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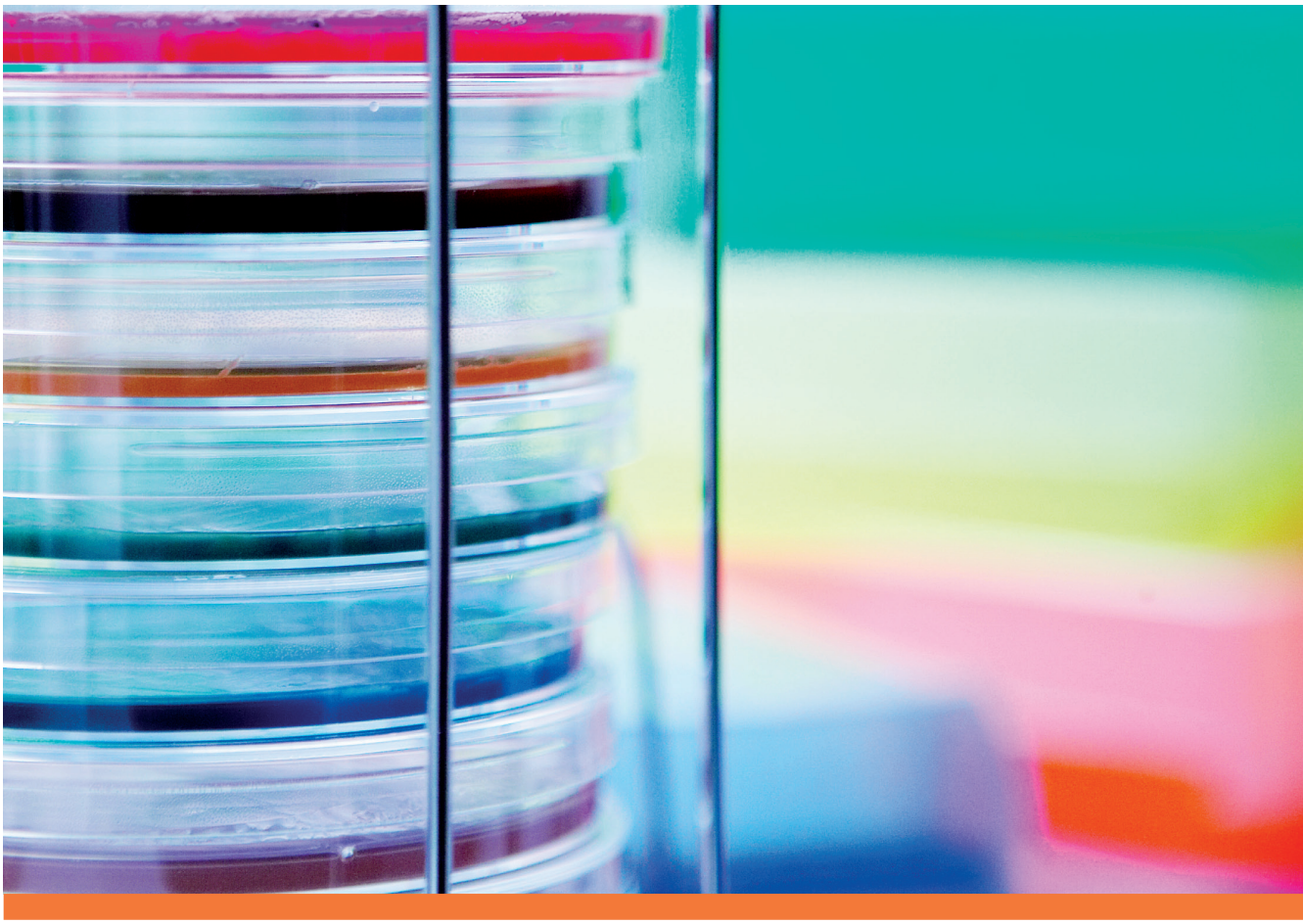

9th EQAsia External Quality Assessment Trial:

Shigella spp., Enterococcus spp., Campylobacter spp. and Neisseria gonorrhoeae – 2024

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Executive Summary

This report summarizes the results of the 9th External Quality Assessment (EQA) trial of EQAsia, the Fleming Fund Regional Grant aiming to strengthen the provision of EQA services across the One Health sector among National Reference Laboratories / Centres of Excellence in South and Southeast Asia. The EQAsia project has entered a second phase (2023 to 2025) in which it will continue to deliver the established EQA programme for both the Human Health (HH sector) and Food and Animal Health (AH sector) laboratories in the region.

