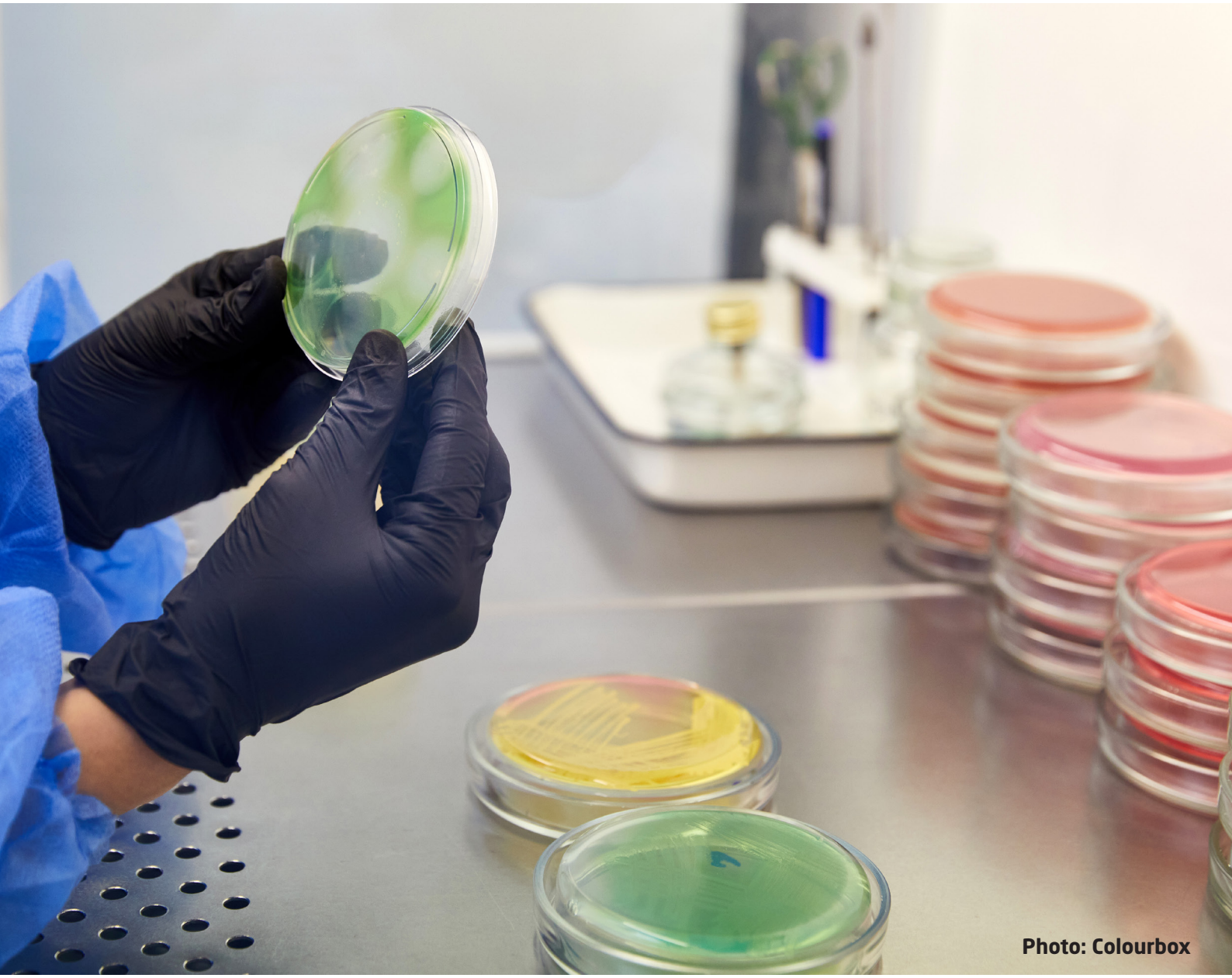
The 5th EQAsia External Quality Assessment trial: Campylobacter jejuni / C. coli, Enterococcus faecium / E. faecalis and Streptococcus pneumoniae - 2022



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The 5th EQAsia External Quality Assessment trial: *Campylobacter jejuni* / *C. coli, Enterococcus faecium*/ *E. faecalis* and *Streptococcus pneumoniae* – 2022

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Executive Summary

This report summarizes the results of the 5th EQA trial of EQAsia, a Fleming Fund Regional Grant 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 has been granted a 2nd phase (2023 to 2025) to continue to deliver the established EQA for both the Human Health (HH sector) and Food and Animal Health (AH sector) laboratories in the region.

The trial was carried out in September-November 2022 and included bacterial identification and antimicrobial susceptibility testing (AST) of *Campylobacter* (*C. jejuni* and *C. coli*), *Enterococcus* (*E. faecium* and *E. faecalis*) and *Streptococcus pneumoniae*.

A total of 15 HH and six AH laboratories participated and submitted results for the EQA, corresponding to 19 participating laboratories in the *E. faecium/ E. faecalis* trial, 17 for the *S. pneumoniae* trial, and three for the *C. jejuni/ C. coli* trial. These laboratories are from 12 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka and Timor-Leste).

The bacterial identification component consisted in identifying the five strains of the organism in question (target organism) among a total of seven strains. Only three AH laboratories participated and submitted results for the bacterial identification component of the *C. jejuni/ C. coli* trial, which revealed no issues with correctly identifying the tested strains. For the *E. faecium/ E. faecalis* trial, misidentification of the non-*E. faecium/ E. faecalis* strains was the major contributor for the deviations observed in this component, whereas misidentification of the *S. pneumoniae* strains was generally problematic, especially among the AH laboratories.

C. jejuni/ C. coli strains were only tested by two laboratories, where ciprofloxacin and gentamicin presented quite high deviations.

E. faecium/ E. faecalis AST results revealed that apart from few exceptions, the majority of the laboratories are proficient at testing ampicillin, teicoplanin and tetracycline, as well as chloramphenicol, linezolid and tigecycline (median deviation of 0%, with some outliers and dispersed deviations). On the contrary, the median deviation was ≥ 10% for gentamicin and daptomycin, the latter tested by only four laboratories, which presented varying deviations.

In the *S. pneumoniae* trial, amoxicillin/clavulanic acid, ertapenem, levofloxacin and linezolid, as well as tetracycline and vancomycin had median deviations of 0% (the last two antimicrobials with a few outliers), whereas azithromycin, cefotaxime, ceftriaxone, chloramphenicol, clindamycin, erythromycin, penicillin and trimethoprim/sulfamethoxazole generated higher and more dispersed deviations. Cefepime, cefuroxime and meropenem were tested by less than five laboratories.

In general, the median deviation was below the acceptance level of 5% deviation from expected results in the *S. pneumoniae* trial, whereas the *E. faecium/ E. faecalis* trial presented a median deviation close to 10%. For the *C. jejuni/ C. coli* trial, the two laboratories that performed AST presented deviations of 0 and 25.0%. It is noticeable that the deviations observed for the trials are disperse, suggesting that the level of proficiency varies among the participating laboratories.

The two participating laboratories in the *C. jejuni/ C. coli* trial submitted results concerning the reference strain, 16 laboratories for the *E. faecium/ E. faecalis* trial, and 13 laboratories for the *S. pneumoniae* trial, meaning that in the *E. faecium/ E. faecalis* trial, results from three laboratories were missing (two HH laboratories did not submit results and results from one AH

laboratory could not be assessed), and four laboratories, two from each sector, did not submit results for the reference strain in the *S. pneumoniae* trial. None of the participants in the *C. jejuni/ C. coli* trial reported deviations from the expected results. For the *E. faecium/ E. faecalis* trial the median deviation was 0%, whereas the median deviation for the *S. pneumoniae* trial was above 15%, with quite disperse deviations in both of the trials.

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]. EQAsia has been granted a 2nd phase to continue to deliver the established EQA for both the Human Health (HH) sector and Food and Animal Health (AH) sector in the region from 2023 to 2025.

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. As mentioned, the EQA trials have focused 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*), *Enterococcus* (*E. faecium* and *E. faecalis*) and *Streptococcus pneumoniae*. In addition, a Matrix EQA trial is offered twice (one in each year), consisting of a complex food sample spiked with AmpC beta-lactamases (AmpC), extended-spectrum beta-lactamases (ESBLs) or carbapenemase-producing *E. coli* for surveillance purposes. The aim is to align with the scope of WHO Tricycle and suggested by FAO, to assess the veterinary laboratories' ability to detect multi-resistant bacteria 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 fifth EQA trial of the EQAsia project (EQA5) carried out in September-November 2022. The trial encompasses the testing of a total of seven test strains of a given organism. Of these, five of the test strains are of the organism in question (target organism), whereas two test strains are different from the targeted species (reported as non-[organism], e.g., non-*S. pneumoniae*). For each of the seven test strains, participants are requested to report which five strains belong to the expected target organism. For the two organisms different from the expected, no further testing is required. For the remaining five test strains of the target organism, results in relation to AST are requested.

This fifth EQA trial includes identification and AST of *Campylobacter* (*C. jejuni* and *C. coli*), *Enterococcus* (*E. faecium* and *E. faecalis*) and

S. 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 Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Interpretative criteria referring to both disk diffusion and MIC determination are listed in the EQA5 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 EQA5 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 root 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 ± 3 mm, respectively. If the expected MIC / Zone Diameter is close to the threshold for categorising the strain as susceptible, intermediate or resistant, a one-fold dilution / ± 3 mm difference may result in different interpretations. As 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 / ± 3 mm 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 HH or 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), Monica Lahra (WHO Collaborating Centre for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia) and Russel Cole (Pacific Pathology Training Centre, New Zealand).

2. Materials and Methods

2.1 Participants in EQAsia EQA5

A total of 21 laboratories participated in the fifth EQA trial of the EQAsia project: 15 laboratories belonging to the HH Sector and six belonging to the AH Sector, originating from: Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka and Timor-Leste (**Figure 1**).

2.2 Strains

Participating laboratories could register for any of the trials. For each registration, the laboratory received seven bacterial strains of which only

five 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 five 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 EQA5, 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). Expected MIC values (**Appendix 2a-c**) of the selected strains for this EQA were further confirmed by NIH (*Enterococcus* and *S. pneumoniae*) and CUVET (*Campylobacter*).

The reference strains *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 3a-c**) were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-32nd Ed., tables 4A-1 and 5A-1 [3], and in document VET06-1st Ed., tables 21A, 21B and 21C [4].

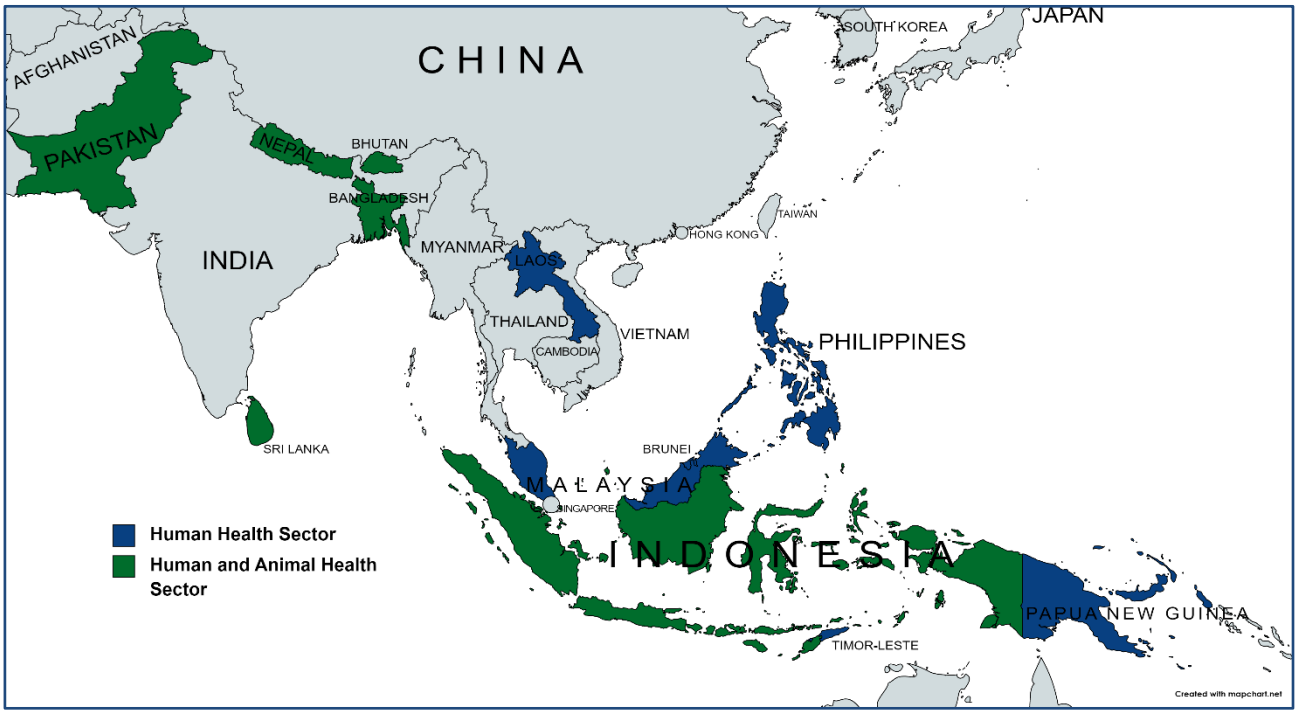

Figure 1: Countries participating in the 5th EQA of the EQAsia project on antimicrobial susceptibility testing. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all three organisms are listed in the EQA5 protocol (**Appendix 1**) and summarized in **Table 1**. These antimicrobials correspond to several antimicrobial class representatives important for surveillance.

