboratories #18 and #33, whereas the non-*E. coli* strains Ec EQASIA 21.4, Ec EQASIA 21.6 and Ec EQASIA 21.10 were reported by laboratories #33, #18 and #20, respectively, as *E. coli*.

Table 34. 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 AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ec EQASIA 21.1	*E. coli*	9/9
Ec EQASIA 21.2	*E. coli*	9/9
Ec EQASIA 21.3	*E. coli*	9/9
Ec EQASIA 21.4	Non- *E. coli* (*Klebsiella pneumoniae*)	8/9
Ec EQASIA 21.5	*E. coli*	8/8
Ec EQASIA 21.6	Non- *E. coli* (*Shigella flexneri*)	8/9
Ec EQASIA 21.7	*E. coli*	9/9
Ec EQASIA 21.8	*E. coli*	7/9
Ec EQASIA 21.9	*E. coli*	9/9
Ec EQASIA 21.10	Non- *E. coli* (*Salmonella*)	8/9
Ec EQASIA 21.11	*E. coli*	9/9

Ec, *E. coli*

4.3.2 AST performance

In this subsection, the AST performance was 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/I/S) ranged from 89.0% (strain Ec EQASIA 21.11) to 98.6% (strains Ec EQASIA 21.7) for each strain (**Table 35**). Only strain Ec EQASIA 21.11 revealed a deviation above 10% (**Table 35**). This high deviation was mostly caused by the results submitted by laboratory #33, which reported a quite susceptible strain (Appendix 2b) as resistant to several antimicrobials.

Table 35. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 9 AH laboratories for the *E. coli* trial.

Strain	AST in total	% Correct
Ec EQASIA 21.1	125	96.4
Ec EQASIA 21.2	125	94.2
Ec EQASIA 21.3	125	96.2
Ec EQASIA 21.5	122	95.7
Ec EQASIA 21.7	125	98.6
Ec EQASIA 21.8	99	95.7
Ec EQASIA 21.9	124	94.4
Ec EQASIA 21.11	123	89.0

Ec, *E. coli*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were colistin (14.7%) and meropenem (12.2%). In reverse, sulfamethoxazole, tigecycline and trimethoprim/ sulfamethoxazole revealed no deviation from the expected results (**Figure 26**).

Colistin was tested by only two laboratories, meaning that even few incorrect results would result in a high percentage of deviation. In this case, the deviation could be explained once again (also observed in the *Salmonella* trial) by AST results being interpreted as susceptible, when intermediate or resistant are the only possible options, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1).

Regarding meropenem's deviation, most of the incorrect results were observed for laboratories #21 and #33, which reported smaller Inhibition Zone Diameters than what would be expected.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for five out of the nine participants (**Figure 27**). In average, the

deviation was 5.1% (ranging from 0.0 to 9.6%). As the acceptance level was set to 5% deviation, four laboratories (#18, #33, #21 and #47) did not perform within the expected range for the *E. coli* trial.

Laboratory #18 (highest deviation) presented occasional deviations when testing, for example, imipenem and chloramphenicol; laboratories #33 and #21 deviations were, as abovementioned, mostly due to the incorrect results reported for strain Ec EQASIA 21.11 and for the antimicrobial meropenem, respectively. In addition, laboratory #21 also reported strain Ec EQASIA 21.9 as resistant to several antimicrobials, opposite to what would be expected.

Lastly, laboratory #47 performance deviation resulted in part from the misinterpretation of colistin results.

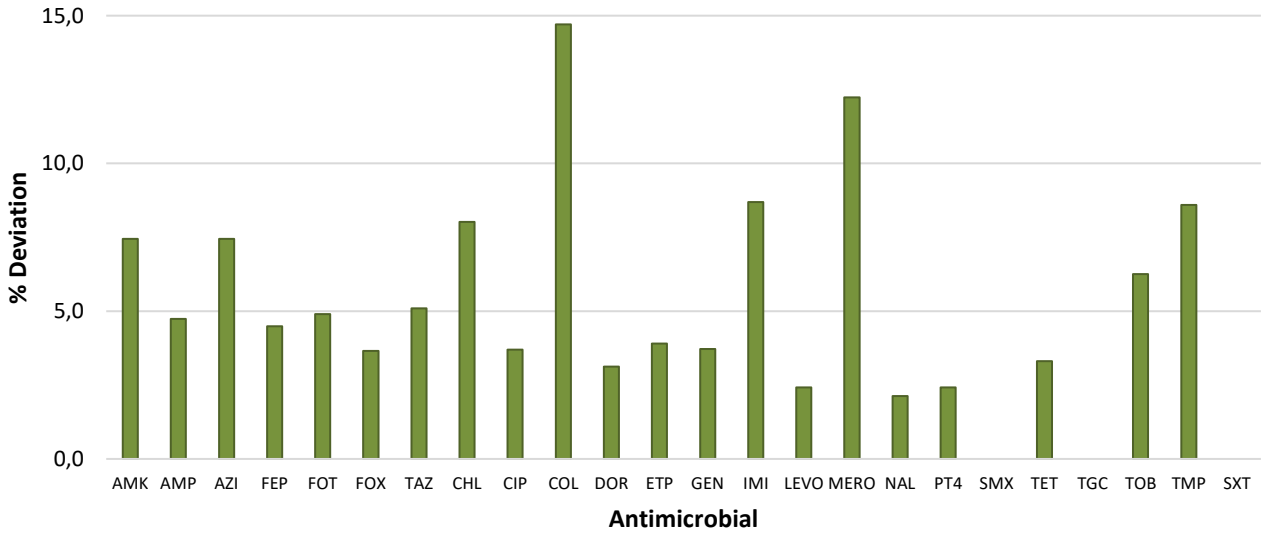

Figure 26. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=9) participating in the 3rd EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

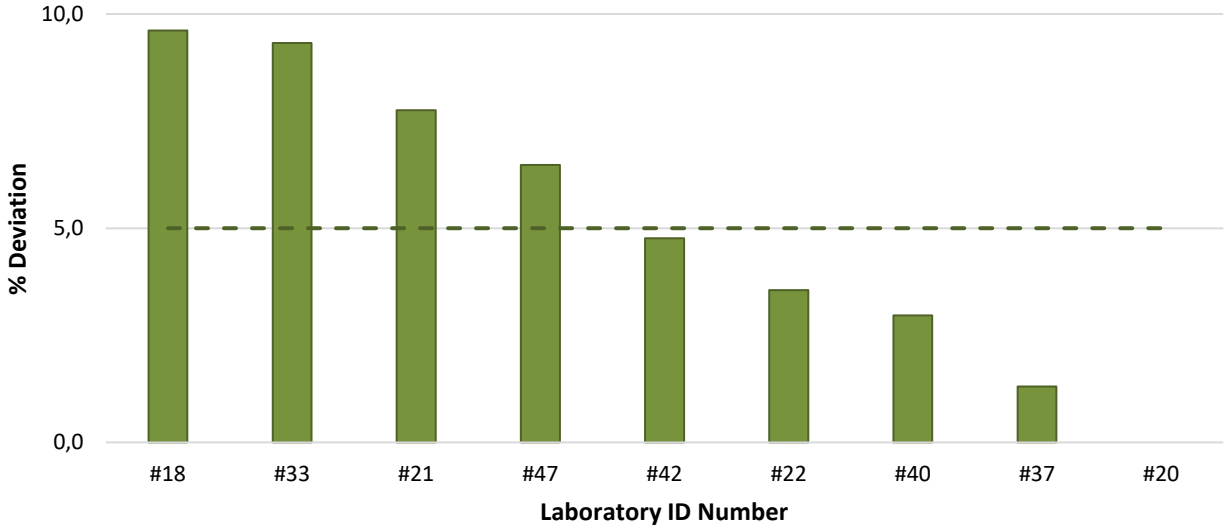

Figure 27. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=9) participating in the 3rd EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 β-lactamase producing *E. coli*

Five of the nine participating laboratories uploaded results for this component of the *E. coli* trial (laboratories #21, #22, #33, #37 and #40). Yet, for strain Ec EQASIA 21.8, only four laboratories tested for ESBL-production. Laboratory #40 was the only participant that correctly identified all the different ESBL/ AmpC/ carbapenemase phenotypes among the eight *E. coli* strains. The remaining reported some results that were not in accordance with what would be expected. Discrepancies from the expected results are summarized in **Table 36**.

The ESBL-producing *E. coli* strains, Ec EQASIA 21.1 and Ec EQASIA 21.7, were the only strains correctly classified by all five laboratories (**Table 36**).

In reverse, the highest deviation from the expected results was obtained for strains Ec EQASIA 21.3 and Ec EQASIA 21.5 (**Table 36)**. Three of the laboratories wrongly identified these carbapenemase-producing *E. coli* strains as either AmpC- (#21) or ESBL+AmpC-producers (#22 and #37), even though all three laboratories obtained Inhibition Zone Diameter values for meropenem < 25 mm, indicating that these two strains should be classified as carbapenemase-producing *E. coli* strains.

The other two carbapenemase-producing *E. coli* strains (Ec EQASIA 21.2 and Ec EQASIA 21.8) were classified as 'Other phenotypes' by laboratories #22 and #37 (**Table 36**), which seems to be due to considering the strains as both AmpC and carbapenemase-producers. According to Figure 1 of the EQA3 protocol (Appendix 1), a strain presenting values for meropenem of > 0.12 µg/mL or < 25 mm should be classified as a carbapenemase-producing *E. coli*.

Regarding the susceptible (no AmpC, ESBL and carbapenemase) isolates (strains Ec EQASIA 21.9 and Ec EQASIA 21.11), a few incorrect results were reported (**Table 36**). Laboratory #21 reported Ec EQASIA 21.9 as resistant towards cefotaxime and cefoxitin and, thus, classified it as ESBL+AmpC; the same laboratory classified Ec EQASIA 21.11 as 'Other phenotypes', even though no easy explanation can be found based on the reported AST results. Laboratory #33, as mentioned in the sub-section '*Strain-based analysis*' found strain Ec EQASIA 21.11 resistant to several antimicrobials, including cefotaxime, cefoxitin, ceftazidime and meropenem, leading to the classification of the strain as carbapenemase-producer.

Table 36. 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 5 AH laboratories.

Strain code		Ec EQASIA 21.1	Ec EQASIA 21.2	Ec EQASIA 21.3	Ec EQASIA 21.5	Ec EQASIA 21.7	Ec EQASIA 21.8	Ec EQASIA 21.9	Ec EQASIA 21.11
Expected results		ESBL	Carbapene-mase	Carbapene-mase	Carbapene-mase	ESBL	Carbapene-mase	Susceptible	Susceptible
Obtained results (n/N)	ESBL	**5/5 (100.0%)**	--	--	--	**5/5 (100.0%)**	--	--	--
	AmpC	--	--	1/5 (20.0%)	1/5 (20.0%)	--	--	--	--
	ESBL + AmpC	--	--	2/5 (40.0%)	2/5 (40.0%)	--	--	1/5 (20.0%)	--
	Carbapenemase	--	**3/5 (60.0%)**	**2/5 (40.0%)**	**2/5 (40.0%)**	--	**2/4 (50.0%)**	--	1/5 (20.0%)
	Other	--	2/5 (40.0%)	--	--	--	2/4 (50.0%)	--	1/5 (20.0%)
	Susceptible*	--	--	--	--	--	--	**4/5 (80.0%)**	**3/5 (60.0%)**

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

4.3.4 Quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for both *Salmonella* and *E. coli* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the nine participating laboratories, seven submitted results for the reference strain *E. coli* ATCC 25922 and only one (#37) performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by broth microdilution; for testing *E. coli* NCTC 13846, MIC was determined by broth microdilution (**Table 37**). The highest proportion of test results outside of the expected range were observed for cefotaxime (2 out of 6), cefepime (1 out of 4), sulfamethoxazole (1 out of 4) and trimethoprim (1 out of 4) (**Table 37**). Moreover, the inaccurate results were obtained when disk diffusion was applied.

Regarding the laboratories' performance, laboratories #20, #22, #33 and #47 presented no deviation (**Figure 28**). While laboratories #20 and #47 applied broth microdilution, laboratories #22 and #33 used disk diffusion. The remaining three laboratories (#18, #37 and #40) presented deviations ranging from 11.1 to 42.9% (**Figure 28**). Laboratory #40 presented deviations for two antimicrobial agents, whereas laboratories #18 and #37 presented three deviations. Despite the same number of deviations (n=3), laboratory #18 tested fewer antimicrobials (n=7) than laboratory #37 (n=23) and thus, the deviation observed was larger (**Figure 28**).

Laboratories #40 and #18 reported Inhibition Zone Diameters both above and below the expected range, whereas laboratory #37 deviations were slightly (1-2mm) below the acceptance interval. These overall deviations imply a need to strengthen the laboratories' performance on applying disk diffusion, a well-known and routinely used method.