The EQA trial was carried out in October - November 2024 and included bacterial identification and antimicrobial susceptibility testing (AST) of several prominent WHO and FAO priority pathogens: *Shigella spp, Enterococcus faecalis, Enterococcus faecium, Campylobacter coli, Campylobacter jejuni,* and *Neisseria gonorrhoeae*. The latter isolate was introduced for the second time in this EQA programme since the start of the EQAsia project.

A total of 35 HH and 16 AH laboratories participated in this EQA trial. The participating laboratories were from 14 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam). Similarly to previous EQAsia EQAs, participating laboratories could choose one or more panels among the ones offered in the current EQA round. In total, data were submitted by 42 laboratories for the *Shigella* spp. panel, 30 laboratories for the *E. faecalis/E. faecium* panel, 18 – for *Campylobacter* spp., and 15 – for *N. gonorrhoeae*.

A major challenge for several laboratories in this EQA trial appeared to be the reconstitution and isolation of a number of strains from the *Campylobacter* spp. and *N. gonorrhoeae* panels. This led to fewer isolates reported per panel and ultimately to a lower performance score.

The bacterial identification component required laboratories to correctly identify five target strains among a total of seven provided strains. For the *Shigella* spp. panel, identification results from nearly all laboratories aligned with the baseline results. However, identification proved more challenging in the other three panels.

While the trial successfully evaluated laboratory performance across most panels, challenges arose with the *Neisseria gonorrhoeae* strains, as none of the participating laboratories were able to successfully revive them. A review of the process identified preservation-related challenges that affected strain viability. Although data could not be generated for *Neisseria gonorrhoeae* in this round, ongoing efforts are focused on refining preservation and shipment strategies to strengthen future EQA trials and ensure the reliability of distributed materials.

On average, the AST performance of participating laboratories was the best in the *Shigella* spp. panel (93.8.6%), followed by enterococci (96.9%) and *Campylobacter* spp. (86.3%).

Laboratories were ranked from #1 to #36 based on their based on their average AST score across the panels in which they participated. One laboratory did not submit any data and was not ranked. Several laboratories received the same rank due to identical scores, resulting in 36 total ranking positions. The average score varied between 68.1% (rank #36) and 100% (rank #1). The total average score among all 48 laboratories that submitted results was 91.8%.

As with previous EQAsia EQAs, many of the laboratories were struggling the most with quality control strain testing. Several laboratories (6 in the *Shigella* spp. Panel, 4 in the *enterococci* panel and 6 in the *Campylobacter* spp. Panel, did not submit results from reference strain testing at all. The rate of laboratories whose tested the QC strains and whose results was conform the expected range of QC values varied across the three panels, as follows – *Shigella*

spp. (36.1%), enterococci (66.7%), and *Campylobacter* spp. (100%).

Several reference strains for the microbiology diagnostics of gonococci were sent to participating laboratories for the second time within this EQA round. Laboratories need to make sure they have all necessary quality control strains that should be tested on a regular basis. EQAsia has also prioritized quality control of AST as a training topic and is offering continuous support on this matter.

Overall, the results from this EQAsia EQA flag once more the need to focus on both basic and more advance methodologies for culture, identification, and antimicrobial susceptibility testing within a training curriculum for the participating laboratories. Quality control testing and the use of the appropriate reference strains, as well as the translation of the QC results into corrective action by laboratories is of utmost importance to ensure a decent level of quality in a microbiology laboratory. Providing and maintaining a standardized level of credible diagnostic services would allow laboratories to generate reliable results that would ultimately feed in a pool of reliable data for surveillance purposes.