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,

32nd Ed. and VET06, 1st Ed.) [3, 4]. When not available, EUCAST clinical breakpoints (Tables v. 12.0, 2022) [5] or epidemiological cut off values [6] were used instead.

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

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA5 2022.

Campylobacter	*Enterococcus*	*S. pneumoniae*
Chloramphenicol (CHL)	Ampicillin (AMP)	Amoxicillin/clavulanic acid (AUG2)
Ciprofloxacin (CIP)	Chloramphenicol (CHL)	Azithromycin (AZI)
Ertapenem (ETP)	Ciprofloxacin (CIP)	Cefepime (FEP)
Erythromycin (ERY)	Daptomycin (DAP)	Cefotaxime (FOT)
Gentamicin (GEN)	Erythromycin (ERY)	Ceftriaxone (AXO)
Tetracycline (TET)	Gentamicin (GEN)	Cefuroxime (FUR)
	Linezolid (LZD)	Chloramphenicol (CHL)
	Quinupristin/dalfopristin (SYN)	Clindamycin (CLI)
	Teicoplanin (TEI)	Ertapenem (ETP)
	Tetracycline (TET)	Erythromycin (ERY)
	Tigecycline (TGC)	Levofloxacin (LEVO)
	Vancomycin (VAN)	Linezolid (LZD)
		Meropenem (MERO)
		Penicillin (PEN)
		Tetracycline (TET)
		Trimethoprim/sulfamethoxazole (SXT)
		Vancomycin (VAN)

2.4 Distribution

The bacterial strains were dispatched as lyophilized strains in September 2022 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 [7], 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.

Laboratories used procedures such as disk diffusion, gradient test, agar dilution and broth dilution. 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.

2.6 Data management

In past EQA trials, antimicrobial susceptibility

testing of some of the reference strains revealed a number of incorrect results outside the acceptance interval for MIC determination. This is due to the use of automated instruments, which often test 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 (automated)' have previously reported an MIC ≤ 1. As this is a method limitation and the laboratories cannot test for lower antimicrobial concentrations, the informatics module was adapted to score these specific occurrences as '1' (correct). **Table 2** contains the only situation where this adaptation in the informatics module was applied in this trial.

Table 2. Adjusted scores for reported MIC values for *S. pneumoniae* ATCC 49619 reference strain. Adjustments were made due to the limitation of the broth microdilution (automated) method applied.

***S. pneumoniae* ATCC 49619**		
Antimicrobial	**MIC Quality Control Range**	**MIC reported**
Clindamycin	0.03-0.12	≤ 0.25

3. Results – Human Health Laboratories

3.1 Overall participation

The 15 Human Health laboratories participating in the 5th EQA of the EQAsia Programme submitted results for the *E. faecium / E. faecalis* trial and 14 for the *S. pneumoniae* trial, whereas no laboratories submitted results for the *C. jejuni / C. coli* trial (**Figure 2**). Regarding the methodologies applied by the laboratories, most of the participants opted for disk diffusion alone, followed by the use of broth microdilution (automated) or a mixture of the two methodologies. The remaining laboratories applied disk diffusion in combination with other methodologies, such as gradient test, broth microdilution (conventional) and agar dilution (**Figure 2**).

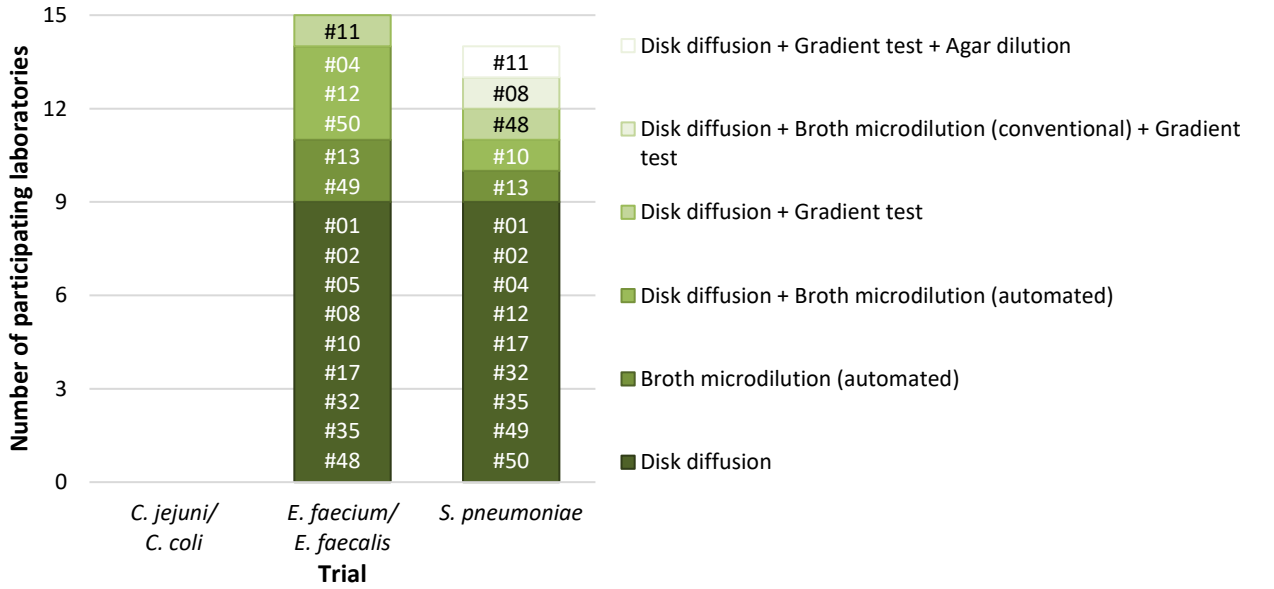

Figure 2. Methodologies applied by the HH laboratories participating in each of the trials. The numbers represent the laboratory identification number (i.e. #01).

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 drugs (**Table 1**). As mentioned above, no HH laboratories submitted results for the *C. jejuni/ C. coli* trial. For the *E. faecium / E. faecalis* trial, ampicillin, chloramphenicol, ciprofloxacin, erythromycin, tetracycline and vancomycin were tested by at least 80% of the participating laboratories (**Table 3**). In contrast, daptomycin, gentamicin, quinupristin/dalfopristin and tigecycline were tested by less than half of the laboratories (**Table 3**). For the *S. pneumoniae* trial, the most tested antimicrobials were azithromycin, chloramphenicol, erythromycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin (more than 80% of the laboratories), whereas amoxicillin/ clavulanic acid, cefepime, cefuroxime, ertapenem and meropenem were chosen for testing by less than 30% of the laboratories (**Table 3**).

Scattering of missing data or incomplete AST results entries were observed in the two trials (**Tables 4 and 5**). Four of the 15 laboratories selecting *E. faecium/ E. faecalis* did not submit complete results: laboratory #02 did not report results for ampicillin for strain Ef EQAsia 22.4 and laboratory #13 for linezolid for strain Ef EQAsia 22.1; laboratories #04 and #49 also missed to report results for gentamicin for strain Ef EQAsia 22.2 and strains Ef EQAsia 22.6 and Ef EQAsia 22.7, respectively (**Table 4**). In the case of laboratory #49, the laboratory reported MIC values, but no interpretation was submitted. As only the categorisation as R, I or S is evaluated, the results for these antimicrobials could not be scored.

Regarding the *S. pneumoniae* trial, two out of the 14 participating laboratories revealed incomplete results of their own available antimicrobial agents (**Table 5**). Laboratory #02 did not report trimethoprim/sulfamethoxazole results for three of the five strains, and laboratory #13 for cefuroxime (strain Sp EQAsia 22.5) and for chloramphenicol (strains Sp EQAsia 22.1 and Sp EQAsia 22.4).

Table 3. Antimicrobial agents tested by the HH laboratories for each trial. For a given trial (Ef, Sp), the number of participating laboratories that tested each antimicrobial is shown (n), as well as the percentage (%) of laboratories out of the total number of participating laboratories (N) for the trial (% of n/N). The antimicrobials not included in a given trial are represented as --.

Antimicrobial	Laboratories in total: n (% of n/N)	
	Ef	Sp
AMP	15 (100.0)	--
AUG2	--	3 (21.4)
AZI	--	12 (85.7)
FEP	--	3 (21.4)
FOT	--	7 (50.0)
AXO	--	8 (57.1)
FUR	--	2 (14.3)
CHL	13 (86.7)	12 (85.7)
CIP	12 (80.0)	--
CLI	--	10 (71.4)
DAP	3 (20.0)	--
ETP	--	3 (21.4)
ERY	12 (80.0)	13 (92.9)
GEN	7 (46.7)	--
LEVO	--	9 (64.3)
LZD	11 (73.3)	8 (57.1)
MERO	--	4 (28.6)
PEN	--	8 (57.1)
SYN	4 (26.7)	--
TEI	8 (53.3)	--
TET	12 (80.0)	13 (92.9)
TGC	6 (40.0)	--
SXT	--	12 (85.7)
VAN	14 (93.3)	13 (92.9)
Total (N)	**15**	**14**

Ef, *E. faecium/ E. faecalis*; Sp, *S. pneumoniae*
(n) number of laboratories that reported results for the antimicrobial; (N) total number of participating laboratories for the trial

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by HH laboratories (n=15) participating in the 5th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 22.1	Ef EQAsia 22.2	Ef EQAsia 22.4	Ef EQAsia 22.6	Ef EQAsia 22.7
#02	--	--	AMP	--	--
#04	--	GEN	--	--	
#13	LZD	--	--	--	--
#49	--	--	--	GEN	GEN

Ef, *E. faecium/ E. faecalis*; blue shade, strain not tested

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *S. pneumoniae* strains reported by HH laboratories (n=14) participating in the 5th EQA of the EQAsia project.

Lab ID No.	Sp EQAsia 22.1	Sp EQAsia 22.3	Sp EQAsia 22.4	Sp EQAsia 22.5	Sp EQAsia 22.6
#02	SXT	SXT	SXT	--	--
#13	CHL	--	CHL	FUR	--

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

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

A total of 15 laboratories from 12 countries uploaded results for the *E. faecium/ E. faecalis* trial.

3.2.1 Bacterial identification

Eight out of 15 participating laboratories correctly identified the five *E. faecium/ E. faecalis* strains and the two non-*E. faecium/ E. faecalis*. Among the five *E. faecium/ E. faecalis* strains, two were *E. faecium* and the other three were *E. faecalis* (**Table 6**).

Table 6. Bacterial identification of each of the seven 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 22.1	*E. faecalis*	15/15
Ef EQAsia 22.2	*E. faecium*	14/15
Ef EQAsia 22.3	Non-*E. faecium/ E. faecalis* (*E. gallinarum*)	13/15
Ef EQAsia 22.4	*E. faecium*	13/15
Ef EQAsia 22.5	Non-*E. faecium/ E. faecalis* (*E. casseliflavus*)	9/15
Ef EQAsia 22.6	*E. faecalis*	14/15
Ef EQAsia 22.7	*E. faecalis*	14/15

Ef, *E. faecium/ E. faecalis*

The *E. faecium* strains Ef EQAsia 22.2 and Ef EQAsia 22.4 were both incorrectly identified as *E. faecalis* by laboratory #32, and the latter was also misidentified as non-*E. faecium/ E. faecalis* by laboratory #01.

The *E. faecalis* strain Ef EQAsia 22.1 was correctly identified by all participating laboratories; the two other *E. faecalis* strains, Ef EQAsia 22.6 and Ef EQAsia 22.7, were considered as non-*E. faecium/ E. faecalis* by laboratory #32 and laboratory #04, respectively.

Regarding the non-*E. faecium/ E. faecalis* strains, Ef EQAsia 22.3 (*E. gallinarum*) was identified as *E. faecium* by laboratory #48, and as *E. faecalis* by laboratory #32; strain Ef EQAsia 22.5 (*E. casseliflavus*) generated the highest number of incorrect results: laboratory #32 identified it as *E. faecalis* whereas five other laboratories (#01, #02, #05, #35 and #48) identified it as *E. faecium*.

3.3.2 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 86.0% (strain Ef EQAsia 22.6) to 97.1% (strain Ef EQAsia 22.2) for each strain (**Table 7**). The AST results submitted for the five *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 highest deviation was seen for strain Ef EQAsia 22.6 (14.0%) and was caused by several instances of results' misinterpretation of the obtained results for chloramphenicol, ciprofloxacin and erythromycin. Strains Ef EQAsia 22.4 and Ef EQAsia 22.7 also presented quite high deviations (close to 10%) that resulted from several incorrect results reported by laboratory #50.

Table 7. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 15 HH laboratories for the *E. faecium /E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQAsia 22.1	116	92.5
Ef EQAsia 22.2	113	97.1
Ef EQAsia 22.4	105	90.7
Ef EQAsia 22.6	114	86.0
Ef EQAsia 22.7	104	90.9

Ef, *E. faecium/ E. faecalis*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were daptomycin (43.8%), tigecycline (25.9%) and gentamicin (23.4%), whereas teicoplanin revealed no deviation from the expected results (**Figure 3**).

Daptomycin was tested by only three laboratories (#04, #12 and #49) and could only be scored for three of the strains (**Appendix 2b**), which resulted in a total of only eight tests performed towards this antimicrobial. The three laboratories reported strain Ef EQAsia 22.6, as susceptible to the drug, even though it was expected to be resistant, resulting in the highest score penalty possible (score of 0).

Tigecycline's deviation was mostly caused by the results submitted by laboratories #17 and #50, which reported all five strains and three of the strains, respectively, as resistant to the drug, even though all were expected to be susceptible.