Table 37. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) 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.	MIC	Total
AMK	0/5	-	**0/5**
AMP	0/4	0/1	**0/5**
FEP	1/4	-	**1/4**
FOT	2/5	0/1	**2/6**
FOX	0/4	-	**0/4**
TAZ	0/5	0/1	**0/6**
CHL	1/4	0/1	**1/5**
CIP	1/5	0/1	**1/6**
COL	-	0/1	**0/1**
DOR	0/2	-	**0/2**
ETP	0/3	-	**0/3**
GEN	0/4	0/1	**0/5**
IMI	0/5	-	**0/5**
LEVO	0/3	-	**0/3**
MERO	0/4	-	**0/4**
NAL	0/3	0/1	**0/4**
PT4	0/2	-	**0/2**
SMX	1/3	0/1	**1/4**
TET	1/5	0/2	**1/7**
TGC	0/1	0/1	**0/2**
TOB	0/1	-	**0/1**
TMP	1/3	0/1	**1/4**
SXT	0/3	-	**0/3**

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

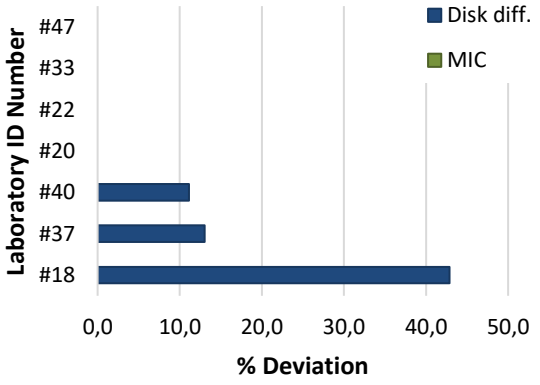

Figure 28. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *E. coli* trial by the HH laboratories.

4.4 *Pseudomonas aeruginosa* trial

A total of 3 laboratories from 2 countries uploaded results for the *P. aeruginosa* trial.

4.4.1 Bacterial identification

All three participating laboratories submitted results for bacterial identification (**Table 38**), and all three correctly identified the eight *P. aeruginosa* strains and the three non-*P. aeruginosa*.

Table 38. Bacterial identification of each of the 11 test strains provided related to the *P. aeruginosa* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Pa EQASIA 21.1	*P. aeruginosa*	3/3
Pa EQASIA 21.2	*P. aeruginosa*	3/3
Pa EQASIA 21.3	*P. aeruginosa*	3/3
Pa EQASIA 21.4	*P. aeruginosa*	3/3
Pa EQASIA 21.5	*P. aeruginosa*	3/3
Pa EQASIA 21.6	*P. aeruginosa*	3/3
Pa EQASIA 21.7	Non- *P. aeruginosa* (*Acinetobacter baumannii*)	3/3
Pa EQASIA 21.8	Non- *P. aeruginosa* (*Acinetobacter lowffii*)	3/3
Pa EQASIA 21.9	*P. aeruginosa*	3/3
Pa EQASIA 21.10	*P. aeruginosa*	3/3
Pa EQASIA 21.11	Non- *P. aeruginosa* (*Acinetobacter pittii*)	3/3

Pa, *P. aeruginosa*

4.4.2 AST 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/I/S) ranged from 89.4% (strain Pa EQASIA 21.2) to 99.0% (strains Pa EQASIA 21.4) for each strain (**Table 39**). The results from only one strain revealed more than 10% deviation (Pa EQASIA 21.2) and half of the strains had a deviation below or equal to 5% (**Table 39**).

The deviation observed for Pa EQASIA 21.2 was caused by few incorrect results for some antimicrobials, including imipenem, which the strain was expected to be considered resistant towards it, but all three laboratories obtained quite large Inhibition Zone Diameters that led to the interpretation of the results as either intermediate or susceptible.

Table 39. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 3 AH laboratories for the *P. aeruginosa* trial.

Strain	AST in total	% Correct
Pa EQASIA 21.1	26	95.2
Pa EQASIA 21.2	26	89.4
Pa EQASIA 21.3	26	94.2
Pa EQASIA 21.4	26	99.0
Pa EQASIA 21.5	26	95.2
Pa EQASIA 21.6	26	96.2
Pa EQASIA 21.9	26	93.3
Pa EQASIA 21.10	26	92.3

Pa, *P. aeruginosa*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were colistin (34.4%), imipenem (13.5%) and meropenem (10.9%), whereas amikacin, aztreonam, levofloxacin and tobramycin revealed no deviation from the expected results (**Figure 29**).

In the case of colistin, only laboratory #37 tested for it. Since the laboratory misinterpreted the results by reporting susceptible instead of intermediate, it caused a score penalty that resulted in the high deviation observed in **Figure 29**. This deviation however, should not be considered as a performance issue.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for only one laboratory (#37), even though laboratory #33 presented a deviation just slightly above the acceptance level (5.1%) (**Figure 30**). In average, the deviation was 6.5% (ranging from 4.3 to

10.0%).

Laboratory #18 underperformance seems to be caused by just few incorrect results when testing ciprofloxacin, gentamicin and imipenem. The laboratory tested the strains against a total of five antimicrobials, meaning that even few incorrect results will cause a high deviation. In this case, the incorrect results seem to be random and not associated with a specific strain or antimicrobial.

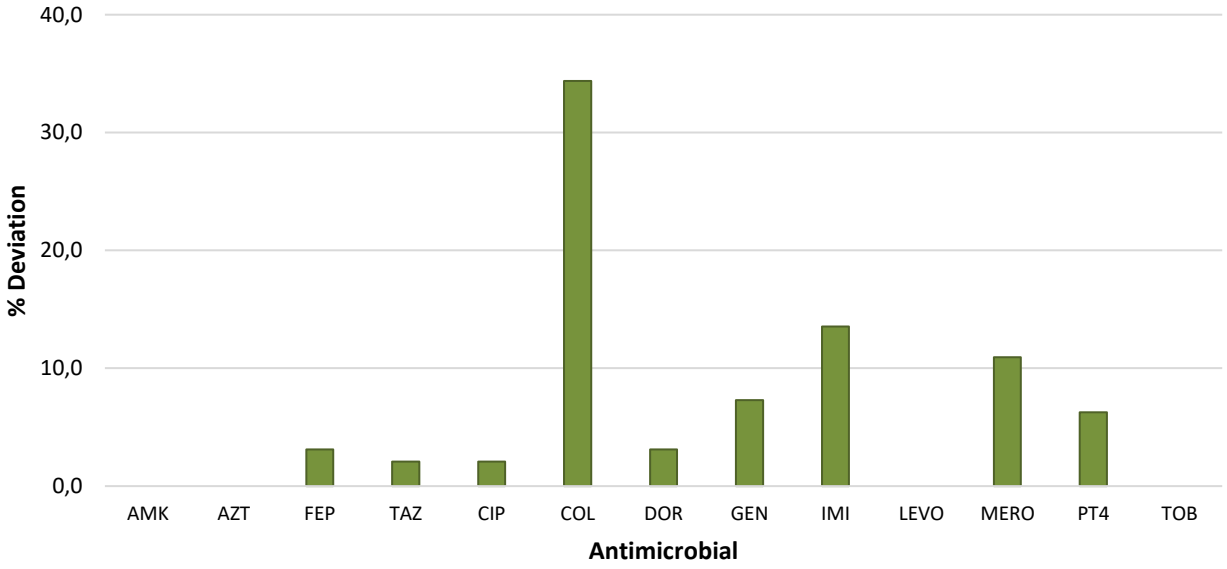

Figure 29. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=3) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

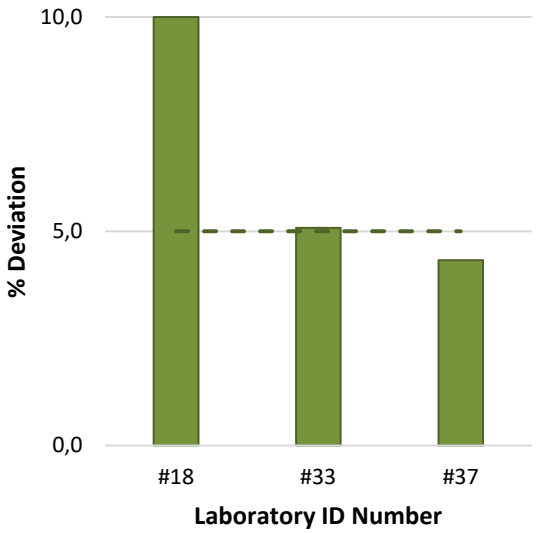

Figure 30. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=3) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strain *P. aeruginosa* ATCC 27853

The quality control strain *P. aeruginosa* ATCC 27853 was sent to all participating laboratories free of charge (in this trial or in previous trials) to be used as a reference strain for the *P. aeruginosa* trial.

All three laboratories submitted results regarding AST of *P. aeruginosa* ATCC 27853 reference strain and disk diffusion was the methodology applied by all three laboratories, except for colistin testing by laboratory #37 that was performed by broth microdilution (**Table 40**).

Only laboratory #37 presented deviations (**Figure 31**), meaning that the proportion of test results outside of the expected range consist in all of the incorrect results reported by this laboratory (**Table 40**). All deviations (n=9) were above the acceptance interval, which could be explained by, for example, the use of a too sparse inoculum or even contamination of the reference strain.

Table 40. AST of the reference strain *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMK	1/3	--	**1/3**
AZT	1/1	--	**1/1**
FEP	1/2	--	**1/2**
TAZ	1/3	--	**1/3**
CIP	1/3	--	**1/3**
COL	--	0/1	**0/1**
DOR	1/1	--	**1/1**
GEN	0/3	--	**0/3**
IMI	0/3	--	**0/3**
LEVO	0/2	--	**0/2**
MERO	1/2	--	**1/2**
P/T4	1/1	--	**1/1**
TOB	1/1	--	**1/1**

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

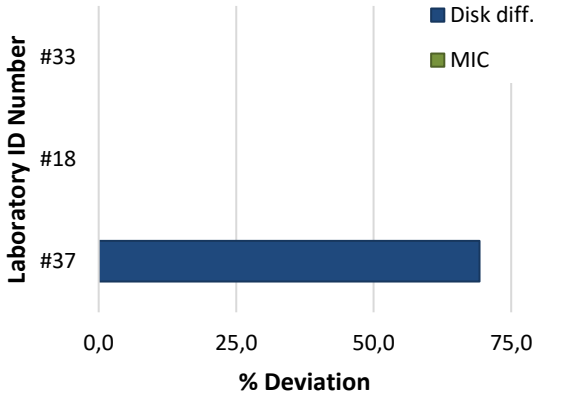

Figure 31. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial by the AH laboratories.

4.5 *Campylobacter jejuni* / *C. coli* trial

Four laboratories from three countries uploaded results for the *C. jejuni/ C. coli* trial.

4.5.1 Bacterial identification

All four participating laboratories submitted results for bacterial identification; however, laboratory #40 submitted results for only one strain (Campy EQASIA 21.5) (**Table 41**). While the remaining three laboratories (#33, #37 and #47) correctly identified the eight *C. jejuni/ C. coli* strains and the three non-*C. jejuni/ C. coli*, laboratory #40 misidentified Campy EQASIA 21.5 (*C. coli*) as *C. jejuni*.

Table 41. Bacterial identification of each of the 11 test strains provided related to the *C. jejuni/ C. coli* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Campy EQASIA 21.1	*C. coli*	3/3
Campy EQASIA 21.2	Non-*C. jejuni/ C. coli (C. lari)*	3/3
Campy EQASIA 21.3	*C. coli*	3/3
Campy EQASIA 21.4	Non-*C. jejuni/ C. coli (C. lari)*	3/3
Campy EQASIA 21.5	*C. coli*	3/4
Campy EQASIA 21.6	*C. jejuni*	3/3
Campy EQASIA 21.7	*C. jejuni*	3/3
Campy EQASIA 21.8	Non-*C. jejuni/ C. coli (C. lari)*	3/3
Campy EQASIA 21.9	*C. coli*	3/3
Campy EQASIA 21.10	*C. coli*	3/3
Campy EQASIA 21.11	*C. jejuni*	3/3

Campy, *C. jejuni/ C. coli*

4.5.2 AST 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

Laboratory #47 did not submit AST results for this trial, as mentioned in the section '4.1 Overall participation'. In addition, laboratory #40 only submitted results for strain Campy EQASIA 21.5, and laboratory #37 did not submit results for strains Campy EQASIA 21.6, Campy EQASIA 21.7 and Campy EQASIA 21.10.

Based on this observation, the percentage of results in agreement with expected interpretative results (R/I/S) varied greatly and ranged from 43.8% (Campy EQASIA 21.10) to 100% (strain Campy EQASIA 21.7) for each strain (**Table 42**).

As strain Campy EQASIA 21.10 was only tested

by laboratory #33, the strain deviation is solely caused by this laboratory's performance. Indeed, the laboratory reported the strain as quite resistant, opposite to what would be expected (Appendix 2d). On the contrary, Campy EQASIA 21.7 presented no deviation, which shows that the laboratory had no performance issues testing this specific strain.