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 transitioned to a second phase and will continue to deliver the established EQA programme for both the Human Health (HH) sector and Food and Animal Health (AH) sector in the region until the end of 2025.

The EQAsia Consortium includes the Technical University of Denmark, National Food Institute (DTU Food) as the Lead Grantee, the International Vaccine Institute (IVI) in South Korea, 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 CUVET Thailand, an existing regional provider. The EQAsia 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 used.

A total of eight EQA trials have taken place since 2021, all of which focused on the WHO GLASS [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*), *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. In addition, a Matrix EQA trial was offered four times, 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 was to align with the scope of WHO Tricycle and, as suggested by FAO, to assess the veterinary laboratories' ability to detect multidrug-resistant bacteria from food matrices.

For a given organism, candidate strains are assessed and validated by DTU Food and an external partner (The Peter Doherty Institute for Infection and Immunity, Australia). The validation includes both phenotypic determination of minimum inhibitory concentration (MIC) 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 ninth EQA trial of the EQAsia project (EQA9) carried out in October – November 2024. The trial included four EQA panels, each containing seven test strains. Of these, five were the organism in question (target organism, i.e., *Shigella* spp.), whereas the other two test strains were different from the targeted species (reported as non-[organism], i.e., non-*Shigella* spp.). For each of the seven test strains, participants were requested to report which five strains belong to the expected target organism. For the two organisms different from the expected, no further testing was required. For the remaining five test strains of the target organism, AST results were requested.

This ninth EQA trial includes identification and AST of *Shigella* spp., *E. faecalis/E. faecium, Campylobacter coli/C. jejuni* and *N. gonorrhoeae*. The aim of this EQA trial was 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 EQA9 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 error), '1' (incorrect: major error) or '3' (incorrect: minor error), as explained in the EQA9 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 the 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 disclosed only to 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 EQA9

A total of 50 laboratories participated in the ninth EQA trial of the EQAsia project: 35 laboratories belonging to the HH Sector and 16 belonging to the AH Sector, located in 14 countries: Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam (**Figure 1**).

2.2 Strains

Participating laboratories could register for any of the four EQA panels. For each registration, laboratories received seven bacterial strains of which only five strains were the target 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 EQA9, 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 the *Shigella*, enterococci, and *Campylobacter* panels 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 CUVET. The isolates part of the *Neisseria gonorrhoeae* panel were tested and selected by University of New South Wales, Melbourne, Australia (UNSW). The expected MIC values are available in the appendix of this report (**Appendix 2d**).

Reference strains for the *Shigella*, enterococci, and *Campylobacter* panels [*Escherichia coli* ATCC 25922/CCM 3954 (for disk diffusion of Salmonella strains), *E. coli* NCTC 13846/CCM 8874 (for testing colistin), *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)] were supplied during previous EQA rounds. The QC strains provided within EQA9 included *Neisseria gonorrhoeae* ATCC49226, WHO G, WHO L, WHO O and WHO P and were sent along with the *N. gonorrhoeae* test strains to all the laboratories that requested to participate in this panel.

The expected quality control ranges for the reference strains (**Appendix 3a-d**) were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-34th Ed., tables 4A-1 and 5A-1 [3] and WHO guidelines [4].

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all four panels are outlined in the EQA9 protocol (**Appendix 1**) and 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, 34th Ed. and VET06, 1st Ed.) [3]. When not available, EUCAST clinical breakpoints (Tables v. 13.0, 2023) [5] or epidemiological cut off values [6] were used instead.