Regarding gentamicin, the majority of the incorrect results were observed for strain Ef EQAsia 22.6, which some laboratories reported as susceptible when resistant was expected.

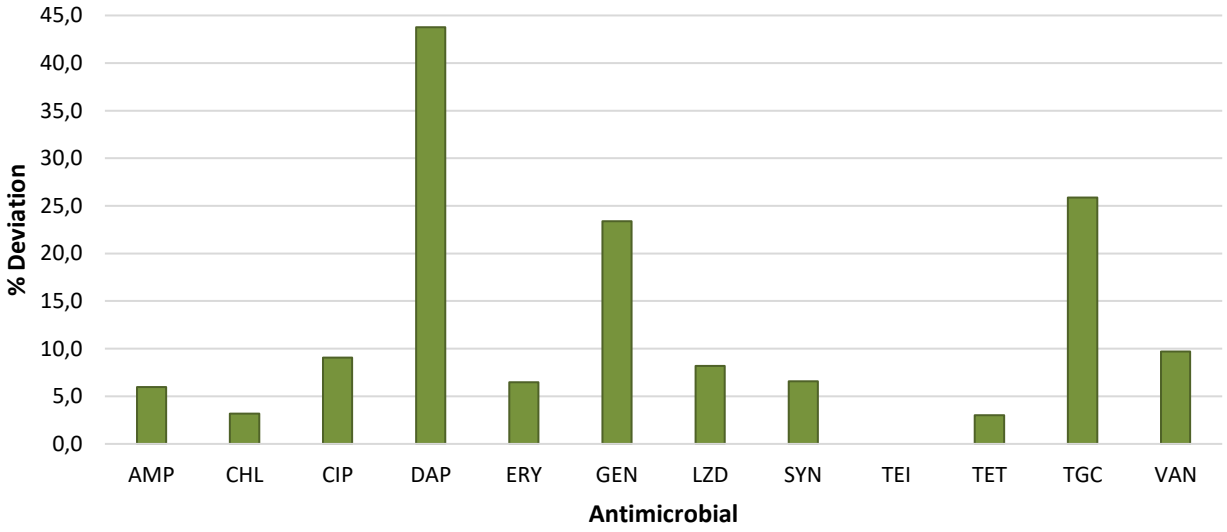

Figure 3. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by HH laboratories (n=15) participating in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

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 for six of the 15 participants (**Figure 4**). In average, the deviation was 10.4% (ranging from 1.3 to 34.4%). As the acceptance level was set to 5% deviation, nine laboratories did not perform within the expected range for the *E. faecium/ E. faecalis* trial (**Figure 4**).

Laboratory #32 presented the highest deviation observed for this trial. This laboratory submitted results for ampicillin and vancomycin only, resulting in a total of eight antibiotic discs tested (four strains). Half of those results were not in accordance with the expected outcome,

resulting in penalties (score of 0, 1 or 3 instead of 4) and in the observed deviation. Similarly, laboratory #02 only submitted results for the same two antimicrobials and reported incorrect results for vancomycin.

Laboratory #50, as mentioned above in subsection '*Strain-based analysis*' reported several incorrect results for strains Ef EQAsia 22.4 and Ef EQAsia 22.7, which is the main contributor for the deviation observed for this participant; laboratory #48 owes its deviation to the incorrect results reported for linezolid for all five strains (resistant or intermediate instead of susceptible); likewise, laboratory #17 reported incorrect results for tigecycline for all five strains (resistant instead of susceptible).

The remaining laboratories with deviations above 5% presented dispersed incorrect results, not necessarily related to a specific antimicrobial or strain.

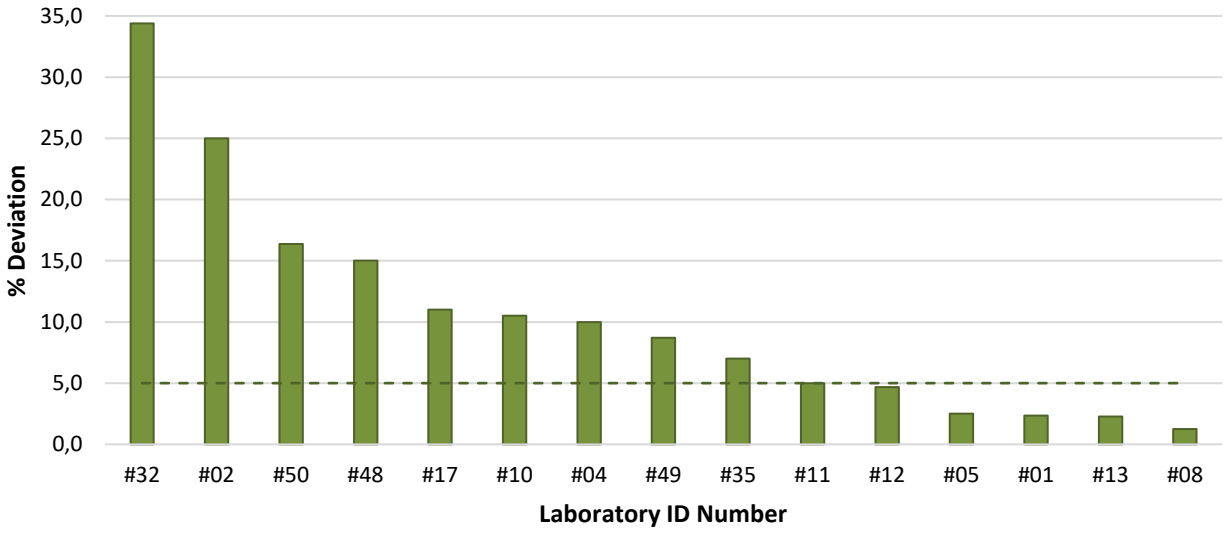

Figure 4. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by HH laboratories (n=15) participating in the 5th EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 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 15 participating laboratories, 13 submitted results for the reference strains. Different methodologies for testing the reference strain *E. faecalis* ATCC 29212 were applied: MIC was determined by either gradient test or broth microdilution (**Table 8**, **). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine Inhibition Zone Diameters by disk diffusion (**Table 8**, *).

The highest proportion of test results outside of the expected range was observed for gentamicin (5 out of 9) (**Table 8**). Considering the overall performance, eight laboratories (#01, #08, #11, #12, #17, #35, #48 and #49) presented no deviation; of those, laboratories #01, #08, #17, #35 and #48 applied disk diffusion and laboratories #12 and #49 applied broth microdilution (automated) as the sole method, whereas laboratory #11 applied a mixture of disk diffusion and gradient test.

The remaining five laboratories (#04, #50, #10, #02 and #13) presented deviations that ranged from 10.0% to 77.8% (**Figure 5**). Laboratories #04 and #50 presented the same number of

deviations (n=1), but laboratory #50 tested fewer antimicrobials and thus, the deviation observed was higher (**Figure 5**); in both cases, the MIC value reported for gentamicin was below the expected interval. Laboratories #10 and #02 had two and three deviations each, respectively, some above the acceptance interval and others below. Lastly, laboratory #13 presented seven deviations (tested nine antimicrobials); this laboratory reported that broth microdilution was the methodology applied for testing the test strains and the reference strain; however, the values submitted for at least six of the tested antimicrobials when testing *E. faecalis* ATCC 29212 seemed to be Inhibition Zone Diameters and not MIC values, which led to the 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.

Table 8. AST of the reference strains *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.

Antimi-crobial	Proportion outside of range			
	Disk Diff. *	Gradient **	MIC **	Total
AMP	1/7	0/1	1/4	**2/12**
CHL	0/7	0/1	1/3	**1/11**
CIP	0/6	0/1	1/5	**1/12**
DAP	--	--	0/3	**0/3**
ERY	1/6	0/1	1/5	**2/12**
GEN	2/5	--	3/4	**5/9**
LZD	0/5	0/1	1/5	**1/11**
SYN	--	--	0/3	**0/3**
TEI	0/4	--	0/4	**0/8**
TET	0/8	--	0/3	**0/11**
TGC	0/1	--	0/2	**0/3**
VAN	1/6	0/1	1/5	**2/12**

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

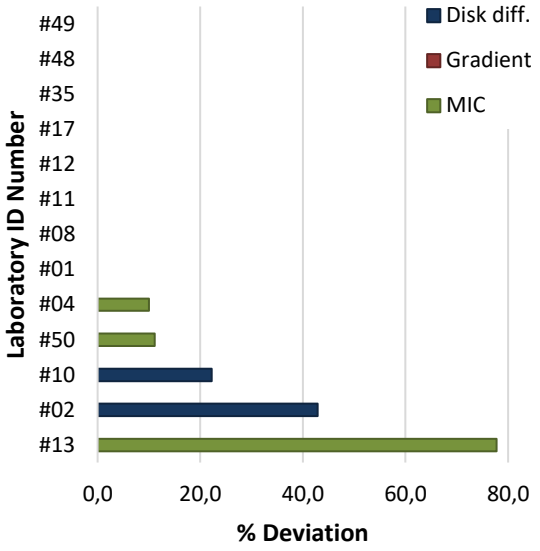

Figure 5. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* trial by the HH laboratories.

3.3 *Streptococcus pneumoniae* trial

Fourteen laboratories from 11 countries uploaded results for the *S. pneumoniae* trial.

3.3.1 Bacterial identification

Of the 14 participating laboratories, 10 correctly identified the tested *S. pneumoniae* and non-*S. pneumoniae* strains (**Table 9**). Laboratories #01, #32 and #50 did not test all the strains: laboratory #01 did not test strain Sp EQAsia 22.5, laboratory #32 strains Sp EQAsia 22.2 and Sp EQAsia 22.6, and laboratory #50 strains Sp EQAsia 22.3 and Sp EQAsia 22.4. In addition, laboratory #32 misidentified strains Sp EQAsia 22.1, Sp EQAsia 22.3 and Sp EQAsia 22.5 (meaning that only data submitted for strain Sp EQAsia 22.4 could actually be evaluated), laboratory #35 identified strain Sp EQAsia 22.4 as non-*S. pneumoniae*, and laboratory #50 also misidentified strains Sp EQAsia 22.5 and Sp EQAsia 22.6 (only data submitted for strain Sp EQAsia 22.1 could actually be evaluated).

Table 9. Bacterial identification of each of the seven 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 22.1	*S. pneumoniae*	13/14
Sp EQAsia 22.2	Non-*S. pneumoniae* (*S. pyogenes*)	13/13
Sp EQAsia 22.3	*S. pneumoniae*	12/13
Sp EQAsia 22.4	*S. pneumoniae*	12/13
Sp EQAsia 22.5	*S. pneumoniae*	11/13
Sp EQAsia 22.6	*S. pneumoniae*	12/13
Sp EQAsia 22.7	Non-*S. pneumoniae* (*S. dysgalactiae*)	14/14

Sp, *S. pneumoniae*

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 91.4% (strain Sp EQAsia 22.5) to 98.0% (strain Sp EQAsia 22.4) for each strain (**Table 10**).

Table 10. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 14 HH laboratories for the *S. pneumoniae* trial.

Strain	AST in total	% Correct
Sp EQAsia 22.1	135	97.6
Sp EQAsia 22.3	119	97.5
Sp EQAsia 22.4	110	98.0
Sp EQAsia 22.5	110	91.4
Sp EQAsia 22.6	120	93.8

Sp, *S. pneumoniae*

Only a couple of strains revealed a deviation higher than 5%. Strain Sp EQAsia 21.5 owes its deviation to some incorrect results mostly reported by laboratory #10, whereas strain Sp EQAsia 22.6 deviation comes from the testing of cefotaxime, ceftriaxone, erythromycin and penicillin, for which the expected categorisation was intermediate, but some of the laboratories found it to be susceptible or resistant, resulting in a slight score penalty (score of 3 instead of 4).

Antimicrobial-based analysis

Antimicrobials with highest deviation from the expected result were cefuroxime (20.0%) and trimethoprim/sulfamethoxazole (8.8%), whereas amoxicillin/clavulanic acid, ertapenem, levofloxacin and linezolid revealed no deviation from the expected results (**Figure 6**).

Cefuroxime was tested by only two laboratories (**Table 3**): #13 (four strains) and #50 (one strain), resulting in a total of only five tests performed towards this antimicrobial. The incorrect result reported for strain Sp EQAsia 22.6 by laboratory #13 (expected to be resistant instead of the reported susceptible) contributed to the observed deviation.

In the case of trimethoprim/sulfamethoxazole, strain Sp EQAsia 22.3 was reported by several laboratories as resistant to the drug, even though it was expected to be intermediate, resulting in a slight score penalty (score of 3 instead of 4).

Laboratory-based analysis

For the *S. pneumoniae* trial, four out of the 14 HH laboratories presented a deviation above the acceptance level of 5% (#50, #35, #10 and #01), and therefore did not perform within the expected range for the trial. The average deviation was 4.6% (ranging from 0.0 to 13.2%) (**Figure 7**).

Laboratory #50, as already mentioned in subsection ‘3.3.1 Bacterial identification’, misidentified or did not test several of the test strains; thus, only the data submitted for strain Sp EQAsia 22.1 could be evaluated. For three of the 17 tested antimicrobials (azithromycin, clindamycin and erythromycin), the laboratory reported an Inhibition Zone Diameter of 0mm and categorisation as resistant, whereas susceptible would be the expected outcome.

Laboratory #35 deviation was mostly caused by the results submitted for strains Sp EQAsia 22.5 and Sp EQAsia 22.6, which were reported as

more susceptible than what would be expected.