Table 42. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 3 AH laboratories for the *C. jejuni/ C. coli* trial.

Strain	AST in total	% Correct
Campy EQASIA 21.1	9	50.0
Campy EQASIA 21.3	9	72.2
Campy EQASIA 21.5	13	82.7
Campy EQASIA 21.6	4	81.3
Campy EQASIA 21.7	4	100.0
Campy EQASIA 21.9	9	72.2
Campy EQASIA 21.10	4	43.8
Campy EQASIA 21.11	9	63.9

Campy, *C. jejuni/ C. coli*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were erythromycin (48.2%), tetracycline (39.3%) and ciprofloxacin (35.7%), whereas chloramphenicol revealed no deviation from the expected results (**Figure 32**).

Chloramphenicol was tested by laboratory #37 only, and therefore only against five strains, and all results were in accordance with the expected. In reverse, ciprofloxacin, erythromycin and tetracycline presented several incorrect results.

Laboratory #33 reported all strains as resistant to ciprofloxacin, which was not expected for half of them (Appendix 2d). Laboratory #37 reported incorrect ciprofloxacin results for three of the five strains.

When it comes to erythromycin, laboratory #33 also reported all strains as resistant (Inhibition Zone Diameter = 6mm for all strains), which was only true for two of them (Appendix 2d). Laboratory #37 reported three incorrect results as well.

For tetracycline, the situation was similar: all strains were reported as resistant by laboratory #33 (only three were), and two incorrect results reported by laboratory #37.

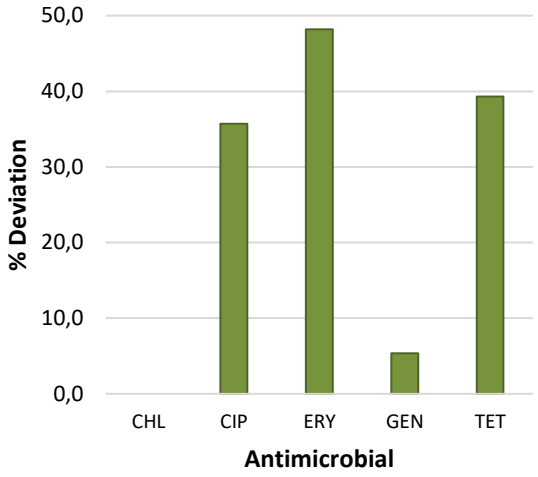

Figure 32. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=3) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed only for laboratory #40, which tested only one of the eight strains (**Figure 33**). In average, the deviation was 20.7% (ranging from 0.0 to 35.2%). As the acceptance level was set to 5% deviation, the laboratories #33 and #37 did not perform within the expected range for the trial.

The explanation for the deviation observed for laboratory #33 was already presented in the previous sub-section. This laboratory reported all strains as resistant towards ciprofloxacin, erythromycin and tetracycline, which was not correct in several instances (Appendix 2d).

Laboratory #37 deviation is not so straightforward. The incorrect results were seen for all antimicrobials (except chloramphenicol) and for all five strains reported.

The general poor performance observed for the *C. jejuni/ C. coli* trial demonstrates the need for

capacity building in terms of culturing/growth and AST of *Campylobacter* strains.

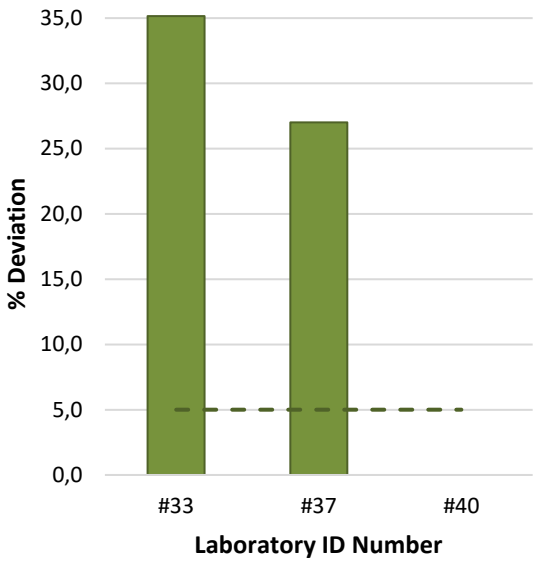

Figure 33. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by HH laboratories (n=3) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.5.3 Quality control strain *C. jejuni* ATCC 33560

The quality control strain *C. jejuni* ATCC 33560 was sent to all participating laboratories free of charge to be used as a reference strain for the *C. jejuni/ C. coli* trial. Even though three laboratories submitted results, only the data reported by laboratory #37 could be assessed. In fact, laboratories #33 and #40 submitted disk diffusion results for *C. jejuni* ATCC 33560 when grown at 36-37°C for 48h; for these conditions, however, acceptance intervals for disk diffusion are not available on the CLSI manuals [4].

In its turn, laboratory #37 tested the reference strain and submitted broth microdilution results for only one antimicrobial (chloramphenicol) (**Table 22**). Even though laboratory #37 tested the test strains against five antimicrobials (chloramphenicol by broth microdilution and the remaining four by disk diffusion), only results for chloramphenicol were submitted for the reference strain when grown at 42°C for 24h. Since disk diffusion acceptance intervals are available for ciprofloxacin and erythromycin when *C. jejuni* ATCC 33560 is grown under those condition, it would have been relevant for the laboratory to have included this quality control assessment.

Regarding the laboratory's performance, the reported MIC for chloramphenicol was one-fold dilution above the acceptance interval, which resulted in a deviation (**Table 43**) and an underperformance of 100% (**Figure 34**).

Table 43. AST of the reference strain *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	MIC	Total
CHL	1/1	**1/1**

MIC – MIC determination by broth microdilution

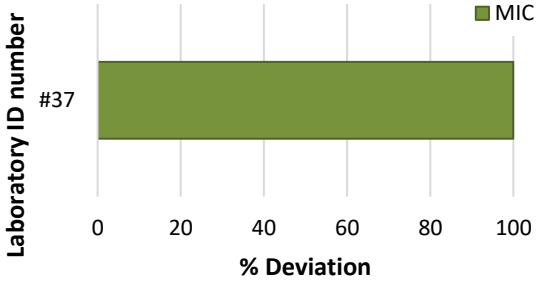

Figure 34. Percentage of deviation in the AST of *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial by the AH laboratory.

4.6 *Enterococcus faecium* / *E. faecalis* trial

Seven laboratories from five countries uploaded results for the *E. faecium/ E. faecalis* trial.

4.6.1 Bacterial identification

All seven participating laboratories submitted results for bacterial identification (**Table 44**). Only one laboratory (#37) correctly identified the eight *E. faecium*/ *E. faecalis* strains and the three non-*E. faecium*/ *E. faecalis*. In addition, laboratory #22 correctly identified the eight *E. faecium*/ *E. faecalis* strains. Laboratory #44 was unable to perform bacterial identification and reported all 11 strains as *E. faecium*. Taking that into consideration, all four *E. faecalis* strains (Ef

EQASIA 21.1, Ef EQASIA 21.2, Ef EQASIA 21.7 and Ef EQASIA 21.8) were correctly identified by the remaining six laboratories (**Table 44**).

Inversely, the *E. faecium* strains generated some incorrect results: strains Ef EQASIA 21.4, Ef EQASIA 21.5 and Ef EQASIA 21.10 were misidentified as *E. faecalis* by laboratory #21, and as non-*E. faecium*/ *E. faecalis* by laboratory #40; moreover, laboratory #33 considered strain Ef EQASIA 21.4 as non-*E. faecium*/ *E. faecalis*, and laboratory #18 considered the remaining three *E. faecium* strains as non-*E. faecium*/ *E. faecalis* as well.

The non-*E. faecium/ E. faecalis* strains were the most problematic ones. Besides laboratory #44, Ef EQASIA 21.3 (*E. mundtii*) was identified as *E. faecium* by laboratories #22 and #40, while laboratories #18 and #21 identified it as *E. faecalis*; similarly, Ef EQASIA 21.6 (also *E. mundtii*) was identified as *E. faecium* by laboratory #44 and four other laboratories (#18, #22, #33 and #40), and as *E. faecalis* by laboratory #21; lastly, Ef EQASIA 21.9 (*E. gallinarum*) was incorrectly identified as *E. faecium* by laboratories #21 and #40 (and #44), and as *E. faecalis* by laboratory #18.

Table 44. Bacterial identification of each of the 11 test strains provided related to the *E. faecium/ E. faecalis* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ef EQASIA 21.1	*E. faecalis*	6/7
Ef EQASIA 21.2	*E. faecalis*	6/7
Ef EQASIA 21.3	Non-*E. faecium/ E. faecalis* (*E. mundtii*)	2/7
Ef EQASIA 21.4	*E. faecium*	4/7
Ef EQASIA 21.5	*E. faecium*	4/7
Ef EQASIA 21.6	Non-*E. faecium/ E. faecalis* (*E. mundtii*)	1/7
Ef EQASIA 21.7	*E. faecalis*	6/7
Ef EQASIA 21.8	*E. faecalis*	6/7
Ef EQASIA 21.9	Non-*E. faecium/ E. faecalis* (*E. gallinarum*)	3/7
Ef EQASIA 21.10	*E. faecium*	4/7
Ef EQASIA 21.11	*E. faecium*	6/7

Ef, *E. faecium/ E. faecalis*

4.6.2 AST performance

In this subsection, the AST performance is analysed from a strain-, antimicrobial-, and laboratory-based perspective.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 83.5% (strain Ef EQASIA 21.10) to 97.5% (strains Ef EQASIA 21.2) for each strain (**Table 45**). The AST results submitted for the eight *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species.

The results from three of the eight strains revealed more than 10% deviation (Ef EQASIA 21.4, Ef EQASIA 21.7 and Ef EQASIA 21.10): strain Ef EQASIA 21.4 owes its deviation to several incorrect results for quinupristin/ dalfopristin, which should be found resistant to the drug, but instead was reported as susceptible; strain Ef EQASIA 21.7, a quite resistant strain (Appendix 2e), was considered susceptible towards all tested antimicrobials by laboratory #18, resulting in a high score penalty; in its turn, Ef EQASIA 21.10, also a fairly resistant strain (Appendix 2e), was considered susceptible towards several antimicrobials by laboratory #22.

Table 45. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 7 AH laboratories for the *E. faecium /E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQASIA 21.1	61	94.3
Ef EQASIA 21.2	61	97.5
Ef EQASIA 21.4	45	86.1
Ef EQASIA 21.5	47	96.8
Ef EQASIA 21.7	61	88.9
Ef EQASIA 21.8	61	95.1
Ef EQASIA 21.10	47	83.5
Ef EQASIA 21.11	55	95.0

Ef, *E. faecium/ E. faecalis*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were quinupristin/dalfopristin (15.6%), teicoplanin (13.8%), ampicillin (13.3%) and vancomycin (10.4%), whereas linezolid revealed no deviation from the expected results (**Figure 35**).

The incorrect results reported for quinupristin/ dalfopristin were mostly seen for strain Ef EQASIA 21.4, as abovementioned. The expected MIC for this antimicrobial/strain pair was 4 µg/mL, just on the limit to be interpreted as resistant. The incorrect Inhibition Zone Diameters reported were a few mm above the breakpoint, which led to the interpretation of the strain as susceptible.

For teicoplanin and vancomycin, the incorrect results varied amongst strains and laboratories.

The greatest contribution for ampicillin deviation was laboratory #21 results. This laboratory reported all strains as resistant to ampicillin, opposite to what would be expected for most of them (Appendix 2e).

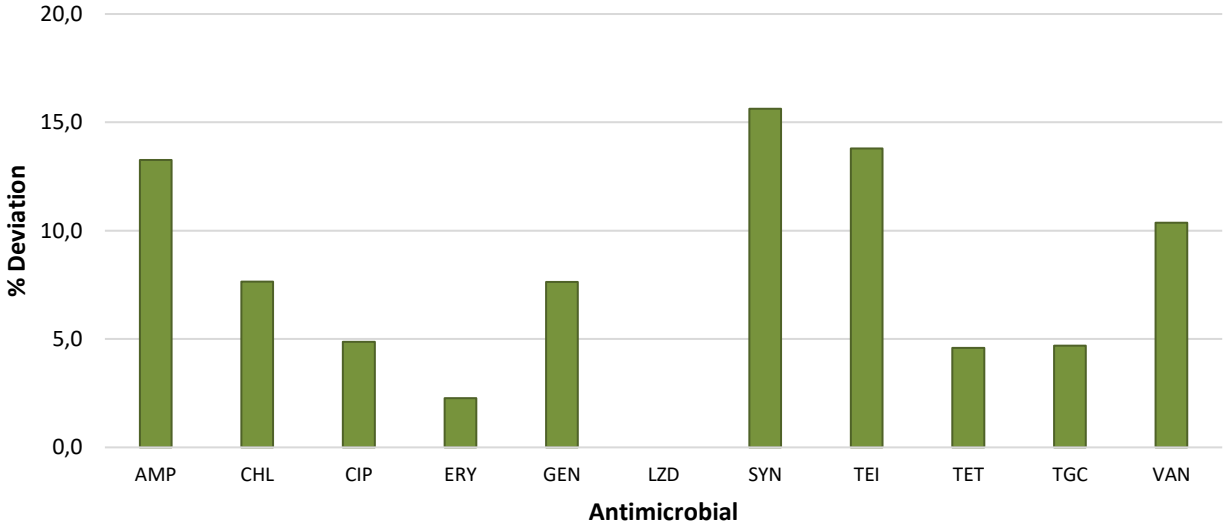

Figure 35. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=7) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for three out of the seven participants (**Figure 36**). In average, the deviation was 8.2% (ranging from 0.9 to 22.5%). As the acceptance level was set to 5% deviation, four laboratories did not perform within the expected range for the trial; however, the deviation for laboratory #44 was just slightly above the acceptance level (5.2%).