Similarly, laboratory #10 presented some incorrect results for the same two strains, where strain Sp EQAsia 22.5 was reported as more susceptible than expected, and strain Sp EQAsia 22.6 as more resistant than expected.

Laboratory #01 presented a few incorrect results when testing azithromycin, erythromycin and trimethoprim/sulfamethoxazole that contributed to the observed deviation.

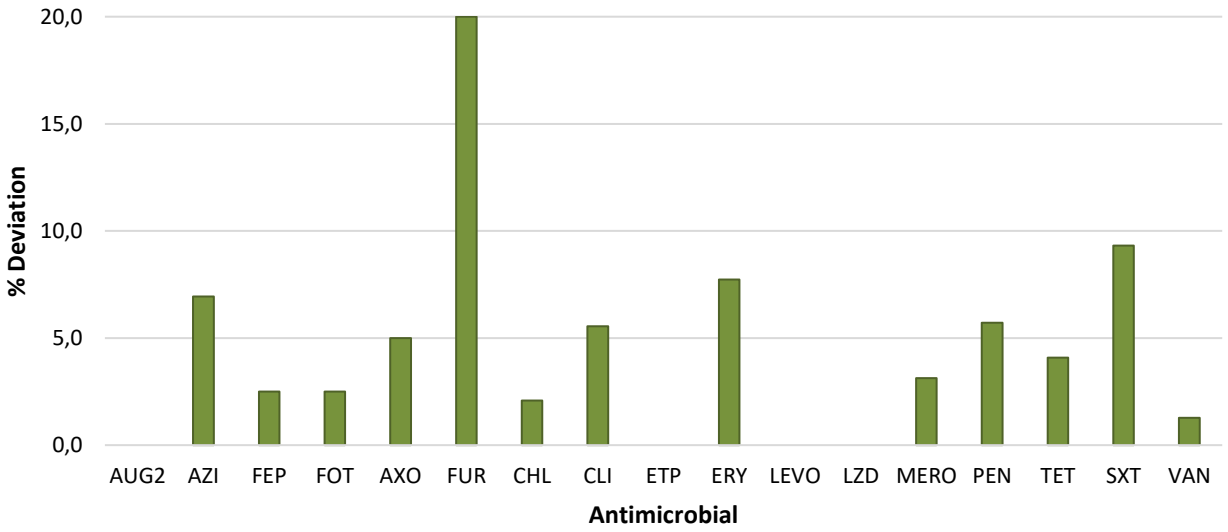

Figure 6. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by HH laboratories (n=14) participating in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

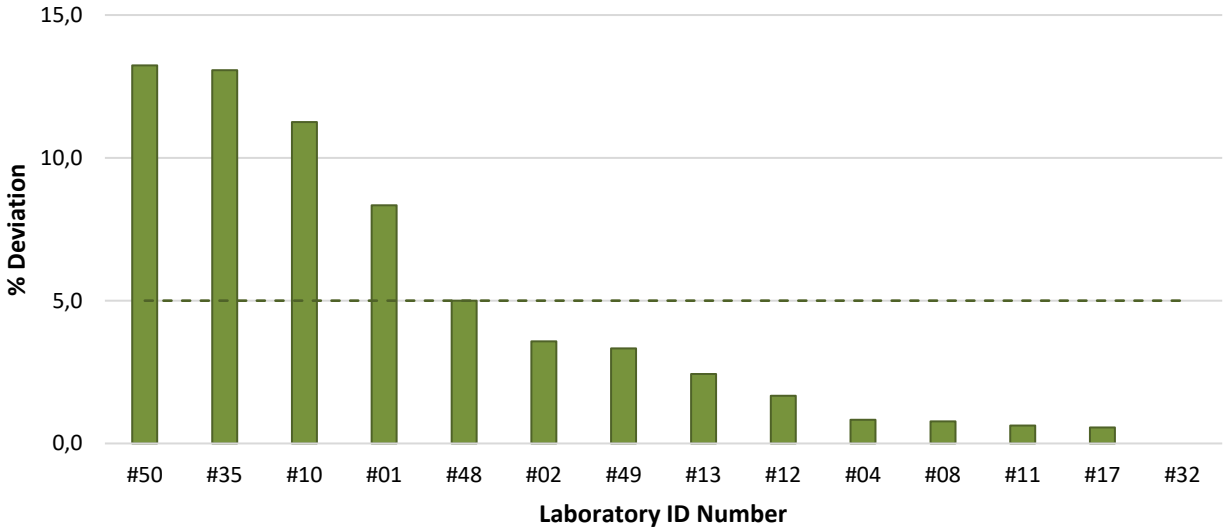

Figure 7. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by HH laboratories (n=14) participating in the 5th EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.3.3 Quality control strain *S. pneumoniae* ATCC 49619

The quality control strain *S. pneumoniae* ATCC 49619 was sent to all participating laboratories free of charge (in this trial or in previous trials) to be used as a reference strain for the *S. pneumoniae* trial.

Among the 14 participating laboratories, 12

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 (conventional and automated) (**Table 11**).

The highest proportion of test results outside of the expected range was observed for trimethoprim/sulfamethoxazole (5 out of 11), and for ertapenem and penicillin (1 out of 3 or 2 out of 6) (**Table 11**).

Table 11. 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.

Antimi-crobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	**Total**
AUG2	--	0/1	--	**0/1**
AZI	1/10	--	--	**1/10**
FEP	1/3	0/1	--	**1/4**
FOT	1/2	0/2	0/1	**1/5**
AXO	1/3	1/3	0/1	**2/7**
FUR	--	--	--	**--**
CHL	2/8	0/1	0/1	**2/10**
CLI	2/7	0/1	0/1	**2/9**
ETP	1/2	0/1	--	**1/3**
ERY	2/9	0/1	0/1	**2/11**
LEVO	2/5	0/2	0/1	**2/8**
LZD	1/5	0/1	0/1	**1/7**
MERO	1/2	0/1	0/1	**1/4**
PEN	1/2	0/2	1/2	**2/6**
TET	2/10	--	0/1	**2/11**
SXT	4/9	0/1	1/1	**5/11**
VAN	3/9	0/1	0/1	**3/11**

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

A closer look at the laboratories' performance (**Figure 8**) shows that four laboratories (#01, #11, #17 and #35) had no deviations. Of those, laboratories #01, #17 and #35 opted for disk diffusion as the sole methodology, whereas laboratory #11 applied disk diffusion, gradient test and agar dilution. In reverse, the remaining eight laboratories had deviations ranging from 7.7 to 100.0% (**Figure 8)**.

Laboratories #08, #48 and #32 presented one deviation each, and laboratories #10, #04 and #49 presented two deviations each. The deviations were both above and below the expected range.

Laboratory #12 presented four deviations, where the Inhibition Zone Diameters reported were all below the expected range.

Laboratory #50 reported that disk diffusion was the methodology applied for testing the test strains and the reference strain; however, the values submitted for the majority of the antimicrobials (12 out of 15) when testing *S. pneumoniae* ATCC 49619 seemed to be MIC values and not Inhibition Zone Diameters, which led to the observed deviations. For the remaining three antimicrobials, the Inhibition Zone Diameters were below the acceptance interval.

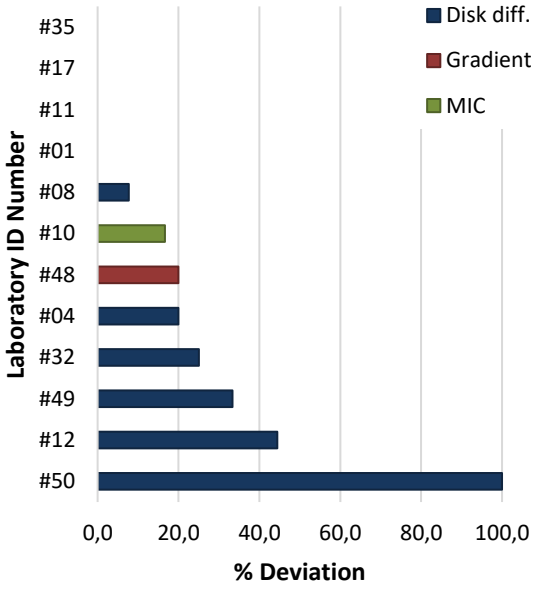

Figure 8. Percentage of deviation in the AST of *S. pneumoniae* ATCC 49619 in the *S. pneumoniae* trial by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the six Animal Health laboratories participating in the 5th EQA of the EQAsia Programme, four laboratories submitted results for the *E. faecium/ E. faecalis* trial, and three for each of the *C. jejuni/ C. coli* and *S. pneumoniae* trials (**Figure 9**). Applied AST methodologies for the three trials are presented in **Figure 9**. Disk diffusion as the sole method was the preferred choice for all the trials. Laboratory #18 was the only participant that reported MIC values obtained solely by broth microdilution method. Laboratory #47 performed bacterial identification but did not submit AST results for the *C. jejuni / C. coli* trial (**Figure 9**).

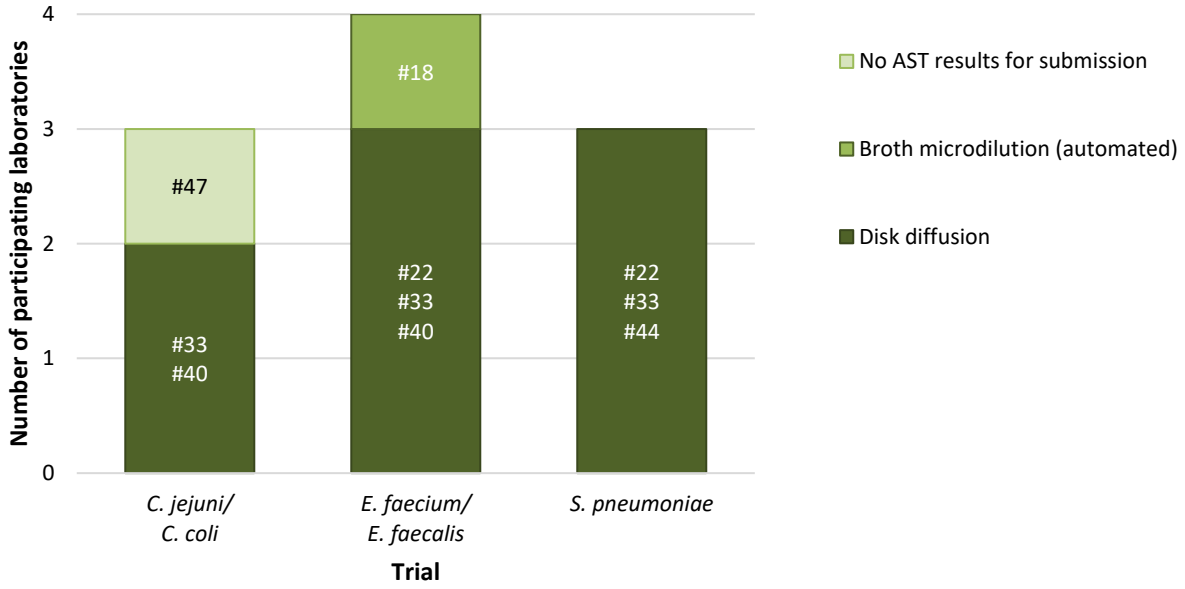

Figure 9. Methodologies applied by the AH laboratories participating in each of the trials.

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 drugs (**Table 1**). Among the antimicrobial agents included in the *C. jejuni/ C. coli* trial, chloramphenicol and ertapenem were not tested by the AH laboratories, whereas ciprofloxacin and gentamicin were tested by the two participating laboratories, and erythromycin and tetracycline were tested by one of the laboratories (**Table 12**). For the *E. faecium / E. faecalis* trial, ampicillin, erythromycin, linezolid and vancomycin were tested by all four participating laboratories; in contrast, daptomycin and quinupristin/dalfopristin were tested by only one laboratory (**Table 12**). Lastly, in the *S. pneumoniae* trial, the most tested antimicrobials were azithromycin, chloramphenicol, clindamycin, erythromycin, levofloxacin, tetracycline and trimethoprim/sulfamethoxazole (tested by two of the three laboratories), whereas amoxicillin/ clavulanic acid, cefepime, cefotaxime, cefuroxime, ertapenem, meropenem and penicillin were not tested by the AH laboratories (**Table 12**).

Table 12. Antimicrobial agents tested by the AH laboratories for each trial. For a given trial (Campy, Ef, Sp), the number of participating laboratories that tested each antimicrobial is shown (n), as well as the percentage (%) of laboratories out of the total number of participating laboratories (N) for the trial (% of n/N). The antimicrobials not included in a given trial are represented as --.

Antimicrobial	Laboratories in total: n (% of n/N)		
	Campy	Ef	Sp
AMP	--	4 (100.0)	--
AUG2	--	--	0
AZI	--	--	2 (66.7)
FEP	--	--	0
FOT	--	--	0
AXO	--	--	1 (33.3)
FUR	--	--	0
CHL	0	3 (75.0)	2 (66.7)
CIP	2 (100.0)	3 (75.0)	--
CLI	--	--	2 (66.7)
DAP	--	1 (25.0)	--
ETP	0	--	0
ERY	1 (50.0)	4 (100.0)	2 (66.7)
GEN	2 (100.0)	3 (75.0)	--
LEVO	--	--	2 (66.7)
LZD	--	4 (100.0)	1 (33.3)
MERO	--	--	0
PEN	--	--	0
SYN	--	1 (25.0)	--
TEI	--	3 (75.0)	--
TET	1 (50.0)	3 (75.0)	2 (66.7)
TGC	--	3 (75.0)	--
SXT	--	--	2 (66.7)
VAN	--	4 (100.0)	1 (33.3)
Total (N)	**2**	**4**	**3**

Campy, *C.jejuni/ C. coli*; Ef, *E. faecium/ E. faecalis*; Sp, *S. pneumoniae*

(n) number of laboratories that reported results for the antimicrobial; (N) total number of participating laboratories for the trial

Scattering of missing data or incomplete AST results entries were observed for the *E. faecium/ E. faecalis* trial only (**Table 13**). Laboratory #18 did not report daptomycin results for strain Ef EQAsia 22.1 and gentamicin results for strains Ef EQAsia 22.4, Ef EQAsia 22.6 and Ef EQAsia 22.7.

Table 13. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by AH laboratories (n=4) participating in the 5th EQA of the EQAsia project.

Lab ID No.	#18
Ef EQAsia 22.1	DAP
Ef EQAsia 22.2	--
Ef EQAsia 22.4	GEN
Ef EQAsia 22.6	GEN
Ef EQAsia 22.7	GEN

Ef, *E. faecium/ E. faecalis*

4.2 Campylobacter jejuni / C. coli trial trial

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

4.2.1 Bacterial identification

While laboratory #47 submitted results for bacterial identification for all seven strains, laboratory #33 submitted data for only three strains (Campy EQAsia 22.2, Campy EQAsia 22.5 and Campy EQAsia 22.7), and laboratory #40 for only two (Campy EQAsia 22.3 and Campy EQAsia 22.6). All tested strains were correctly identified (**Table 14**).

Table 14. Bacterial identification of each of the seven 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 22.1	Non-*C. jejuni/ C. coli* (*C. lari*)	1/1
Campy EQAsia 22.2	*C. jejuni*	2/2
Campy EQAsia 22.3	*C. coli*	2/2
Campy EQAsia 22.4	Non-*C. jejuni/ C. coli* (*C. lari*)	1/1
Campy EQAsia 22.5	*C. jejuni*	2/2
Campy EQAsia 22.6	*C. coli*	2/2
Campy EQAsia 22.7	*C. coli*	2/2

Campy, *C. jejuni/ C. coli*

4.2.2 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

Laboratory #47 did not submit AST results for this trial, as mentioned in the section '*4.1 Overall participation*'. In addition, only results from three and two strains could be evaluated for laboratories #33 and #40, respectively.

Based on this observation, the percentage of results in agreement with expected interpretative results (R/I/S) varied greatly and ranged from 0%

(Campy EQAsia 22.2) to 100% (strains Campy EQAsia 22.3, Campy EQAsia 22.6 and Campy EQAsia 22.7) for each strain (**Table 15**).

Strains Campy EQAsia 22.2 and Campy EQAsia 22.5 were only tested by laboratory #33, thus the strains' deviation was solely caused by this laboratory's performance. The laboratory tested for two antimicrobials (ciprofloxacin and gentamicin) and the reported results were only correct for strain Campy EQAsia 22.5 when tested against gentamicin. On the contrary, Campy EQAsia 22.7 presented no deviation, which shows that the laboratory had no performance issues testing this specific strain.

Table 15. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from two AH laboratories for the *C. jejuni/ C. coli* trial.

Strain	AST in total	% Correct
Campy EQAsia 22.2	2	0.0
Campy EQAsia 22.3	4	100.0
Campy EQAsia 22.5	2	50.0
Campy EQAsia 22.6	4	100.0
Campy EQAsia 22.7	2	100.0

Campy, *C. jejuni/ C. coli*

Antimicrobial-based analysis

As mentioned in section '*4.1 Overall participation*', chloramphenicol and ertapenem were not tested by the AH laboratories. Of the remaining antimicrobials, the highest deviations from the expected result were seen for ciprofloxacin (40.0%) and gentamicin (20.0%), whereas erythromycin and tetracycline revealed no deviation from the expected results (**Figure 10**).

For both antimicrobials, laboratory #33 was the responsible for the incorrect results as previously mentioned. This laboratory tested three strains and two antimicrobials, resulting in a total of six tests performed. The obtained results were all reported as susceptible, which was incorrect for ciprofloxacin for strains Campy EQAsia 22.2 and Campy EQAsia 22.5, and for gentamicin for strain Campy EQAsia 22.2.

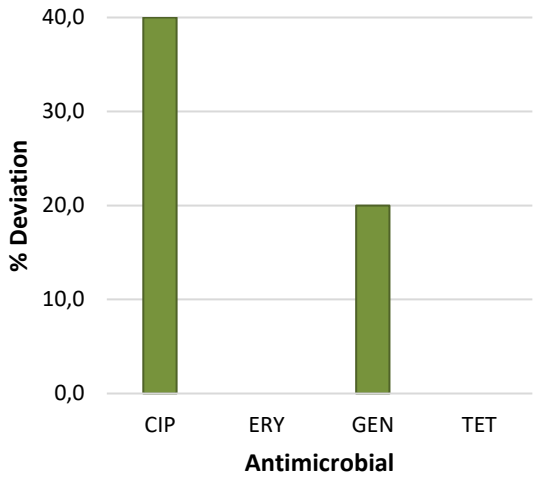

Figure 10. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=2) participating in the 5th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed only for laboratory #40, whereas laboratory #33 did not perform within the expected range for the trial (**Figure 11**). The explanation for the deviation observed for laboratory #33 was already presented in the previous sub-sections.

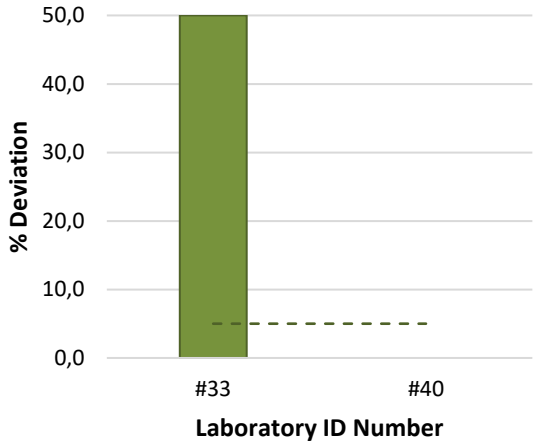

Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=2) participating in the 5th EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 Quality control strain *C. jejuni* ATCC 33560

The quality control strain *C. jejuni* ATCC 33560 was sent to all participating laboratories free of charge (in this trial or in previous trials) to be used as a reference strain for the *C. jejuni/ C. coli* trial.

Both participating laboratories (#33 and #40) submitted disk diffusion results for *C. jejuni* ATCC 33560 when grown at 42°C for 24h; for these conditions, acceptance intervals for disk diffusion are only available for ciprofloxacin and erythromycin (**Appendix 3a**). Therefore, even though the laboratories submitted results for other antimicrobials, those results could not be assessed. Laboratory #33 was then assessed for ciprofloxacin and laboratory #40 for ciprofloxacin and erythromycin. The reported Inhibition Zone Diameters were within the acceptance intervals (**Table 16**).

Table 16. AST of the reference strains *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	0/2	**0/2**
ERY	0/1	**0/1**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion.

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

Four laboratories from four countries uploaded results for the *E. faecium/ E. faecalis* trial.

4.3.1 Bacterial identification

Of the four participating laboratories, only laboratory #40 correctly identified the five *E. faecium*/ *E. faecalis* strains and the two non-*E. faecium*/ *E. faecalis*. Laboratory #33 did not test four of the strains (Ef EQAsia 22.2, Ef EQAsia 22.3, Ef EQAsia 22.4 and Ef EQAsia 22.5), but the remaining three were correctly identified (all *E. faecalis*). Laboratory #18 misidentified strain Ef EQAsia 22.5 as *E. faecalis*, and laboratory #22 identified incorrectly strains Ef EQAsia 22.2 and Ef EQAsia 22.3 as *E. faecalis*, and strain Ef EQAsia 22.4 as non-*E. faecium*/ *E. faecalis* (**Table 17**).

Table 17. Bacterial identification of each of the seven 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 22.1	*E. faecalis*	4/4
Ef EQAsia 22.2	*E. faecium*	2/3
Ef EQAsia 22.3	Non-*E. faecium*/ *E. faecalis* (*E. gallinarum*)	2/3
Ef EQAsia 22.4	*E. faecium*	2/3
Ef EQAsia 22.5	Non-*E. faecium*/ *E. faecalis* (*E. casseliflavus*)	2/3
Ef EQAsia 22.6	*E. faecalis*	4/4
Ef EQAsia 22.7	*E. faecalis*	4/4

Ef, *E. faecium/ E. faecalis*

4.3.2 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 79.6% (strain Ef EQAsia 22.2) to 97.9% (strain Ef EQAsia 22.7) for each strain (**Table 18**). The strains with less AST performed correspond to the strains with higher deviation (Ef EQAsia 22.2 and Ef EQAsia 22.4) (**Table 18**). The AST results submitted for the five *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.

Strain Ef EQAsia 22.2 deviation was caused by laboratories #18 and #22; laboratory #18 reported the strain as resistant to linezolid, teicoplanin and vancomycin, opposite to what was expected; laboratory #22 reported incorrect

results for half of the antimicrobials tested.

Strain Ef EQAsia 22.4 was tested by only two laboratories (#18 and #40) and the deviation observed was caused by two incorrect results (erythromycin and linezolid reported as resistant instead of susceptible) submitted by laboratory #18.

Table 18. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 4 AH laboratories for the *E. faecium/ E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQAsia 22.1	35	97.1
Ef EQAsia 22.2	27	79.6
Ef EQAsia 22.4	16	90.6
Ef EQAsia 22.6	35	96.4
Ef EQAsia 22.7	35	97.9

Ef, *E. faecium/ E. faecalis*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were daptomycin (25.0%), quinupristin/dalfopristin (18.8%), vancomycin (11.8%) and gentamicin (11.1%), whereas chloramphenicol and tigecycline revealed no deviation from the expected results (**Figure 12**).

In the case of daptomycin, only laboratory #18 tested for it. The laboratory reported daptomycin results for two strains and both were categorised as intermediate, where resistant (strain Ef EQAsia 22.6) and susceptible (strain Ef EQAsia 22.7) were the expected outcome. This contributed to a slight score penalty (score of 3 instead of 4) and to the deviation observed in **Figure 12**.

Similarly, quinupristin/dalfopristin was tested by only one laboratory (#22). The laboratory tested the drug against four strains and reported them as resistant, which was not true for strain Ef EQAsia 22.2.

The vancomycin deviation was mostly caused by minor errors (score of 3 instead of 4), whereas the gentamicin deviation was caused by one very major error (score of 0 instead of 4).

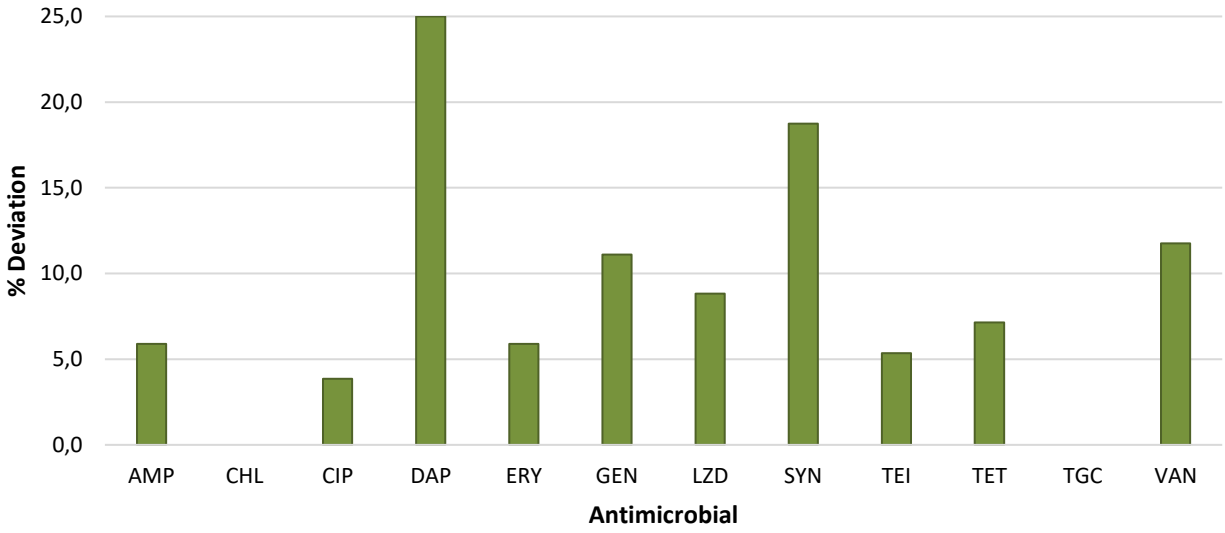

Figure 12. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=4) participating in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for two participants (**Figure 13**). In average, the deviation was 6.2% (ranging from 0.0 to 11.4%). As the acceptance level was set to 5% deviation, laboratories #18 and #22 did not perform within the expected range for the trial.

Laboratory #18 underperformance, as already mentioned in the previous sections, seems to

have been caused by incorrect results when testing antimicrobials such as daptomycin, linezolid and vancomycin.

Laboratory #22 deviation was caused by the several incorrect results reported for strain Ef EQAsia 22.2, as well as a few incorrect results for tetracycline and vancomycin.