Laboratory #18 presented the highest deviation, which can be in part explained by the incorrect results reported for strain Ef EQASIA 21.7, as explained before. In addition, the laboratory interpreted incorrectly a couple of chloramphenicol results.

Similarly, laboratory #22 deviation is mostly due to the incorrect results reported for strain Ef EQASIA 21.10, as well as some other occasional mistakes.

For laboratory #21, the deviation was caused by the incorrect ampicillin results as previously mentioned, and also some incorrect results for other antimicrobials, such as ciprofloxacin, teicoplanin and vancomycin.

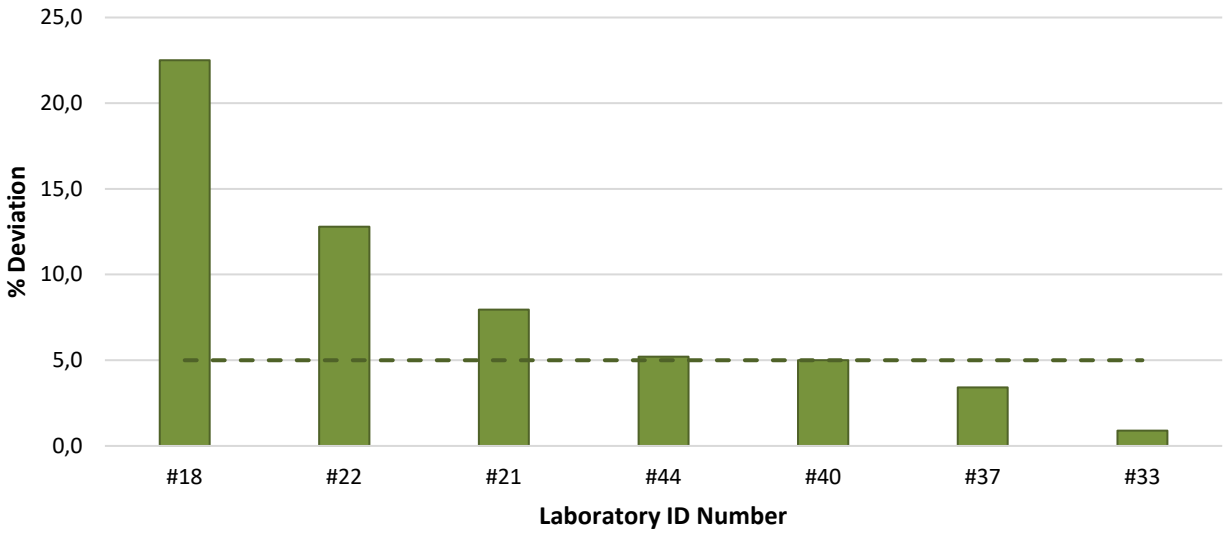

Figure 36. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=7) participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.6.3 Quality control strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212

The quality control strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 for testing when disk diffusion or MIC determination methodologies are applied, respectively, were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *E. faecium/ E. faecalis* trial.

All seven participating laboratories submitted results for the reference strains, but the data from only four could be assessed. Laboratories #21, #40 and #44 submitted disk diffusion for the reference strain *E. faecalis* ATCC 29212, which is not recommended and could not be evaluated, as no acceptance intervals are available in the CLSI manuals (Appendix 2e). The remaining four laboratories also applied the disk diffusion methodology, but tested the reference strain *S. aureus* ATCC 25923 (**Table 46**). The highest proportion of test results outside of the expected range was observed for vancomycin (2 out of 4) and erythromycin (1 out of 3) (**Table 46**).

A closer look at the laboratories' performance (**Figure 37**) shows that two laboratories had no deviation (#22 and #33). Inversely, laboratories #18 and #37 had deviations of 66.7% and 27.3%, respectively, corresponding to four and three deviations each.

Laboratory #37 deviations were all quite below the acceptance interval; a similar scenario was observed for laboratory #18, even though one of the deviations seems to be a typo, since an Inhibition Zone Diameter of just 2 mm was reported for ciprofloxacin.

Table 46. AST of the reference strain *S. aureus* ATCC 25923 in the *E. faecium/ E. faecalis* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diff.	**Total**
AMP	1/4	**1/4**
CHL	1/4	**1/4**
CIP	1/4	**1/4**
ERY	1/3	**1/3**
GEN	0/4	**0/4**
LZD	0/3	**0/3**
SYN	0/2	**0/2**
TEI	0/2	**0/2**
TET	1/4	**1/4**
TGC	0/2	**0/2**
VAN	2/4	**2/4**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

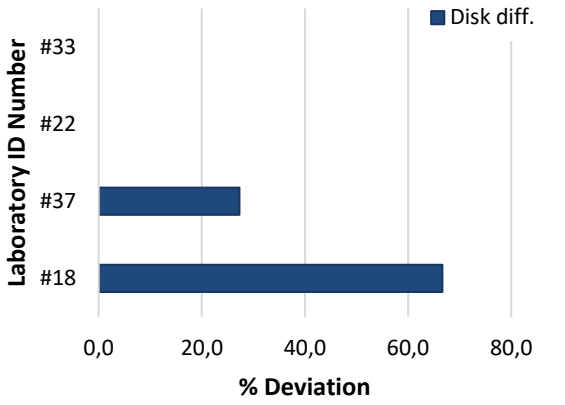

Figure 37. Percentage of deviation in the AST of *S. aureus* ATCC 25923 in the *E. faecium/ E. faecalis* trial by the AH laboratories.

4.7 *Streptococcus pneumoniae* trial

Only one laboratory (#33) participated and uploaded results for the *S. pneumoniae* trial.

4.7.1 Bacterial identification

The results for bacterial identification are shown in **Table 47**. Laboratory #33 submitted data for all 11 strains, but revealed some difficulties in correctly identifying the *S. pneumoniae* and non-*S. pneumoniae* strains: while the *S. pneumoniae* strains Sp EQASIA 21.1, Sp EQASIA 21.6 and Sp EQASIA 21.11 were misidentified as non-*S. pneumoniae*, all three non-*S. pneumoniae* (Sp EQASIA 21.4, Sp EQASIA 21.5 and Sp EQASIA 21.9) were incorrectly identified as *S. pneumoniae*.

Table 47. Bacterial identification of each of the 11 test strains provided related to the *S. pneumoniae* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Sp EQASIA 21.1	*S. pneumoniae*	0/1
Sp EQASIA 21.2	*S. pneumoniae*	1/1
Sp EQASIA 21.3	*S. pneumoniae*	1/1
Sp EQASIA 21.4	Non-*S. pneumoniae* (*E. faecalis*)	0/1
Sp EQASIA 21.5	Non-*S. pneumoniae* (*Listeria innocua*)	0/1
Sp EQASIA 21.6	*S. pneumoniae*	0/1
Sp EQASIA 21.7	*S. pneumoniae*	1/1
Sp EQASIA 21.8	*S. pneumoniae*	1/1
Sp EQASIA 21.9	Non-*S. pneumoniae* (*E. faecalis*)	0/1
Sp EQASIA 21.10	*S. pneumoniae*	1/1
Sp EQASIA 21.11	*S. pneumoniae*	0/1

Sp, *S. pneumoniae*

4.7.2 AST 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

Due to the incorrect identification of the test strains provided, only the data submitted for five strains could be assessed (**Table 48**).

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 57.1% (strains Sp EQASIA 21.3, Sp EQASIA 21.8 and Sp EQASIA 21.10) to 100.0% (strains Sp EQASIA 21.7) for each strain (**Table 48**).

Table 48. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from one AH laboratory for the *S. pneumoniae* trial.

Strain	AST in total	% Correct
Sp EQASIA 21.1	--	--
Sp EQASIA 21.2	7	75.0
Sp EQASIA 21.3	7	57.1
Sp EQASIA 21.6	--	--
Sp EQASIA 21.7	7	100.0
Sp EQASIA 21.8	7	57.1
Sp EQASIA 21.10	7	57.1
Sp EQASIA 21.11	--	--

Sp, *S. pneumoniae*

Antimicrobial-based analysis

Laboratory #33 tested the strains against seven antimicrobials and presented deviations for three of them: azithromycin (75.0%), erythromycin (75.0%) and tetracycline (65.0%) (**Figure 38**).

Azithromycin, erythromycin and tetracycline results were incorrect for all strains except Sp EQASIA 21.

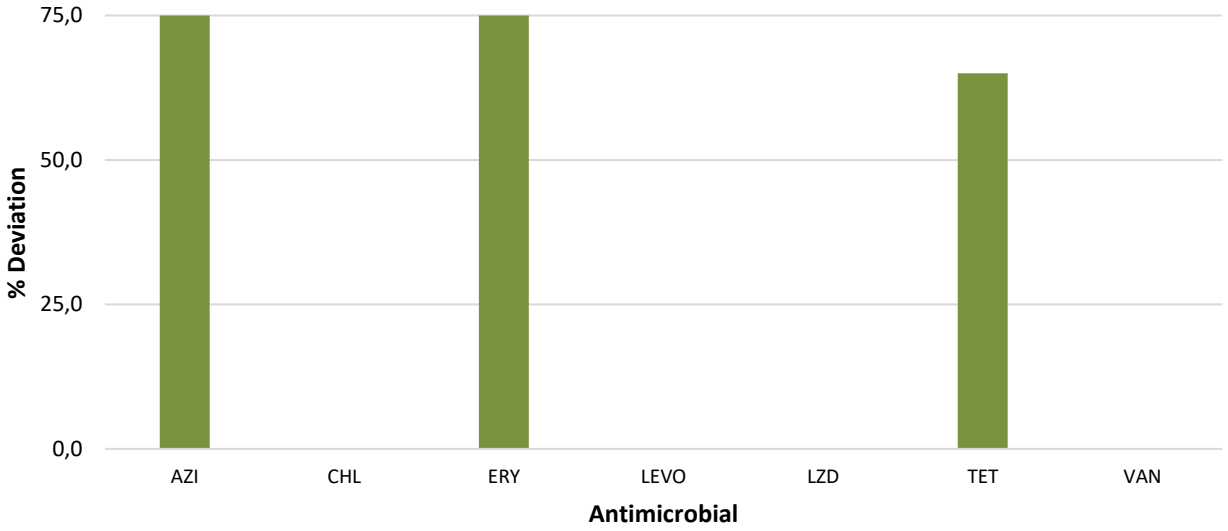

Figure 38. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by AH laboratories (n=1) participating in the 3rd EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

Laboratory #33 presented a deviation from the expected results of 30.7% (**Figure 39**), due to the incorrect results mentioned in the previous sub-section. In fact, the laboratory reported strains Sp EQASIA 21.3, Sp EQASIA 21.7, Sp EQASIA 21.8 and Sp EQASIA 21.10 has completely susceptible to all seven tested antimicrobials, which was only true for strain Sp EQASIA 21.7 (Appendix 2f).

In its turn, strain Sp EQASIA 21.2, which was expected to be susceptible to these seven antimicrobials (Appendix 2f), was reported as intermediate and resistant to some of the antimicrobials, namely azithromycin, erythromycin and tetracycline.

These observed results for laboratory #33 could indicate that the strains were switched around for example, but also that a light bacterial inoculum was used for plating some of the strains, resulting in the very susceptible profiles observed. Another possibility could be inappropriate growth conditions and handling of the *S. pneumoniae* strains, leading to the use of inviable cells.

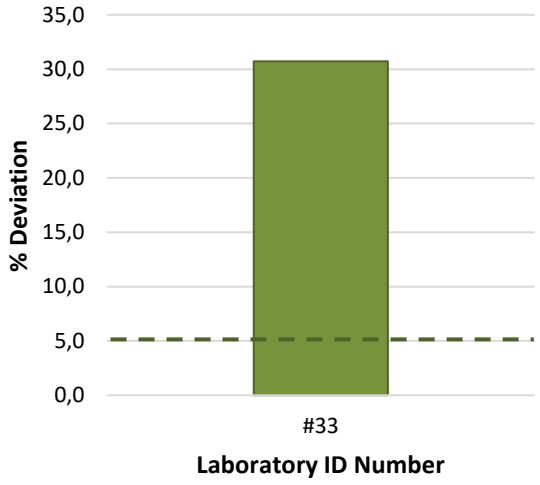

Figure 39. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by one AH laboratory participating in the 3rd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.7.3 Quality control strain *S. pneumoniae* ATCC 49619

The quality control strain *S. pneumoniae* ATCC 49619 was sent to all participating laboratories free of charge to be used as a reference strain for the *S. pneumoniae* trial.