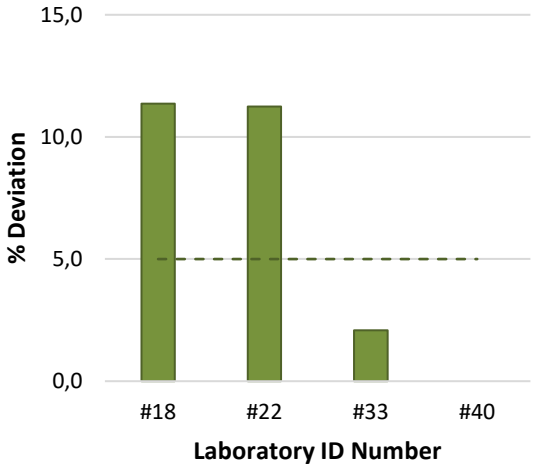

Figure 13. Percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=4) participating in the 5th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 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 four participating laboratories submitted results for the reference strains, but the data from laboratory #40 could not be assessed. This participant 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 3b**). The remaining three laboratories applied either disk diffusion (laboratories #22 and #33) and tested the reference strain *S. aureus* ATCC 25923 (**Table 19**, *), or applied broth microdilution (laboratory #18) and tested *E. faecalis* ATCC 29212 (**Table 19**, **). The highest proportion of test results outside of the expected range was observed for daptomycin (1 out of 1) and ampicillin (2 out of 3) (**Table 19**).

Table 19. 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. *	MIC **	Total
AMP	1/2	1/1	**2/3**
CHL	0/2	--	**0/2**
CIP	0/1	0/1	**0/2**
DAP	--	1/1	**1/1**
ERY	0/2	0/1	**0/3**
GEN	1/2	--	**1/2**
LZD	0/2	0/1	**0/3**
SYN	0/1	--	**0/1**
TEI	0/1	0/1	**0/2**
TET	1/1	0/1	**1/2**
TGC	0/1	0/1	**0/2**
VAN	0/2	1/1	**1/3**

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

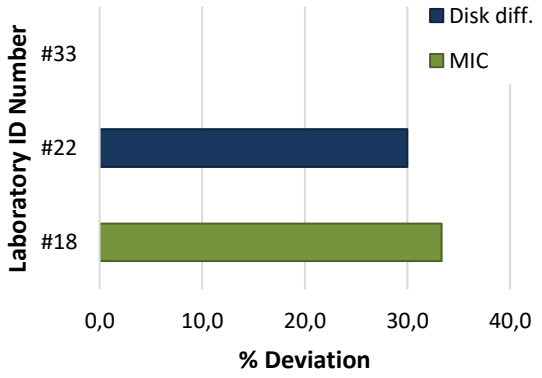

Figure 14. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* trial by the AH laboratories.

In terms of performance, laboratory #33 presented no deviation for the seven antimicrobials tested. Inversely, laboratories #22

and #18 had deviations of 30.0% and 33.3%, respectively, corresponding to three deviations each (**Figure 14**). Laboratory #18 deviations (ampicillin, daptomycin and vancomycin) were all above the acceptance interval; on the contrary, laboratory #22 reported Inhibition Zone Diameters for ampicillin, gentamicin and tetracycline below the expected range.

4.4 *Streptococcus pneumoniae* trial

Three laboratories from three countries uploaded results for the *S. pneumoniae* trial.

4.4.1 Bacterial identification

The results for bacterial identification are shown in **Table 20**. None of the participating laboratories correctly identified the tested *S. pneumoniae* and non-*S. pneumoniae* strains. Laboratory #22 misidentified strain Sp EQAsia 22.1 as non-*S. pneumoniae*, and strain Sp EQAsia 22.2 as *S. pneumoniae*; laboratory #33 incorrectly identified strains Sp EQAsia 22.2, Sp EQAsia 22.3, Sp EQAsia 22.5 and Sp EQAsia 22.7; laboratory #44 misidentified Sp EQAsia 22.5 and, in addition, did not submit results for strains Sp EQAsia 22.3 and Sp EQAsia 22.6 (**Table 20**).

Table 20. Bacterial identification of each of the seven 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 22.1	*S. pneumoniae*	2/3
Sp EQAsia 22.2	Non-*S. pneumoniae* (*S. pyogenes*)	1/3
Sp EQAsia 22.3	*S. pneumoniae*	1/2
Sp EQAsia 22.4	*S. pneumoniae*	3/3
Sp EQAsia 22.5	*S. pneumoniae*	1/3
Sp EQAsia 22.6	*S. pneumoniae*	2/2
Sp EQAsia 22.7	Non-*S. pneumoniae* (*S. dysgalactiae*)	2/3

Sp, *S. pneumoniae*

4.4.2 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 53.1% (strain Sp EQAsia 22.3) to 97.2% (strain Sp EQAsia 22.1) for each strain (**Table 21**).

Strains Sp EQAsia 22.3 and Sp EQAsia 22.4, both very susceptible strains (**Appendix 2c**), were reported by laboratory #22 as resistant towards several antimicrobials, resulting in the very high deviations observed (**Table 21**). The same laboratory together with laboratory #33 contributed for the deviation observed for strain Sp EQAsia 22.6.

Table 21. 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 *S. pneumoniae* trial.

Strain	AST in total	% Correct
Sp EQASIA 22.1	9	97.2
Sp EQASIA 22.3	8	53.1
Sp EQASIA 22.4	17	72.1
Sp EQASIA 22.5	8	90.6
Sp EQASIA 22.6	13	65.4

Sp, *S. pneumoniae*

Antimicrobial-based analysis

The majority of antimicrobials tested by the AH laboratories presented a deviation equal or higher than 25.0% (**Figure 15**). The exception were ceftriaxone, levofloxacin and linezolid, which revealed no deviation from the expected results (**Figure 15**).

Chloramphenicol presented the highest deviation (50.0%). Laboratory #33 reported all three tested strains as susceptible to the drug, which was not correct for one of the strains. Laboratory #22 reported the correct result for only one of the four tested strains.

Vancomycin, the second highest deviation, was only tested by laboratory #22. Of the four tests performed, two were incorrect (strains reported as resistant instead of susceptible). Similarly, all strains were reported as resistant to clindamycin, whereas only one was in fact resistant.

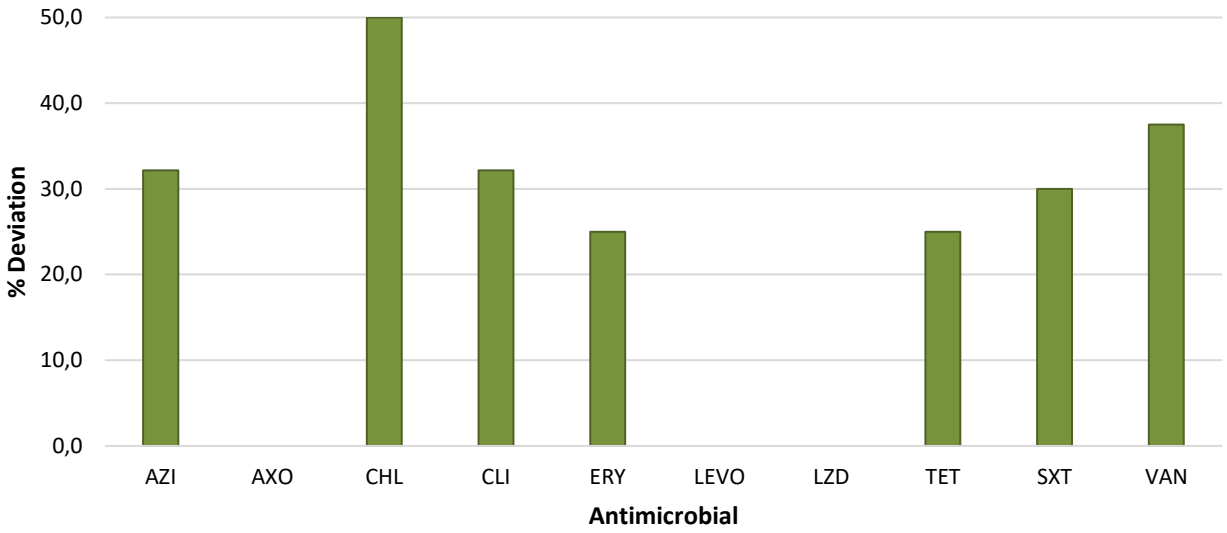

Figure 15. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by AH laboratories (n=3) participating in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

None of the participating laboratories presented a deviation below or equal to 5% in terms of interpretation of the result (R/I/S) (**Figure 16**), meaning that the three laboratories did not perform within the expected range for the *S. pneumoniae* trial. In average, the deviation was 19.4% (ranging from 6.3 to 33.16%).

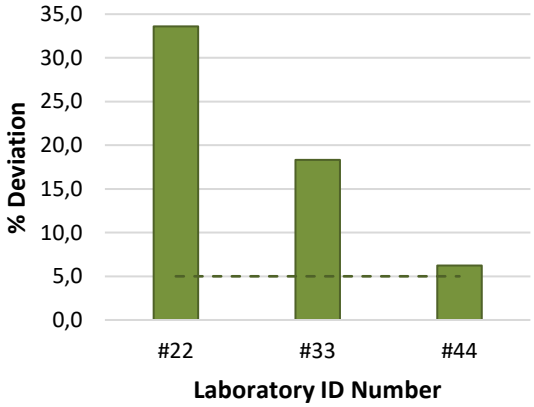

Figure 16. Percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by AH laboratories (n=3) participating in the 5th EQA of the EQAsia project. Results are categorized by laboratory ID number.

Laboratory #22 presented the highest deviation, which can be explained by the already mentioned incorrect results reported for strains Sp EQAsia 22.3, Sp EQAsia 22.4 and Sp EQAsia 22.6 for several antimicrobials (azithromycin, chloramphenicol, clindamycin, erythromycin, tetracycline and vancomycin).

Laboratory #33 results could only be assessed for three strains. For each strain, the laboratory reported results for five antimicrobials. Even though the results were only incorrect for three of the performed tests (two very major and one major error), it caused the observed deviation.

Lastly, laboratory #44 presented a deviation just slightly above the acceptance level of 5 %. Due to misidentified and not tested strains, only results from two strains could be assessed. Besides, the laboratory reported results for just four antimicrobials, corresponding to a total of eight tests performed. Of those, the only incorrect results were observed for trimethoprim/ sulfamethoxazole, where the participant reported the strains as intermediate instead of the expected susceptible outcome. This resulted in a slight score penalty (score of 3 instead of 4).

4.4.3 Quality control strain *S. pneumoniae* ATCC 49619

The quality control strain *S. pneumoniae* ATCC 49619 was sent to all participating laboratories free of charge (in this trial or in previous trials) to be used as a reference strain for the *S. pneumoniae* trial.

Only laboratory #33 submitted results regarding AST of *S. pneumoniae* ATCC 49619 reference strain. The laboratory tested the strain against 10 antimicrobials, even though only five were tested against the test strains. All reported inhibition zone diameters were within the acceptance interval (**Table 22**).

Table 22. 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.

Antimi-crobial	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	0/1	**0/1**
ETP	--	**--**
ERY	--	**--**
LEVO	0/1	**0/1**
LZD	0/1	**0/1**
MERO	0/1	**0/1**
PEN	--	**--**
TET	--	**--**
SXT	0/1	**0/1**
VAN	--	**--**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

5. Results – Overall

5.1 Bacterial identification

In this fifth EQA round, a total of 19 laboratories participated and submitted results for the *E. faecium/ E. faecalis* trial, 17 for the *S. pneumoniae* trial, and three for the *C. jejuni/ C. coli* trial. Considering the test strains tested by each laboratory in each of the trials, it is possible to calculate the percentage of incorrectly identified isolates. **Figure 17** shows the distribution of the deviation for each of the trials.

No deviation is observed for the few laboratories (n=3) that participated and submitted results for the bacterial identification component of the *C. jejuni/ C. coli* trial. For both the *E. faecium/ E. faecalis* and *S. pneumoniae* trials, the median deviation is also 0%, but the results are a bit more dispersed, meaning that some of the laboratories have higher deviations.

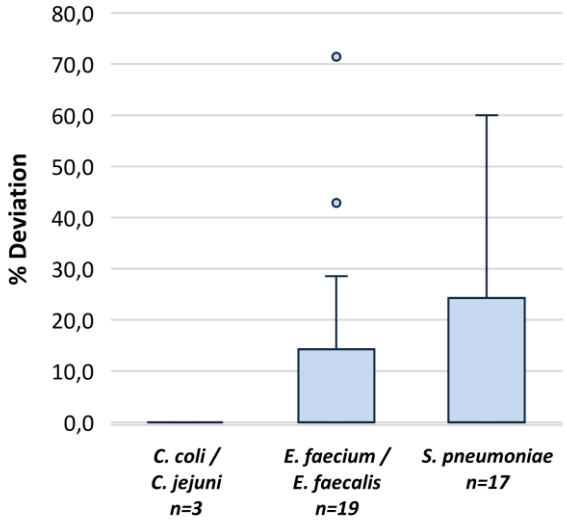

Figure 17. Percentage of deviation in the bacterial identification of *C. jejuni/ C. coli*, *E. faecium/ E. faecalis* and *S. pneumoniae* isolates by the participating laboratories.

The majority of laboratories that submitted results for bacterial identification of *E. faecium/ E. faecalis* strains had either a deviation of 0% (n=10) or 14.3% (n=5), but the remaining (n=4) had higher deviations; misidentification of one of the non-*E. faecium/ E. faecalis* strains (*E. casseliflavus*) was the major contributor for the deviations observed.

Regarding bacterial identification of *S. pneumoniae* strains, similar results are observed; in this case, it appears that correct identification of *S. pneumoniae* was mostly problematic among the AH participating laboratories.

5.2 AST performance

To better understand the overall performance of the participating laboratories, the distribution of the deviations observed for each antimicrobial in each of the trials, and for each trial in general, is presented in this section.

5.2.1 Antimicrobials

In each of the trials, the antimicrobials were tested by a varying number of laboratories. **Figures 18-20** show the distribution of deviations presented by the laboratories submitting results for the respective antimicrobial (number of laboratories is indicated under each antimicrobial abbreviated name).

Distributions are difficult to visualize for antimicrobials tested by few laboratories (n<5), which is the case in the *C. jejuni/ C. coli* trial (**Figure 18**). The deviation for erythromycin (n=1) and tetracycline (n=1) was 0%, whereas the deviation for ciprofloxacin (n=2) was 0 and 66.7%, and for gentamicin (n=2) 0 and 33.3%.

In the *E. faecium/ E. faecalis* trial (**Figure 19**), ampicillin, teicoplanin and tetracycline showed deviations of 0%, with the exception of a few outliers. Some other antimicrobials (chloramphenicol, linezolid and tigecycline) also had a median deviation of 0%, but with more dispersed results. The remaining antimicrobials presented median deviations below 10%, except daptomycin and gentamicin. As already mentioned, antimicrobials tested by less than five laboratories are more difficult to be analysed for their deviations' distribution, which is the case of daptomycin; this drug was tested by four participants, which presented deviations of 25.0% (n=1), 41.7% (n=2) or 50.0% (n=1). Therefore, gentamicin seems to be the antimicrobial causing more issues among the participating laboratories in the *E. faecium/ E. faecalis* trial.

In the *S. pneumoniae* trial (**Figure 20**), amoxicillin/clavulanic acid, ertapenem, levofloxacin and linezolid presented no deviations, whereas tetracycline and vancomycin had median deviations of 0% with a few outliers; the remaining antimicrobials presented higher median deviations and/or more dispersed deviations suggesting that these antimicrobials may have been found more difficult to test by the participating laboratories and therefore generated more incorrect results.

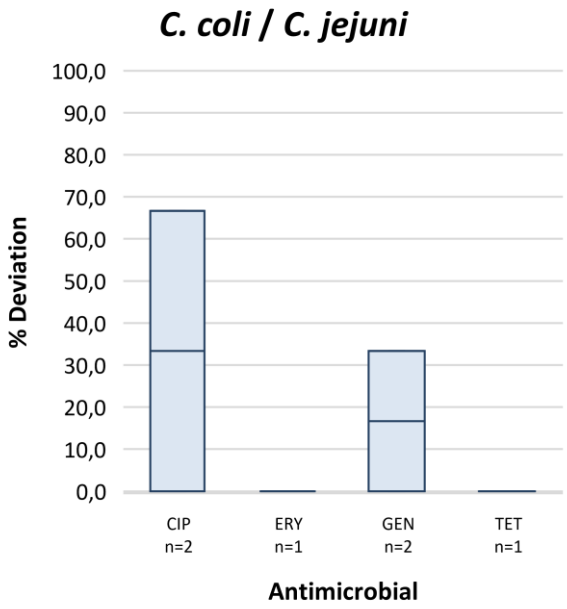

Figure 18. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by the participating laboratories (n=3) in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

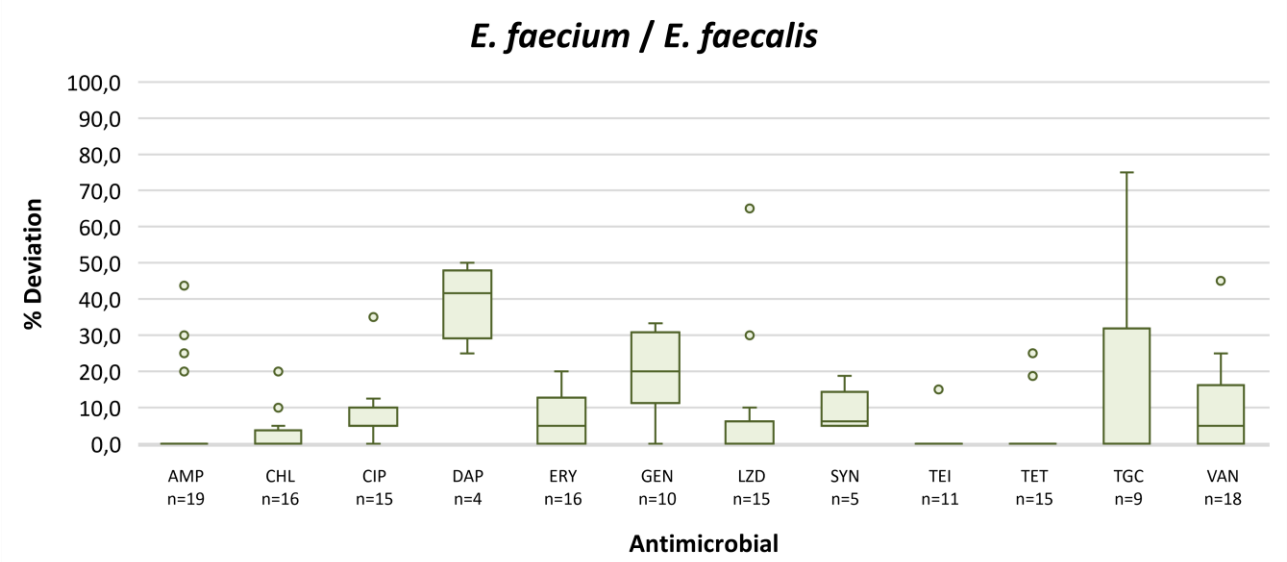

Figure 19. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. faecium/ E. faecalis* strains by the participating laboratories (n=19) in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

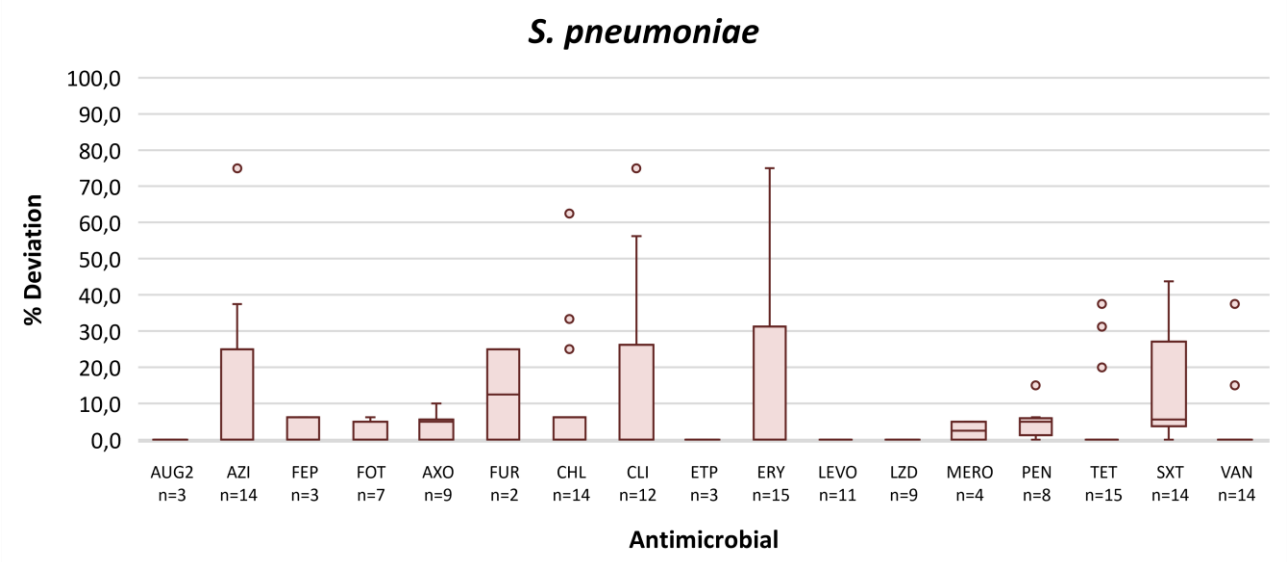

Figure 20. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *S. pneumoniae* strains by the participating laboratories (n=17) in the 5th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

5.2.2 Laboratories performance

In each of the trials, the laboratories performance varied. **Figure 21** presents the distribution of the deviations obtained for the laboratories participating in each of the trials (number of laboratories is indicated under each trial name). Only two laboratories performed AST of the *C. jejuni/ C. coli* strains and presented deviations of 0 and 25.0%.

For the other two trials, it is clear that in the *S. pneumoniae* trial the median deviation is lower (below the acceptance level of 5% deviation from expected results) than the median deviation in the *E. faecium/ E. faecalis* trial (close to 10%), suggesting that more than half of the participating laboratories in the *S. pneumoniae* trial performed within the expected, in contrast to the laboratories submitting results for the *E.*

faecium/ E. faecalis trial.

Nevertheless, it can be observed that the deviations observed for both trials are disperse, suggesting that the level of proficiency varies among the participating laboratories.

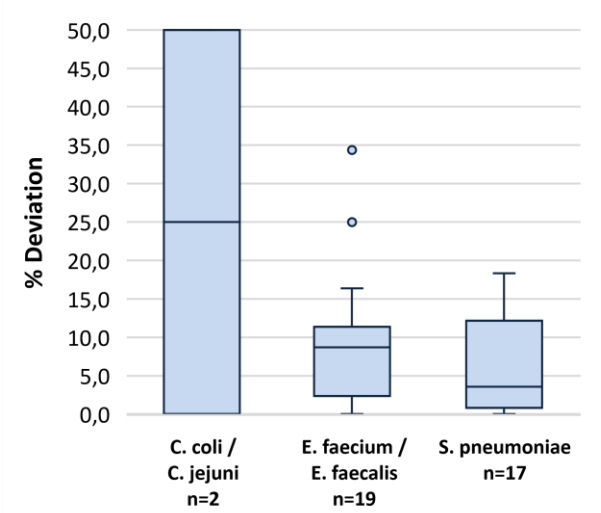

Figure 21. Distribution of the percentage of deviation in the AST interpretation (R/I/S) of obtained results by laboratories participating in the 5th EQA of the EQAsia project. Results are categorized by trial.

5.3 Quality control strains

Relevant quality control strains were tested for each of the trials: *C. jejuni* ATCC 33560 was used as reference strain for the *C. jejuni/ C. coli* trial, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 for testing when disk diffusion or MIC determination methodologies were applied, respectively, for the *E. faecium/ E. faecalis* trial, and *S. pneumoniae* ATCC 49619 for the *S. pneumoniae* trial. Only two laboratories submitted results concerning the reference strain for the *C. jejuni/ C. coli* trial, 16 laboratories for the *E. faecium/ E. faecalis* trial, and 13 laboratories for the *S. pneumoniae* trial.

Figure 22 presents the distribution of the deviations obtained by the participating laboratories for the reference strains included in each of the trials. None of the participants in the *C. jejuni/ C. coli* trial reported deviations from the expected results.

For the *E. faecium/ E. faecalis* trial the median deviation is 0%, whereas the median deviation for the *S. pneumoniae* trial is above 15%. The deviations observed are quite disperse in both of the trials, suggesting that the laboratories proficiency when testing the reference strains seems to vary greatly among the participants.

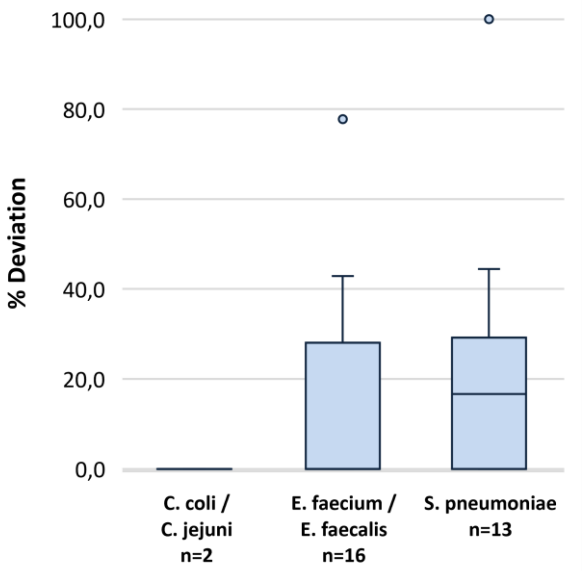

Figure 22. Distribution of the percentage of deviation in the AST of obtained results for the reference strains by laboratories participating in the 5th EQA of the EQAsia project. Results are categorized by trial.

6. Discussion

6.1 Human Health Laboratories

A total of 15 Human Health laboratories participated in the 5th EQA of the EQAsia programme. Disk diffusion as the only methodology was chosen by the majority of the participants for testing the recommended antimicrobials in each of the trials. The remaining laboratories opted for broth microdilution alone or disk diffusion combined with other methodologies, such as gradient test, broth microdilution and/or agar dilution.

All laboratories that performed bacterial identification have also submitted AST results for the *E. faecium/ E. faecalis* and *S. pneumoniae* trials, whereas no laboratories submitted results for the *C. jejuni / C. coli* trial. Incomplete AST results' entries were, however, observed in both trials, 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 *E. faecium* and *E. faecalis* strains; in contrast, the laboratories had difficulties in identifying the *non-E. faecium/ E. faecalis* (specially *E. casseliflavus*) strains. In the *S. pneumoniae* trial, some laboratories demonstrated limited capacity to correctly identify the *S. pneumoniae* species among the provided test strains. Continuous guidance and training is therefore required to assure proper pathogen identification, especially in a clinical setting.

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 five *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 both the *E. faecium/ E. faecalis* and *S. pneumoniae* trials, the deviations observed were usually higher for antimicrobials that were tested by fewer laboratories.