Laboratory #33 submitted results regarding AST of *S. pneumoniae* ATCC 49619 reference strain

for 11 antimicrobials, even though only seven were tested against the test strains (**Table 49**).

Disk diffusion was the methodology applied for testing the quality control strain *S. pneumoniae* ATCC 49619 and laboratory #33 presented no deviation (**Table 49**).

Table 49. AST of the reference strain *S. pneumoniae* ATCC 49619 in the *S. pneumoniae* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diff.	Total
AZI	0/1	**0/1**
FEP	0/1	**0/1**
FOT	0/1	**0/1**
AXO	0/1	**0/1**
CHL	0/1	**0/1**
CLI	--	**--**
ETP	--	**--**
ERY	0/1	**0/1**
LEVO	0/1	**0/1**
LZD	0/1	**0/1**
MERO	0/1	**0/1**
PEN	--	**--**
TET	0/1	**0/1**
SXT	--	**--**
VAN	0/1	**0/1**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

5. Discussion

5.1 Human Health Laboratories

A total of 14 Human Health laboratories participated in the 3rd EQA of the EQAsia programme. Disk diffusion and broth microdilution as solo methodologies were chosen by the majority of the participants for testing the recommended antimicrobials in each of the trials. The remaining laboratories opted for a combination of methodologies that included disk diffusion, broth microdilution, gradient test, and/or agar dilution.

All laboratories that performed bacterial identification also submitted AST results. Incomplete AST results' entries were, however, observed in five of the trials (exception for the *C. jejuni/C. coli* trial), meaning that the participating laboratories did not submit complete results of their own available antimicrobial agents. It would be expected that the isolates of each trial would be tested against the same panel of antimicrobials, allowing for a solid assessment of the laboratories' performance and capacity.

Regarding the bacterial identification component, the participants showed high proficiency in correctly differentiating the *S. pneumoniae* from the non-*S. pneumoniae* strains. For the *C. jejuni/C. coli* trial, only one laboratory participated and submitted data for just two strains, whereas only one was correctly identified. In the *Salmonella*, *E. coli* and *P. aeruginosa* trials, most of the laboratories could properly identify the target species. On the contrary, almost half of the participating laboratories in the *E. faecium/ E. faecalis* trial showed limited capacity to correctly identify the 11 test strains provided. In general, the laboratories had more difficulties in identifying the *E. faecalis* strains than the *E. faecium* strains. Accurate pathogen identification is crucial, especially in a clinical setting and proficiency testing is a regulatory requirement in many settings. There is a need to assess the causes for bacterial misidentification, in particular for some of the pathogens, and provide guidance and appropriate training.

The antimicrobial susceptibility testing performance was assessed from different angles to better identify deviations from the expected results. For the *E. faecium/ E. faecalis* trial, the AST results submitted for the eight *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the

laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species.

For the Gram-negative bacteria *Salmonella*, *E. coli* and *P. aeruginosa* trials, some common antimicrobials presented a high deviation from the expected results, such as colistin (24.0%, 15.7% and 12.2% respectively) and doripenem (19.8% in the *E. coli* trial and 13.3% in the *P. aeruginosa* trial). These high deviations for colistin can be explained by the fact that the AST results of this antimicrobial (and also amikacin and gentamicin in the *Salmonella* trial) should only be interpreted as intermediate or resistant, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1). Instead, several laboratories reported the different isolates as susceptible instead of intermediate, which resulted in a score penalty (score of 3 instead of the full score 4). In addition, colistin testing requires the use of broth microdilution, which some laboratories may be lacking the required knowledge and experience for applying this methodology. Doripenem, in its turn, was tested by a limited number of laboratories, meaning that even few incorrect results would result in a high percentage of deviation. In this case, most of the incorrect results were observed for laboratory #01, which reported smaller Inhibition Zone Diameters than what would be expected for both *E. coli* and *P. aeruginosa* trials.

The AST deviations observed in the *C. jejuni/C. coli* trial reflect the performance of only one laboratory. Analysis of the data submitted by this laboratory reveals very large Inhibition Zone Diameter values reported, which could indicate, for example, that a sparse bacterial inoculum was used for plating or that inappropriate growth conditions were applied for the *Campylobacter* strains.

For the Gram-positive bacteria *E. faecium/ E. faecalis* and *S. pneumoniae* trials, the deviations observed were usually higher for antimicrobials that were tested by fewer laboratories. Moreover, a big part of the deviations was seen for situations where the expected results were close to the threshold for categorising the strain as susceptible / intermediate or intermediate / resistant, and a one-fold dilution/±3mm difference resulted in different interpretations. In these cases, the participants should be confident about the good quality of their AST performance. A different situation is the incorrect interpretation of obtained results, because even though this deviation may not reflect an actual performance issue, correct interpretation of the obtained results is as important as testing the antimicrobials, since it can lead to inappropriate clinical treatment.

Regarding the HH laboratories' AST performance, on average, the deviation was 6.3% in the *Salmonella* trial, 3.2% in the *E. coli* trial, 6.1% in the *P. aeruginosa* trial, 33.3% in the *C. jejuni/C. coli* trial, 3.7% in the *E. faecium/ E. faecalis* and 3.9% in the *S. pneumoniae* trial. Despite the average being close to acceptable (below the acceptance level of 5% or just slightly above, except for the *C. jejuni/C. coli* trial), there were some laboratories that had deviations above 5% in multiple trials. Laboratories #04 (*Salmonella* and *P. aeruginosa* trials) and #06 (*C. jejuni/C. coli*) were the only HH participants with deviations above 10%.

Detection and confirmation of presumptive beta-lactamase producing *E. coli* strains was an optional component of EQA3, but highly encouraged due to its importance. Ten out of the 12 participating laboratories submitted results and were able to differentiate the susceptible (no ESBL, AmpC or carbapenemase) from the ESBL/ AmpC/ carbapenemase-producers. However, only laboratory #10 correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the eight *E. coli* strains. The major issue observed was the incorrect classification of the carbapenemase phenotypes, even though the obtained values for meropenem were frequently > 0.12 µg/mL or < 25 mm. According to Figure 1 of the EQA3 protocol (Appendix 1), a strain presenting these values for meropenem should be classified as a

carbapenem-resistant *E. coli* strain.

Serotyping of *Salmonella* was also a component with voluntary participation, for which only four of the eight participating laboratories reported results. Of those, however, only one submitted data for both serogroup and serovars. Based on the results, it is notable that some laboratories could only identify certain serogroups. This could be due to limited technical capacity, but also lack of antisera supply.

Among all laboratories, there were six laboratories that did not submit antimicrobial susceptibility testing results for the quality control strains: laboratory #02 did not submit results for the reference strains in the *E. coli* trial, laboratory #04 for the reference strains in the *Salmonella*, *E. coli* and *P. aeruginosa* trials, laboratory #05 for the *Salmonella* and *E. coli* trials, laboratory #08 for the *E. faecium/ E. faecalis* and *S. pneumoniae* trials, laboratory #13 for the *P. aeruginosa* and *S. pneumoniae* trials, and laboratory #48 for the *E. coli* trial. In addition, laboratories #01 and #10 tested the incorrect reference strain for the methodology applied for the *E. faecium/ E. faecalis* trial. Among the laboratories that tested the quality control strains, it was noticed that at times the reference strain was not tested against the same panel of antimicrobials used for the test strains. For quality control purposes, the participating laboratories should test the same antimicrobials for both the reference strains and the test strains, as well as apply the same methodology in both situations. The submitted results also suggested that there might be poor handling and maintenance of the quality control strains, as some data implied possible contamination or inviable strains. According to the CLSI recommendation, the quality of laboratory performance is determined by the quality control management, indicating accuracy and precision of data produced by an individual laboratory. Therefore, the correct AST results of test strains without quality control may not imply a reliable laboratory AST performance. A systemic performance of internal quality control including testing of reference strains must be implemented to warrant the improvement of laboratory capacity.

5.2 Animal Health Laboratories

For the Animal Health sector, 11 laboratories participated in the 3rd EQA of the EQAsia programme. The participating laboratories mostly applied disk diffusion for determining Inhibition Zone Diameters and few opted for broth microdilution or a combination of the two methods.

The participants were asked to firstly perform bacterial identification and then proceed with AST of the target strains. Although laboratory #47 performed bacterial identification, it did not submit AST results for the *C. jejuni/ C. coli* trial. In addition, laboratory #40 submitted results for only one strain of the same trial. Besides these missing data, incomplete AST results' entries were observed in one of the trials (*E. coli*). For two of the laboratories, it seems that they may have wrongly selected the antimicrobial agents for one of the strains, which can lead to a wrong assessment of the laboratories' performance.

As mentioned above, bacterial identification was the first component in each of the trials. For the *Salmonella*, *P. aeruginosa* and *C. jejuni/ C. coli* trials, there were no major issues with bacterial identification. The remaining three trials suggest limited capacity for performing bacterial identification, since several occasions of bacterial misidentification were observed. In the *E. coli* trial, for instance, a third of the participating laboratories demonstrated difficulties in identifying the non-*E. coli* strains. The identification and differentiation between *E. faecium*, *E. faecalis* and other *Enterococcus* species seems to be difficult for the majority of the AH laboratories. For instance, laboratory #44 was unable to perform bacterial identification and reported all 11 strains as *E. faecium*. Most of the laboratories could properly identify the *E. faecalis* strains, but not the remaining, suggesting that advice and training on the subject may be required among the AH laboratories. Lastly, for the *S. pneumoniae* trial,

only one laboratory participated and submitted data. This laboratory could only properly identify five of the 11 strains provided.

For the antimicrobial susceptibility testing performance, and as already mentioned, the AST results submitted for the eight *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species.

Considering the tested antimicrobials, the ones with the highest deviation from the expected results varied from trial to trial. Colistin susceptibility results, however, presented a high deviation in all the three trials it was included: *Salmonella*, *E. coli* and *P. aeruginosa*. The reason for this deviation has already been identified: the AST results of colistin should only be interpreted as intermediate or resistant, as recommended in the CLSI guidelines and stated in the EQA3 protocol (Appendix 1). Instead, several laboratories reported the isolates as being susceptible to colistin, which resulted in a score penalty. This deviation, however, should not be considered as a performance issue. Another similar situation was observed for azithromycin in the *Salmonella* trial: for some strains, the expected result was close to the threshold for categorising them as susceptible or resistant; thus, obtained results of a one-fold dilution/ ±3mm difference often resulted in different interpretations. This situation was also observed for other antimicrobials occasionally. Again, this type of deviations may not reflect an actual performance issue and should be carefully analysed in a route cause analysis procedure performed by individual participants (self-evaluation).

On the contrary, in the *C. jejuni/ C. coli* and *E. faecium/ E. faecalis* trials, antimicrobials such as ciprofloxacin, erythromycin and tetracycline, as well as ampicillin, teicoplanin and vancomycin presented deviations that seem to be associated with performance issues. This suggests that it is not necessarily related to the antimicrobial itself, but with AST of the abovementioned bacterial species.

The AST deviations observed in the *S. pneumoniae* trial are a special case because only one laboratory submitted results. Nevertheless, the deviations reported by laboratory #33 indicate, for example, that the strains may have been switched around or that a sparse bacterial inoculum was used for plating some of the strains, resulting in the very susceptible profiles observed. Another possibility could be inappropriate growth conditions and handling of the *S. pneumoniae* strains, leading to the use of inviable cells.

Regarding laboratories performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. The average deviation was, in fact, above the acceptance level of 5% for all six trials: 6.1% in the *Salmonella* trial, 5.1% in the *E. coli* trial, 6.5% in the *P. aeruginosa* trial, 20.7% in the *C. jejuni/ C. coli* trial, 8.2% in the *E. faecium/ E. faecalis* trial, and 30.7% in the *S. pneumoniae* trial. In addition, almost all AH laboratories had a deviation above 5% in at least one of the trials that they have participated in.

Five out of the nine participating laboratories in the *E. coli* trial submitted results for the detection and confirmation of presumptive beta-lactamase producing bacteria. Only one laboratory (#40) correctly identified all the different ESBL/ AmpC/ carbapenemase phenotypes among the eight *E. coli* strains. As seen for the HH laboratories, classification of the carbapenemase phenotypes was problematic as well. Even though laboratories obtained Inhibition Zone Diameter values for meropenem < 25 mm, they struggled to identify the correct classification. This observation suggests that further clarification on the classification of the different phenotypes is still required.

For the voluntary serotyping of *Salmonella*, only one AH laboratory submitted data, demonstrating that resources and capacity for

this component may still be scarce among the AH sector laboratories in South and Southeast Asia.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the trials. Laboratory #21 did not submit results for the reference strains in the *E. coli* trial, laboratory #22 for the reference strains in the *Salmonella* trial, laboratory #42 for the *Salmonella* and *E. coli* trials, and laboratory #47 for the *C. jejuni/ C. coli* trial. In addition, laboratories #21, #40 and #44 tested the incorrect reference strain for the methodology applied in the *E. faecium/ E. faecalis* trial. Testing the recommended reference strains is required in terms of quality control and reliability of AST results and performance. For the laboratories reporting data, the deviations in this component were defined as AST results of the reference strain that were outside the quality control acceptance intervals. The deviations originated mostly from disk diffusion, where the Inhibition Zone Diameters determined were either above or below the expected range. In several cases, the deviations suggest a poor handling of reference strains, which definitely needs to be strengthened to assure the laboratories' performance.

6. Conclusions

This report presented the results of the EQAsia 3rd EQA trial in 2021-22, which included *Salmonella*, *E. coli*, *P. aeruginosa*, *C. jejuni/ C. coli*, *E. faecium/ E. faecalis* and *S. pneumoniae*. 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 accurate bacterial identification and antimicrobial susceptibility testing of the offered pathogens with a result deviation level below 5%, and to address underperformance by supporting the laboratories with technical guidance through follow ups and capacity building.

Performance issues in terms of bacterial identification and antimicrobial susceptibility testing were detected for both sectors, in particular for *C. jejuni/ C. coli* (HH sector), *E. faecium/ E. faecalis* (both sectors) and *S. pneumoniae* (AH sector). These observations demonstrate the need for supporting with training and capacity building the reference laboratories in the South and Southeast Asian region.

For this trial, the data submitted, i.e., the interpretation of the obtained results by the participating laboratories, was assessed and scored based on the severity of the error. This type of scoring system helps to detect if the errors/deviations were caused by, for example, a limitation in reproducibility of the methodology applied, which translates into an MIC or Inhibition Zone Diameter value differing by one-fold dilution or ±3mm from the expected result.

As seen in the previous EQAsia EQA surveys, the laboratories still report incorrect interpretation of the MIC/Inhibition Zone Diameter values. Once again, it is recommended to solely use the interpretative criteria available in the EQA protocol, as it is a requirement that all participating laboratories follow the same interpretation criteria to allow for comparison of results. Several other incorrect results were also detected upon submission of the results, such as selection of the wrong antimicrobial or strains that seem to have been switched around. To avoid all these issues in future EQA surveys, it is recommended to implement quality control procedures such as having two different persons reading the results and the respective interpretations, both in the laboratory and when

the data is entered in the informatics system.

Antimicrobial susceptibility testing of the reference strains is also highly important and, therefore, recommended. Relevant reference strains have been sent to the participating laboratories free of charge to be used not only in the EQAsia EQAs, but also in their routine work for quality control purposes. Thus, proper storage and maintenance of these reference strains is recommended. Routine testing is required for quality control purposes, as deviating results for the quality control strains imply invalidation of the AST results for the test strains.

7. 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.

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[3] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 31st ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.

[4] CLSI. *Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated from Animals*. 1st ed. CLSI supplement VET06. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

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

[9] 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: EQA3 Protocol

Protocol for EQAsia EQAS – 3rd round

ID and antimicrobial susceptibility testing of *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter jejuni and C. coli*, *Enterococcus faecium and E. faecalis* and *Streptococcus pneumoniae* test strains
Serotyping of *Salmonella* spp. test strains

1 INTRODUCTION

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

The 3rd iteration of EQAsia EQAS includes the antimicrobial susceptibility testing of eight *Salmonella* spp., eight *Escherichia coli*, eight *Pseudomonas aeruginosa*, eight *Campylobacter jejuni / C. coli*, eight *Enterococcus faecium / E. faecalis* and eight *Streptococcus pneumoniae* strains **identified** among a total of 11 test strains for each microorganism, which include three non-target species strains. It also includes serotyping of the identified *Salmonella* spp. test strains.

Additionally, antimicrobial susceptibility testing of the relevant reference strains for quality control (QC) in relation to antimicrobial susceptibility testing is included. The QC reference strains supplied are: *Escherichia coli* ATCC 25922/CCM 3954, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *Campylobacter jejuni* ATCC 33560/ CCM 6214, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disk diffusion of the enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) and *Streptococcus pneumonia* ATCC 49619/CCM 4501. These reference strains are original CERTIFIED cultures provided free of charge, and should be used 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 identification and antimicrobial susceptibility testing of pathogens, specifically *Salmonella* spp., *E. coli*, *P. aeruginosa*, *C. jejuni* / *C. coli*, *E. faecium* / *E. faecalis* and *S. pneumoniae*. Therefore, the laboratory work for this EQAS should be performed using the methods routinely used in your own laboratory.

3 OUTLINE OF THE EQAS 2021

3.1 Shipping, receipt and storage of strains

In November 2021, it is expected that around 30 laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- 11 test strains of which eight are *Salmonella* spp., in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains (if not already received for EQA1or EQA2).

- 11 test strains of which eight are *E. coli*, in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains (if not already received for EQA1or EQA2).

- 11 test strains of which eight are *P. aeruginosa*, in addition to three non-target species strains. The *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 will be provided as reference strain (if not already received for EQA2).

- 11 test strains of which eight are *C. jejuni* or *C. coli*, in addition to three non-target species strains. The *Campylobacter jejuni* ATCC 33560/ CCM 6214 will be provided as reference strain.

- 11 test strains of which eight are *E. faecium* or *E. faecalis*, in addition to three non-target species strains. The *Staphylococcus aureus* ATCC 25923/CCM 3953 (for disk diffusion - if not already received for EQA2) and *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) will be provided as reference strains.

- 11 test strains of which eight are *S. pneumoniae*, in addition to three non-target species strains. The *Streptococcus pneumonia* ATCC 49619/CCM 4501 will be provided as reference strain.

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

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

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

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

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

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

3.2 Identification of *Salmonella* spp., *E. coli*, *P. aeruginosa*, *C. jejuni / C. coli*, *E. faecium* / *E. faecalis* and *S. pneumoniae* test strains

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

3.3 Serotyping of *Salmonella* spp.

The eight identified *Salmonella* strains should be serotyped by using the method routinely used in your own laboratory. In addition, serogroup results will be evaluated. Therefore, if you do not have all the necessary antisera for 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* spp., *E. coli*, *P. aeruginosa*, *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae* test strains, and of the reference strains

The strains identified as *Salmonella* spp., *E. coli*, *P. aeruginosa*, *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae*, as well as the appropriate reference strains, should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form and in **Tables 1-6**. Note that some of the antimicrobials (highlighted) could be omitted by the Human Health laboratories. Please use the methods routinely used in your own laboratory.

The reference values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 31st Ed.). When not available, EUCAST clinical breakpoints (Tables v. 11.0, 2021) or epidemiological cut off values (https://mic.eucast.org/) are used instead. The breakpoint values for *Salmonella* spp., *E. coli*, *P. aeruginosa*, *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae* can be found in **Tables 1-6**, respectively. **Make sure to use the correct table for the interpretation**.

Interpretation of MIC or disk diffusion results will lead to categorization of the result into one of the categories: **resistant** (R), **intermediate** (I) or **susceptible** (S). In the evaluation report you receive upon the submission deadline, the obtained interpretations in comparison with the expected interpretation will be evaluated/scored as follows:

SCORES		Obtained Interpretation		
		Susceptible	Intermediate	Resistant
Expected Interpretation	Susceptible	4	3	1
	Intermediate	3	4	3
	Resistant	0	3	4

0	Incorrect: very major
1	Incorrect: major
3	Incorrect: minor
4	Correct

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

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	-	≤ 32	≥ 64	-	≥ 15	≤ 14
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.06	0.12-0.5	≥ 1	≥ 31	21-30	≤ 20
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Gentamicin, GEN	-	≤ 8	≥ 16	-	≥ 13	≤ 12
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10

Reference values are based on Enterobacterales breakpoint values from CLSI M100, 31st Ed.

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

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference values MIC (µg/mL)			Reference values Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefotaxime + clavulanic acid, F/C	NA	NA	NA	NA	NA	NA
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Ceftazidime + clavulanic acid, T/C	NA	NA	NA	NA	NA	NA
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.25	0.5	≥ 1	≥ 26	22-25	≤ 21
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Levofloxacin, LEVO	≤ 0.5	1	≥ 2	≥ 21	17-20	≤ 16
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Nalidixic acid, NAL	≤ 16	-	≥ 32	≥ 19	14-18	≤ 13
Piperacillin/tazobactam, PT4	≤ 16/4	32/4-64/4	≥ 128/4	≥ 21	18-20	≤ 17
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11
Tigecycline, TGC*	≤ 0.5	-	≥ 1	≥ 18	-	≤ 17
Tobramycin, TOB	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10
Trimethoprim/sulfamethoxazole, SXT	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10

Reference values are based on Enterobacterales breakpoints from CLSI M100, 31st Ed.

*Reference values are based on Enterobacterales clinical breakpoints from www.eucast.org (Tables v. 11.0, 2021)

Beta-lactam and carbapenem resistance

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

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

- Confirmatory test for ESBL production: it requires the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone, as well as in combination with a β-lactamase inhibitor (clavulanic acid). Synergy can be determined by broth microdilution methods, E-test or Disk 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 Disk 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.

It should be noted that some resistance mechanisms do not always confer clinical resistance. Therefore, the classification of the phenotypic results (**Figure 1** below) should be based on the "EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance", Version 2.0, July 2017, and the most recent EFSA recommendations – 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

1. ESBL-Phenotype	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19
FOT/CLV and/or TAZ/CLV	SYNERGY	SYNERGY

2. AmpC-Phenotype	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19
FOT/CLV and/or TAZ/CLV	NO SYNERGY	NO SYNERGY

3. ESBL + AmpC-Phenotype	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19
FOT/CLV and/or TAZ/CLV	SYNERGY	SYNERGY

4. Carbapenemase-Phenotype	MIC (mg/L)	Zone Diameter (mm)
MERO	> 0.12	< 25

5. Other Phenotypes	MIC (mg/L)	Zone Diameter (mm)
1)		
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19
FOT/CLV and/or TAZ/CLV	NO SYNERGY	NO SYNERGY
2)		
FOT or TAZ	≤ 1	≥ 21 (FOT); ≥ 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19

Susceptible	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	≤ 1	≥ 21 (FOT); ≥ 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19

Figure 1: Adapted from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020 – The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018 – and in accordance with the EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, Version 2.0, July 2017.

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

Table 3. Interpretive criteria for *P. aeruginosa* antimicrobial susceptibility testing

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
Aztreonam, AZT	≤ 8	16	≥ 32	≥ 22	16-21	≤ 15
Cefepime, FEP	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ciprofloxacin, CIP	≤ 0.5	1	≥ 2	≥ 25	19-24	≤ 18
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Imipenem, IMI	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Levofloxacin, LEVO	≤ 1	2	≥ 4	≥ 22	15-21	≤ 14
Meropenem, MERO	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Piperacillin/tazobactam, PT4	≤ 16/4	32/4-64/4	≥ 128/4	≥ 21	15-20	≤ 14
Tobramycin, TOB	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12

Reference values are based on *P. aeruginosa* breakpoints from CLSI M100, 31st Ed.

Table 4. Interpretive criteria for *C. jejuni* / *C. coli* antimicrobial susceptibility testing

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Chloramphenicol, CHL*	≤ 16	-	≥ 32	NA	NA	NA
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 24	21-23	≤ 20
Ertapenem, ETP**	≤ 0.5	-	≥ 1	NA	NA	NA
Erythromycin, ERY	≤ 8	16	≥ 32	≥ 16	13-15	≤ 12
Gentamicin, GEN*	≤ 1	-	≥ 2	≥ 21	-	≤ 20
Tetracycline, TET	≤ 4	8	≥ 16	≥ 26	23-25	≤ 22

Reference values are based on *Campylobacter jejuni/coli* breakpoints from CLSI M45, 3rd Ed.
*Reference values are based on *C. jejuni* and *C. coli* epidemiological cut off values from https://mic.eucast.org/ on September 2021. **Reference values are based on EFSA (European Food Safety Authority) recommendation.