Regarding the HH laboratories' AST performance, on average, the deviation was 10.4% in the *E. faecium/ E. faecalis* and 4.6% in the *S. pneumoniae* trial; the latter, slightly below the acceptance level of 5%. Some laboratories presented deviations above 5% in both *E. faecium/ E. faecalis* and *S. pneumoniae* trials.

Among all laboratories, there were four laboratories that did not submit antimicrobial susceptibility testing results for the quality control strains: laboratories #05 and #32 did not submit results for the reference strains in the *E. faecium/ E. faecalis* trial, and laboratories #02 and #13 for the reference strain in the *S. pneumoniae* trial. In addition, laboratory #13 reported that broth microdilution was the methodology applied for testing the *E. faecium/ E. faecalis* test strains and the reference strain; however, the values submitted for some of the tested antimicrobials when testing *E. faecalis* ATCC 29212 seemed to be Inhibition Zone Diameters and not MIC values. Similarly, laboratory #50 reported that disk diffusion was the methodology applied for testing the *S. pneumoniae* test strains and the reference strain, but the values submitted for the majority of the antimicrobials when testing *S. pneumoniae* ATCC 49619 seemed to be MIC values and not Inhibition Zone Diameters.

For quality control purposes, the participating laboratories should apply the same methodology for both the reference strains and the test strains, test the recommended reference strain for the applied methodology, as well as test the same

antimicrobials in both situations. 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.

6.2 Animal Health Laboratories

For the Animal Health sector, six laboratories participated in the 5th EQA of the EQAsia programme. The participating laboratories mostly applied disk diffusion for determining Inhibition Zone Diameters, though one participant opted for broth microdilution.

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. Besides, incomplete AST results' entries were observed in the *E. faecium/ E. faecalis* trial, where laboratory #18 missed to report daptomycin and gentamicin results for some of the strains.

As mentioned above, bacterial identification was the first component in each of the trials. For the *C. jejuni/ C. coli* trial, there were no issues with correctly identifying the tested strains. The identification and differentiation between *E. faecium*, *E. faecalis* and other *Enterococcus* species revealed some limited capacity of the participating laboratories at performing bacterial identification, where the *E. faecalis* isolates were correctly identified, but not the remaining strains, suggesting that advice and training on the subject may be required among the AH laboratories. Similarly, the three laboratories that participated and submitted results to the *S. pneumoniae* trial demonstrated limitations on differentiating the *S. pneumoniae* strains from the non-*S. pneumoniae*.

For the antimicrobial susceptibility testing performance, chloramphenicol and ertapenem were not tested by the AH laboratories in the *C. jejuni/ C. coli* trial, whereas two (ciprofloxacin and gentamicin) of the four tested antimicrobials presented quite high deviations. In the *E. faecium/ E. faecalis* trial, and as already mentioned, the AST results submitted for the five *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; here, the highest deviations (daptomycin and quinupristin/dalfopristin) can be explained by the fact that these antimicrobials were tested by very few laboratories, as already seen for the HH laboratories. The AST deviations observed in the *S. pneumoniae* trial were very high for some of the tested drugs (chloramphenicol, vancomycin and clindamycin), suggesting performance issues that can be related with inappropriate growth conditions and handling of the *S. pneumoniae* strains.

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 three trials: 25.0% in the *C. jejuni/ C. coli* trial, 6.2% in the *E. faecium/ E. faecalis* trial, and 19.4% in the *S. penumoniae* trial. In addition, four out of five laboratories submitting AST results had a deviation above 5% in at least one of the trials that they have participated in.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the trials. Laboratories #22 and #44 did not submit results for the reference strains in the *S. pneumonae* trial, and laboratory #40 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 from both disk diffusion and broth microdilution methodologies, where the MIC values reported were above the acceptance interval, and the Inhibition Zone Diameters determined were below the expected range, suggesting that handling of reference strains needs to be strengthened to assure the laboratories' good performance.

7. Conclusions

This report presented the results of the EQAsia 5th EQA trial, which included *C. jejuni/ C. coli*, *E. faecium/ E. faecalis* and *S. pneumoniae*. This EQA assessed the performance in 1) bacterial identification, and 2) AST determination and interpretation.

The goal of EQAsia EQAs is to have all participating Human and Food 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, demonstrating the ongoing need for support, 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.

In this EQA trial, the laboratories seem to have reported fewer misinterpretations of the MIC/ Inhibition Zone Diameter values, demonstrating that the participating laboratories have followed the recommendation to solely use the interpretative criteria available in the EQA protocol. It is a requirement that all participating laboratories follow the same interpretation criteria to allow for comparison of results.

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 the routine work. Testing not recommended reference strains for the methodology selected, testing the reference strain against antimicrobials that were not selected for the test strains (and vice-versa), as well as results outside the expected range were observed for both sectors. 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.

8. 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*. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.

[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.

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

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

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

9. Appendices

Appendix 1: EQA5 Protocol

Protocol for EQAsia EQA5 2022

ID and antimicrobial susceptibility testing of *Campylobacter jejuni* and *C. coli*, *Enterococcus faecium* and *E. faecalis*, and *Streptoccocus pneumoniae* test strains

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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 2021-22. The EQA is organized by the consortium of EQAsia and supported by the Fleming Fund.

The EQAsia EQA5 2022 includes the antimicrobial susceptibility testing of five *Campylobacter jejuni* / *C. coli*, five *Enterococcus faecium* / *E. faecalis* and five *Streptococcus pneumoniae* strains **identified** among a total of **seven** test strains for each microorganism, which include two non-target species 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 (or that have been supplied in previous EQAS) are: *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 pneumoniae* 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 control strains' available on the EQAsia website.

2 OBJECTIVES

The main objective of this EQA is to support laboratories to assess and if necessary, improve the identification and antimicrobial susceptibility testing of pathogens, specifically *C. jejuni* / *C. coli*, *E. faecium* / *E. faecalis* and *S. pneumoniae*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3 OUTLINE OF THE EQASIA EQA

3.1 Shipping, receipt and storage of strains

In September 2022, it is expected that National Reference Laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- Seven test strains of which five are *C. jejuni* or *C. coli*, in addition to two non-target species strains. The *Campylobacter jejuni* ATCC 33560/ CCM 6214 will be provided as reference strain (if not already received in previous EQAs).

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

- Seven test strains of which five are *S. pneumoniae*, in addition to two non-target species strains. The *Streptococcus pneumoniae* ATCC 49619/CCM 4501 will be provided as reference strain (if not already received in previous EQAs).

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, dry and 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' on the EQAsia website.

For reconstitution of the QC reference strains, please see the document 'Subculture and maintenance of quality control strains' 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 *C. jejuni / C. coli, E. faecium / E. faecalis* and *S. pneumoniae* test strains

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

3.3 Antimicrobial susceptibility testing of *C. jejuni / C. coli, E. faecium / E. faecalis* and *S. pneumoniae* test strains, and of the reference strains

The strains identified as *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 antimicrobials listed in **Tables 1-3**, but always considering their relevance regarding the laboratory's routine work. 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, 32nd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 12.0, 2022) or epidemiological cut off values (https://mic.eucast.org/) are used instead. The breakpoint values for *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae* can be found in **Tables 1-3**, 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 *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*	≤ 2	-	≥ 4	≥ 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 August 2022.
**Reference values are based on EFSA (European Food Safety Authority) recommendation.

Table 2. 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*	-	-	≥ 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, 32nd Ed.
*Reference values are based on *Enterococcus* spp. clinical breakpoints from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org."

Table 3. 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, 32nd Ed.

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 informatics module. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The informatics module 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; then, 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 November 4th 2022.

If you have trouble in entering your results, please contact the EQA Coordinator directly, explaining the issues that you encountered:

Patrícia T. dos Santos
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK
E-mail: pado@food.dtu.dk

Direct communication with the EQA Coordinator must be in English.

5 HOW TO SUBMIT RESULTS VIA THE INFORMATICS MODULE

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

Access the Informatics Module (**incognito window**) using https://eqasia-pt.dtu.dk. See below how to login to the Informatics Module.

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 Informatics Module.

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 informatics module:

When first given access to login to the Informatics Module, 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 pado@food.dtu.dk.

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Appendix 2: Reference values (MIC) for the test strains

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

	Chloramphenicol CHL		Ciprofloxacin CIP		Ertapenem ETP		Erythromycin ERY		Gentamicin GEN		Tetracycline TET	
Campy EQASIA 22.2 – *C. jejuni*	4	S	16	R	0.25	S	512	R	> 16	R	64	R
Campy EQASIA 22.3 – *C. coli*	8	S	≤ 0.12	S	≤ 0.12	S	512	R	1	S	≤ 0.5	S
Campy EQASIA 22.5 – *C. jejuni*	≤ 2	S	8	R	≤ 0.12	S	≤ 1	S	0.5	S	≤ 0.5	S
Campy EQASIA 22.6 – *C. coli*	8	S	32	R	2	R	4	S	0.5	S	≤ 0.5	S
Campy EQASIA 22.7 – *C. coli*	4	S	≤ 0.12	S	0.25	S	≤ 1	S	1	S	8	I

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2b: Reference values (MIC values and interpretation) – *Enterococcus*

	Ampicillin AMP		Chloramphenicol CHL		Ciprofloxacin CIP		Daptomycin DAP		Erythromycin ERY		Gentamicin GEN	
Ef EQASIA 22.1 – *E. faecalis*	≤ 0.5	S	8	S	1	S	2	S	> 128	R	16	S
Ef EQASIA 22.2 – *E. faecium*	> 64	R	8	S	> 16	R	2	NA	> 128	R	> 1024	R
Ef EQASIA 22.4 – *E. faecium*	> 64	R	≤ 4	S	> 16	R	1	NA	0.25	S	≤ 8	S
Ef EQASIA 22.6 – *E. faecalis*	1	S	128	R	2	I	8	R	> 128	R	1024	R
Ef EQASIA 22.7 – *E. faecalis*	1	S	≤ 4	S	2	I	1	S	2	I	≤ 8	S

R, Resistant; I, Intermediate; S, Susceptible; NA, Not Applicable

	Linezolid LZD		Quinupristin/dalfopristin SYN		Teicoplanin TEI		Tetracycline TET		Tigecycline TGC		Vancomycin VAN	
Ef EQASIA 22.1 – *E. faecalis*	1	S	8	R	≤ 0.5	S	64	R	0.12	S	16	I
Ef EQASIA 22.2 – *E. faecium*	1	S	1	S	≤ 0.5	S	≤ 1	S	0.06	S	≤ 1	S
Ef EQASIA 22.4 – *E. faecium*	2	S	2	I	≤ 0.5	S	≤ 1	S	0.06	S	≤ 1	S
Ef EQASIA 22.6 – *E. faecalis*	2	S	8	R	≤ 0.5	S	128	R	0.06	S	2	S
Ef EQASIA 22.7 – *E. faecalis*	2	S	8	R	≤ 0.5	S	32	R	0.06	S	2	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2c: 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 22.1	≤ 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 22.3	≤ 2/1	S	≤ 0.25	S	≤ 0.5	S	≤ 0.12	S	≤ 0.12	S	≤ 0.5	S	4	S	≤ 0.12	S	≤ 0.5	S
Sp EQASIA 22.4	≤ 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 22.5	≤ 2/1	S	> 2	R	1	S	0.5	S	1	S	4	R	4	S	> 1	R	≤ 0.5	S
Sp EQASIA 22.6	≤ 2/1	S	2	R	2	I	2	I	2	I	> 4	R	16	R	≤ 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 22.1	≤ 0.25	S	1	S	1	S	≤ 0.25	S	≤ 0.03	S	≤ 1	S	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 22.3	≤ 0.25	S	1	S	1	S	≤ 0.25	S	0.25	S	≤ 1	S	2/38	S	≤ 0.5	S
Sp EQASIA 22.4	≤ 0.25	S	1	S	0.5	S	≤ 0.25	S	≤ 0.03	S	≤ 1	S	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 22.5	> 2	R	1	S	1	S	≤ 0.25	S	0.5	S	> 8	R	≤ 0.5/9.5	S	≤ 0.5	S
Sp EQASIA 22.6	2	R	2	S	1	S	0.5	I	4	I	> 8	R	4/76	R	≤ 0.5	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 3: Quality control ranges for the reference strains

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

C. jejuni ATCC 33560 - **36-37°C/48h**		
Antimicrobial	**Agar Dilution MIC (mg/L)**	**Broth Microdilution MIC (mg/L)**
Chloramphenicol, CHL	--	1-8
Ciprofloxacin, CIP	0.12-1	0.06-0.25
Ertapenem, ETP	--	--
Erythromycin, ERY	1-8	0.5-2
Gentamicin, GEN	0.5-2	0.5-2
Tetracycline, TET	--	0.25-2

MIC ranges are according to CLSI VET06 1st edition, Tables 21B and 21C

C. jejuni ATCC 33560 - **42°C/24h**			
Antimicrobial	**Inhibition Zone Diameter (mm)**	**Agar Dilution MIC (mg/L)**	**Broth Microdilution MIC (mg/L)**
Chloramphenicol, CHL	--	--	1-4
Ciprofloxacin, CIP	32-45	0.06-0.5	0.03-0.12
Ertapenem, ETP	--	--	--
Erythromycin, ERY	26-38	1-4	0.25-2
Gentamicin, GEN	--	0.5-4	0.25-2
Tetracycline, TET	--	--	0.25-1

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

Appendix 3b: Quality control ranges for *E. faecalis* ATCC 29212 *and S. aureus* ATCC 25923

	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 and disk diffusion ranges are according to CLSI M100 32nd edition, Tables 4A-1 and 5A-1

Appendix 3c: 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 32nd edition, Tables 4A-1 and 5A-1

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