Table 5. Interpretive criteria for *E. faecium* / *E. faecalis* antimicrobial susceptibility testing

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials		Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
		S	I	R	S	I	R
Ampicillin, AMP		≤ 8	-	≥ 16	≥ 17	-	≤ 16
Chloramphenicol, CHL		≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP		≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Daptomycin, DAP	*E. faecium*	-	≤ 4	≥ 8	NA	NA	NA
	E. faecalis	≤ 2	4	≥ 8	NA	NA	NA
Erythromycin, ERY		≤ 0.5	1-4	≥ 8	≥ 23	14-22	≤ 13
Gentamicin, GEN*		≤ 128	-	≥ 256	≥ 8	-	≤ 7
Linezolid, LZD		≤ 2	4	≥ 8	≥ 23	21-22	≤ 20
Quinupristin/dalfopristin, SYN		≤ 1	2	≥ 4	≥ 19	16-18	≤ 15
Teicoplanin, TEI		≤ 8	16	≥ 32	≥ 14	11-13	≤ 10
Tetracycline, TET		≤ 4	8	≥ 16	≥ 19	15-18	≤ 14
Tigecycline, TGC*	*E. faecium*	≤ 0.25	-	≥ 0.5	≥ 22	-	≤ 21
	E. faecalis	≤ 0.25	-	≥ 0.5	≥ 20	-	≤ 19
Vancomycin, VAN		≤ 4	8-16	≥ 32	≥ 17	15-16	≤ 14

Reference values are based on *Enterococcus* spp. breakpoints from CLSI M100, 31st Ed.

*Reference values are based on *Enterococcus* spp. clinical breakpoints from www.eucast.org (Tables v. 11.0, 2021)

Table 6. Interpretive criteria for *S. pneumoniae* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amoxicillin/clavulanic acid, AUG2$_{(nonmeningitis)}$	≤ 2/1	4/2	≥ 8/4	NA	NA	NA
Azithromycin, AZI	≤ 0.5	1	≥ 2	≥ 18	14-17	≤ 13
Cefepime, FEP$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Cefotaxime, FOT$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Ceftriaxone, AXO$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Cefuroxime, FUR$_{(parenteral)}$	≤ 0.5	1	≥ 2	NA	NA	NA
Chloramphenicol, CHL	≤ 4	-	≥ 8	≥ 21	-	≤ 20
Clindamycin, CLI	≤ 0.25	0.5	≥ 1	≥ 19	16-18	≤ 15
Ertapenem, ETP	≤ 1	2	≥ 4	NA	NA	NA
Erythromycin, ERY	≤ 0.25	0.5	≥ 1	≥ 21	16-20	≤ 15
Levofloxacin, LEVO	≤ 2	4	≥ 8	≥ 17	14-16	≤ 13
Linezolid, LZD	≤ 2	-	-	≥ 21	-	-
Meropenem, MERO	≤ 0.25	0.5	≥ 1	NA	NA	NA
Penicillin, PEN$_{(nonmeningitis)}$	≤ 2	4	≥ 8	NA	NA	NA
Tetracycline, TET	≤ 1	2	≥ 4	≥ 28	25-27	≤ 24
Trimethoprim/sulfamethoxazole, SXT	≤ 0.5/9.5	1/19-2/38	≥ 4/76	≥ 19	16-18	≤ 15
Vancomycin, VAN	≤ 1	-	-	≥ 17	-	-

Reference values are based on *S. pneumoniae* breakpoint values from CLSI M100, 31st Ed.

*Reference values are based on *S. pneumoniae* clinical breakpoints from www.eucast.org (Tables v. 11.0, 2021)

4 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the web database. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The web database will allow you to view and print a report with your reported results. The scores for the results will be released after the result submission deadline where you will be able to access the evaluation of your results. Results in agreement with the expected interpretation are categorised as '4' (correct), while results deviating from the expected interpretation are categorised as '3' (incorrect, minor), '1' (incorrect, major) or '0' (incorrect, very major).

Results must be submitted no later than January 5th 2022.

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 submission of results via webtool' 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 2: Reference values (MIC) for the test strains

Appendix 2a: Reference values (MIC values and interpretation) – *Salmonella*

	Amikacin AMK		Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		Cefoxitin FOX		Ceftazidime TAZ		Chloramphenicol CHL	
Salm EQASIA 21.2	≤ 4	I	4	S	8	S	0.25	S	0.5	S	16	I	1	S	≤ 8	S
Salm EQASIA 21.3	≤ 4	I	> 32	R	8	S	0.12	S	8	R	64	R	16	R	64	R
Salm EQASIA 21.4	≤ 4	I	> 32	R	16	S	8	I	64	R	8	S	4	S	> 64	R
Salm EQASIA 21.5	≤ 4	I	> 32	R	8	S	0.5	S	≤ 0.25	S	4	S	1	S	≤ 8	S
Salm EQASIA 21.6	≤ 4	I	> 32	R	64	R	32	R	64	R	4	S	32	R	≤ 8	S
Salm EQASIA 21.7	≤ 4	I	> 32	R	> 64	R	0.12	S	≤ 0.25	S	8	S	0.5	S	≤ 8	S
Salm EQASIA 21.8	≤ 4	I	> 32	R	8	S	16	R	64	R	4	S	4	S	> 64	R
Salm EQASIA 21.11	≤ 4	I	≤ 1	S	4	S	≤ 0.06	S	≤ 0.25	S	2	S	≤ 0.25	S	≤ 8	S

R, Resistant; I, Intermediate; S, Susceptible

	Ciprofloxacin CIP		Colistin COL		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Meropenem MERO		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP	
Salm EQASIA 21.2	0.03	S	4	R	0.03	S	> 16	R	0.25	S	0.06	S	> 512	R	≤ 2	S	≤ 0.25	S
Salm EQASIA 21.3	0.25	I	2	I	0.03	S	≤ 0.5	I	0.25	S	≤ 0.03	S	> 512	R	32	R	> 16	R
Salm EQASIA 21.4	0.5	I	2	I	≤ 0.015	S	16	R	≤ 0.12	S	≤ 0.03	S	> 512	R	> 32	R	> 16	R
Salm EQASIA 21.5	> 8	R	2	I	≤ 0.015	S	> 16	R	0.25	S	≤ 0.03	S	> 512	R	32	R	≤ 0.25	S
Salm EQASIA 21.6	1	R	2	I	≤ 0.015	S	> 16	R	0.25	S	0.06	S	> 512	R	≤ 2	S	> 16	R
Salm EQASIA 21.7	0.03	S	≤ 1	I	≤ 0.015	S	≤ 0.5	I	0.25	S	0.06	S	> 512	R	> 32	R	> 16	R
Salm EQASIA 21.8	0.12	I	≤ 1	I	≤ 0.015	S	> 16	R	0.25	S	0.06	S	> 512	R	> 32	R	≤ 0.25	S
Salm EQASIA 21.11	0.5	I	≤ 1	I	≤ 0.015	S	≤ 0.5	I	0.25	S	≤ 0.03	S	> 512	R	> 32	R	≤ 0.25	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2b: Reference values (MIC values and interpretation) – *E. coli*

	Amikacin AMK		Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		FOT+Cl F/C	Cefoxitin FOX		Ceftazidime TAZ		TAZ+Cl T/C
Ec EQASIA 21.1	≤ 4	S	> 32	R	8	S	32	R	> 64	R	0.12/4	4	S	4	S	0.25/4
Ec EQASIA 21.2	≤ 4	S	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	> 64	R	> 128	R	> 128/4
Ec EQASIA 21.3	8	S	> 32	R	8	S	> 32	R	> 64	R	32/4	> 64	R	> 128	R	32/4
Ec EQASIA 21.5	≤ 4	S	> 32	R	8	S	> 32	R	> 64	R	32/4	> 64	R	> 128	R	32/4
Ec EQASIA 21.7	≤ 4	S	> 32	R	> 64	R	4	I	> 64	R	0.12/4	8	S	2	S	0.25/4
Ec EQASIA 21.8	> 128	R	> 32	R	8	S	> 32	R	> 64	R	> 64/4	> 64	R	> 128	R	> 128/4
Ec EQASIA 21.9	≤ 4	S	8	S	4	S	0.12	S	≤ 0.25	S	0.12/4	8	S	0.5	S	0.25/4
Ec EQASIA 21.11	≤ 4	S	> 32	R	32	R	≤ 0.06	S	≤ 0.25	S	≤ 0.06/4	2	S	≤ 0.25	S	≤ 0.12/4

R, Resistant; I, Intermediate; S, Susceptible

	Chloramphenicol CHL		Ciprofloxacin CIP		Colistin COL		Doripenem DOR		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Levofloxacin LEVO		Meropenem MERO	
Ec EQASIA 21.1	≤ 8	S	≤ 0.015	S	≤ 0.25	I	≤ 0.12	S	0.03	S	≤ 0.5	S	≤ 0.12	S	≤ 1	S	≤ 0.03	S
Ec EQASIA 21.2	16	I	> 8	R	≤ 0.25	I	> 2	R	> 4	R	> 16	R	8	R	> 8	R	> 16	R
Ec EQASIA 21.3	≤ 8	S	> 8	R	≤ 0.25	I	0.5	S	2	R	> 16	R	2	I	> 8	R	0.5	S
Ec EQASIA 21.5	≤ 8	S	> 8	R	0.5	I	0.25	S	4	R	≤ 0.5	S	0.5	S	> 8	R	0.5	S
Ec EQASIA 21.7	> 64	R	> 8	R	4	R	≤ 0.12	S	0.06	S	> 16	R	0.25	S	> 8	R	≤ 0.03	S
Ec EQASIA 21.8	≤ 8	S	> 8	R	≤ 0.25	I	> 2	R	> 4	R	> 16	R	8	R	> 8	R	8	R
Ec EQASIA 21.9	≤ 8	S	0.03	S	≤ 0.25	I	≤ 0.12	S	≤ 0.015	S	1	S	0.25	S	≤ 1	S	≤ 0.03	S
Ec EQASIA 21.11	≤ 8	S	≤ 0.015	S	≤ 0.25	I	≤ 0.12	S	≤ 0.015	S	1	S	0.25	S	≤ 1	S	≤ 0.03	S

R, Resistant; I, Intermediate; S, Susceptible

	Nalidixic acid NAL		Piperacillin/ tazobactam P/T4		Sulfamethoxazole SMX		Tetracycline TET		Tigecycline TGC		Tobramycin TOB		Trimethoprim TMP		Trimethoprim/ sulfamethoxazole SXT	
Ec EQASIA 21.1	≤ 4	S	≤ 8/4	S	≤ 8	S	≤ 2	S	≤ 0.5	S	≤ 1	S	≤ 0.25	S	≤ 0.5/9.5	S
Ec EQASIA 21.2	> 64	R	> 64/4	R	> 512	R	> 32	R	≤ 0.5	S	> 8	R	> 16	R	> 4/76	R
Ec EQASIA 21.3	> 64	R	> 64/4	R	> 512	R	> 32	R	≤ 0.25	S	> 8	R	> 16	R	> 4/76	R
Ec EQASIA 21.5	> 64	R	> 64/4	R	> 512	R	> 32	R	≤ 0.5	S	≤ 1	S	> 16	R	> 4/76	R
Ec EQASIA 21.7	> 64	R	≤ 8/4	S	> 512	R	> 32	R	≤ 0.5	S	8	I	> 16	R	> 4/76	R
Ec EQASIA 21.8	> 64	R	> 64/4	R	> 512	R	≤ 2	S	≤ 0.25	S	> 8	R	≤ 0.25	S	≤ 0.5/9.5	S
Ec EQASIA 21.9	≤ 4	S	≤ 8/4	S	≤ 8	S	≤ 2	S	≤ 0.25	S	≤ 1	S	≤ 0.25	S	≤ 0.5/9.5	S
Ec EQASIA 21.11	≤ 4	S	≤ 8/4	S	> 512	R	≤ 2	S	≤ 0.25	S	≤ 1	S	> 16	R	> 4/76	R

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2c: Reference values (MIC values and interpretation) – *P. aeruginosa*

	Amikacin AMK		Aztreonam AZT		Cefepime FEP		Ceftazidime TAZ		Ciprofloxacin CIP		Colistin COL		Doripenem DOR	
Pa EQASIA 21.1	> 32	R	> 16	R	> 16	R	> 16	R	> 2	R	4	R	> 4	R
Pa EQASIA 21.2	≤ 4	S	4	S	≤ 2	S	≤ 1	S	≤ 0.25	S	1	I	2	S
Pa EQASIA 21.3	≤ 4	S	> 16	R	16	I	> 16	R	1	I	2	I	1	S
Pa EQASIA 21.4	≤ 4	S	4	S	≤ 2	S	≤ 1	S	≤ 0.25	S	0.5	I	≤ 0.12	S
Pa EQASIA 21.5	> 32	R	> 16	R	> 16	R	> 16	R	> 2	R	1	I	> 4	R
Pa EQASIA 21.6	> 32	R	16	I	> 16	R	> 16	R	> 2	R	2	I	4	I
Pa EQASIA 21.9	> 32	R	> 16	R	> 16	R	> 16	R	> 2	R	2	I	> 4	R
Pa EQASIA 21.10	≤ 4	S	16	I	4	S	4	S	0.5	S	0.5	I	1	S

R, Resistant; I, Intermediate; S, Susceptible

	Gentamicin GEN		Imipenem IMI		Levofloxacin LEVO		Meropenem MERO		Piperacillin/tazobactam P/T4		Tobramycin TOB	
Pa EQASIA 21.1	> 8	R	> 8	R	> 8	R	> 8	R	> 64/4	R	> 8	R
Pa EQASIA 21.2	≤ 1	S	8	R	≤ 1	S	4	I	≤ 8/4	S	≤ 1	S
Pa EQASIA 21.3	2	S	≤ 1	S	2	I	≤ 1	S	> 64/4	R	≤ 1	S
Pa EQASIA 21.4	2	S	≤ 1	S	≤ 1	S	≤ 1	S	≤ 8/4	S	≤ 1	S
Pa EQASIA 21.5	> 8	R	> 8	R	> 8	R	> 8	R	> 64/4	R	> 8	R
Pa EQASIA 21.6	> 8	R	2	S	> 8	R	4	I	16/4	S	> 8	R
Pa EQASIA 21.9	> 8	R	> 8	R	> 8	R	> 8	R	> 64/4	R	> 8	R
Pa EQASIA 21.10	2	S	≤ 1	S	2	I	2	S	32/4	I	≤ 1	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2d: Reference values (MIC values and interpretation) – *Campylobacter*

	Chloramphenicol CHL		Ciprofloxacin CIP		Ertapenem ETP		Erythromycin ERY		Gentamicin GEN		Tetracycline TET	
Campy EQASIA 21.1 – *C. coli*	8	S	≤ 0.12	S	≤ 0.12	S	2	S	0.5	S	≤ 0.5	S
Campy EQASIA 21.3 – *C. coli*	4	S	8	R	1	R	≤ 1	S	0.5	S	≤ 0.5	S
Campy EQASIA 21.5 – *C. coli*	4	S	≤ 0.12	S	≤ 0.12	S	> 512	R	≤ 0.25	S	≤ 0.5	S
Campy EQASIA 21.6 – *C. jejuni*	≤ 2	S	4	R	≤ 0.12	S	≤ 1	S	≤ 0.25	S	64	R
Campy EQASIA 21.7 – *C. jejuni*	32	R	32	R	2	R	512	R	≤ 0.25	S	32	R
Campy EQASIA 21.9 – *C. coli*	8	S	32	R	4	R	4	S	0.5	S	0.5	S
Campy EQASIA 21.10 – *C. coli*	8	S	0.25	S	≤ 0.12	S	2	S	0.5	S	1	S
Campy EQASIA 21.11 – *C. jejuni*	≤ 2	S	≤ 0.12	S	≤ 0.12	S	≤ 1	S	≤ 0.25	S	64	R

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2e: Reference values (MIC values and interpretation) – *Enterococci*

	Ampicillin AMP		Chloramphenicol CHL		Ciprofloxacin CIP		Daptomycin DAP		Erythromycin ERY		Gentamicin GEN	
Ef EQASIA 21.1 – *E. faecalis*	2	S	16	I	> 16	R	0.5	S	> 128	R	> 1024	R
Ef EQASIA 21.2 – *E. faecalis*	1	S	≤ 4	S	> 16	R	1	S	> 128	R	≤ 8	S
Ef EQASIA 21.4 – *E. faecium*	4	S	≤ 4	S	0.5	S	2	I	8	R	≤ 8	S
Ef EQASIA 21.5 – *E. faecium*	> 64	R	≤ 4	S	> 16	R	0.5	I	> 128	R	≤ 8	S
Ef EQASIA 21.7 – *E. faecalis*	1	S	64	R	> 16	R	4	I	> 128	R	> 1024	R
Ef EQASIA 21.8 – *E. faecalis*	≤ 0.5	S	8	S	1	S	2	S	> 128	R	≤ 8	S
Ef EQASIA 21.10 – *E. faecium*	> 64	R	≤ 4	S	> 16	R	2	I	> 128	R	1024	R
Ef EQASIA 21.11 – *E. faecium*	> 64	R	≤ 4	S	> 16	R	8	R	> 128	R	≤ 8	S

R, Resistant; I, Intermediate; S, Susceptible

	Linezolid LZD		Quinupristin/dalfopristin SYN		Teicoplanin TEI		Tetracycline TET		Tigecycline TGC		Vancomycin VAN	
Ef EQASIA 21.1 – *E. faecalis*	1	S	8	R	8	S	64	R	0.25	S	128	R
Ef EQASIA 21.2 – *E. faecalis*	1	S	8	R	≤ 0.5	S	128	R	0.12	S	≤ 1	S
Ef EQASIA 21.4 – *E. faecium*	1	S	4	R	1	S	≤ 1	S	0.06	S	≤ 1	S
Ef EQASIA 21.5 – *E. faecium*	2	S	1	S	8	S	≤ 1	S	0.12	S	> 128	R
Ef EQASIA 21.7 – *E. faecalis*	1	S	8	R	≤ 0.5	S	64	R	0.25	S	64	R
Ef EQASIA 21.8 – *E. faecalis*	1	S	16	R	≤ 0.5	S	64	R	0.12	S	16	I
Ef EQASIA 21.10 – *E. faecium*	1	S	1	S	64	R	64	R	0.12	S	> 128	R
Ef EQASIA 21.11 – *E. faecium*	2	S	1	S	1	S	128	R	0.12	S	> 128	R

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2f: Reference values (MIC values and interpretation) – *S. pneumoniae*

	Amoxicillin/clavulanic acid AUG2		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		Ceftriaxone AXO		Cefuroxime FUR		Chloramphenicol CHL		Clindamycin CLI		Ertapenem ETP	
Sp EQASIA 21.1	≤ 2/1	S	≤ 0.25	S	≤ 0.5	S	0.25	S	0.25	S	≤ 0.5	S	4	S	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 21.2	≤ 2/1	S	≤ 0.25	S	2	I	2	I	2	I	> 4	R	4	S	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 21.3	≤ 2/1	S	> 2	R	1	S	1	S	1	S	4	R	4	S	> 1	R	≤ 0.5	S
Sp EQASIA 21.6	≤ 2/1	S	> 2	R	2	I	2	I	2	I	> 4	R	16	R	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 21.7	≤ 2/1	S	≤ 0.25	S	≤ 0.5	S	≤ 0.12	S	≤ 0.12	S	≤ 0.5	S	2	S	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 21.8	≤ 2/1	S	> 2	R	2	I	1	S	1	S	> 4	R	4	S	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 21.10	≤ 2/1	S	> 2	R	≤ 0.5	S	≤ 0.12	S	≤ 0.12	S	≤ 0.5	S	4	S	> 1	R	≤ 0.5	S
Sp EQASIA 21.11	≤ 2/1	S	2	R	≤ 0.5	S	≤ 0.12	S	0.25	S	≤ 0.5	S	4	S	≤ 0.12	S	≤ 0.5	S

R, Resistant; I, Intermediate; S, Susceptible

	Erythromycin ERY		Levofloxacin LEVO		Linezolid LZD		Meropenem MERO		Penicillin PEN		Tetracycline TET		Trimethoprim/sulfamethoxazole SXT		Vancomycin VAN	
Sp EQASIA 21.1	≤ 0.25	S	1	S	1	S	≤ 0.25	S	0.5	S	≤ 1	S	> 4/76	R	≤ 0.5	S
Sp EQASIA 21.2	≤ 0.25	S	1	S	1	S	0.5	I	4	I	≤ 1	S	> 4/76	R	≤ 0.5	S
Sp EQASIA 21.3	> 2	R	1	S	1	S	≤ 0.25	S	0.5	S	> 8	R	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 21.6	2	R	1	S	1	S	0.5	I	4	I	> 8	R	4/76	R	≤ 0.5	S
Sp EQASIA 21.7	≤ 0.25	S	2	S	0.5	S	≤ 0.25	S	≤ 0.03	S	≤ 1	S	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 21.8	> 2	R	1	S	1	S	0.5	I	2	S	> 8	R	4/76	R	≤ 0.5	S
Sp EQASIA 21.10	> 2	R	1	S	0.5	S	≤ 0.25	S	0.12	S	> 8	R	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 21.11	2	R	2	S	1	S	≤ 0.25	S	0.25	S	≤ 1	S	≤ 0.5/9.5	S	≤ 0.5	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 3: Quality control ranges for the reference strains

Appendix 3a: Quality control ranges for *E. coli* ATCC 25922 and *E. coli* NCTC 13846

E. coli ATCC 25922		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amikacin, AMK	0.5-4	19-26
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 and clavulanic acid, F/C	--	--
Cefoxitin, FOX	2-8	23-29
Ceftazidime, TAZ	0.06-0.5	25-32
Ceftazidime and clavulanic acid, T/C	--	--
Chloramphenicol, CHL	2-8	21-27
Ciprofloxacin, CIP	0.004-0.016	29-38
Doripenem, DOR	0.016-0.06	27-35
Ertapenem, ETP	0.004-0.016	29-36
Gentamicin, GEN	0.25-1	19-26
Imipenem, IMI	0.06-0.25	26-32
Levofloxacin, LEVO	0.008-0.06	29-37
Meropenem, MERO	0.008-0.06	28-35
Nalidixic acid, NAL	1-4	22-28
Piperacillin and tazobactam, P/T4	1-4	24-30
Sulfamethoxazole, SMX	8-32	15-23
Tetracycline, TET	0.5-2	18-25
Tigecycline, TGC	0.03-0.25	20-27
Tobramycin, TOB	0.25-1	18-26
Trimethoprim, TMP	0.5-2	21-28
Trimethoprim and sulfamethoxazole, SXT	≤ 0.5	23-29

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.”

Appendix 3b: Quality control ranges for *P. aeruginosa* ATCC 27853

P. aeruginosa ATCC 27853		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amikacin, AMK	1-4	20-26
Aztreonam, AZT	2-8	23-29
Cefepime, FEP	0.5-4	25-31
Ceftazidime, TAZ	1-4	22-29
Ciprofloxacin, CIP	0.12-1	25-33
Colistin, COL	0.5-4	--
Doripenem, DOR	0.12-0.5	28-35
Gentamicin, GEN	0.5-2	17-23
Imipenem, IMI	1-4	20-28
Levofloxacin, LEVO	0.5-4	19-26
Meropenem, MERO	0.12-1	27-33
Piperacillin and tazobactam, P/T4	1-8	25-33
Tobramycin, TOB	0.25-1	20-26

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

Appendix 3c: Quality control ranges for *C. jejuni* ATCC 33560

C. jejuni ATCC 33560 - **36-37°C/48h**	
Antimicrobial	**MIC (mg/L)**
Chloramphenicol, CHL	1-8
Ciprofloxacin, CIP	0.06-0.25
Ertapenem, ETP	--
Erythromycin, ERY	0.5-2
Gentamicin, GEN	0.5-2
Tetracycline, TET	0.25-2

MIC ranges are according to CLSI VET06 1st edition, Table 21C

C. jejuni ATCC 33560 - **42°C/24h**		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Chloramphenicol, CHL	1-4	--
Ciprofloxacin, CIP	0.03-0.12	32-45
Ertapenem, ETP	--	--
Erythromycin, ERY	0.25-2	26-38
Gentamicin, GEN	0.25-2	--
Tetracycline, TET	0.25-1	--

MIC ranges and disk diffusion ranges are according to CLSI VET06 1st edition, Tables 21A and 21C

Appendix 3d: Quality control ranges for *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213

	E. faecalis ATCC 29212	*S. aureus* ATCC 25923
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Ampicillin, AMP	0.5-2	27-35
Chloramphenicol, CHL	4-16	19-26
Ciprofloxacin, CIP	0.25-2	22-30
Daptomycin, DAP	1-4	--
Erythromycin, ERY	1-4	22-30
Gentamicin, GEN	4-16	19-27
Linezolid, LZD	1-4	25-32
Quinupristin and dalfopristin, SYN	2-8	21-28
Teicoplanin, TEI	0.25-1	15-21
Tetracycline, TET	8-32	24-30
Tigecycline, TGC	0.03-0.12	20-25
Vancomycin, VAN	1-4	17-21

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

Appendix 3e: Quality control ranges for *S. pneumoniae* ATCC 49619

S. pneumoniae ATCC 49619		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amoxicillin and clavulanic acid, AUG2	0.03-0.12	--
Azithromycin, AZI	0.06-0.25	19-25
Cefepime, FEP	0.03-0.25	28-35
Cefotaxime, FOT	0.03-0.12	31-39
Ceftriaxone, AXO	0.03-0.12	30-35
Cefuroxime, FUR	0.25-1	--
Chloramphenicol, CHL	2-8	23-27
Clindamycin, CLI	0.03-0.12	19-25
Ertapenem, ETP	0.03-0.25	28-35
Erythromycin, ERY	0.03-0.12	25-30
Levofloxacin, LEVO	0.5-2	20-25
Linezolid, LZD	0.25-2	25-34
Meropenem, MERO	0.03-0.25	28-35
Penicillin, PEN	0.25-1	24-30
Tetracycline, TET	0.06-0.5	27-31
Trimethoprim and sulfamethoxazole, SXT	0.12-1	20-28
Vancomycin, VAN	0.12-0.5	20-27

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

National Food Institute
Technical University of Denmark
Building 202
Kemitorvet
2800 Lyngby
Denmark

Tel: +45 35 88 70 00

www.food.dtu.dk

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