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

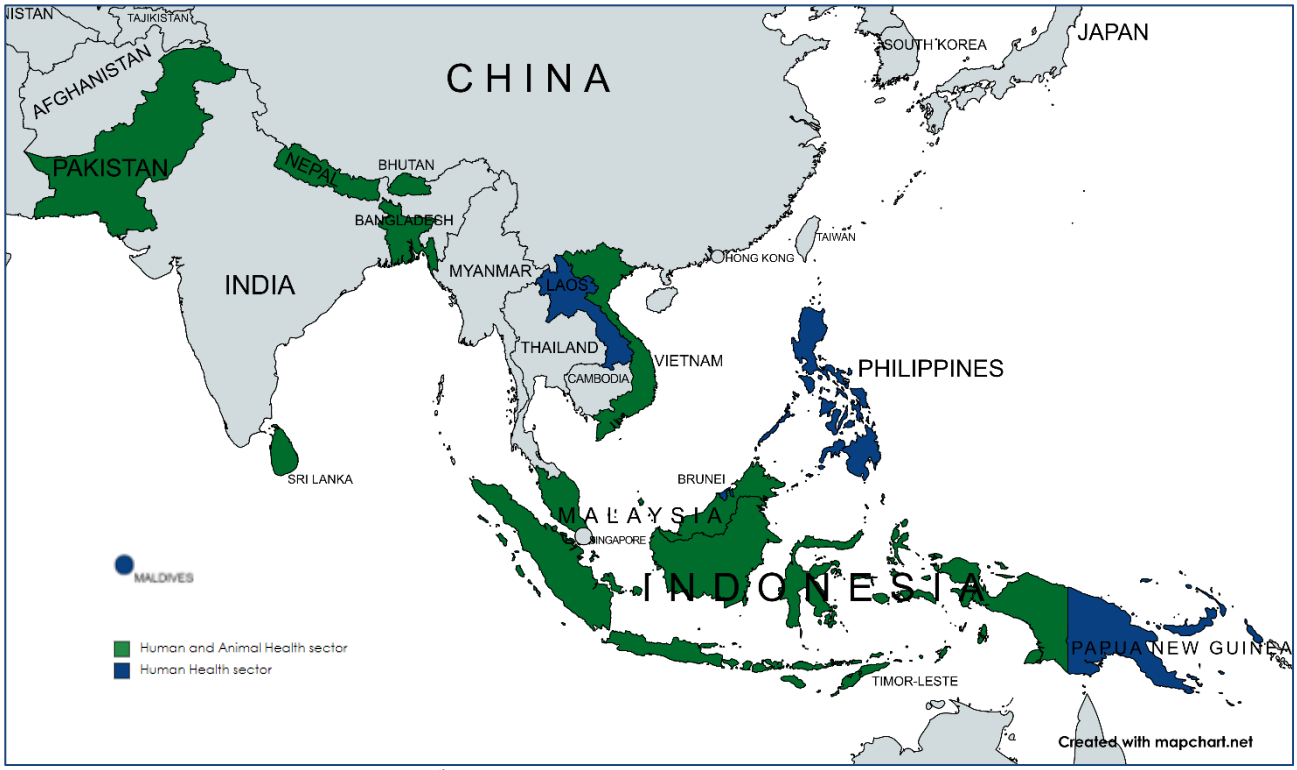

Figure 1. Countries participating in the 9th EQA of the EQAsia project. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA9 2024. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

***Shigella* spp.**	***Campylobacter jejuni* / *C. coli***	***Enterococcus faecium* / *E. faecalis***	***Neisseria gonorrhoeae***
Amikacin	Chloramphenicol	Ampicillin	Azithromycin
Ampicillin	Ciprofloxacin	Chloramphenicol	Cefixime
Azithromycin	Ertapenem	Ciprofloxacin	Cefoxitin
Cefepime	Erythromycin	Daptomycin	Ceftriaxone
Cefotaxime	Gentamicin	Erythromycin	Ciprofloxacin
Cefotaxime/clavulanic acid	Tetracycline	Gentamicin	Penicillin
Cefoxitin		Linezolid	Tetracycline
Ceftazidime		Quinupristin/ dalfopristin	
Ceftazidime/clavulanic acid		Teicoplanin	
Chloramphenicol		Tetracycline	
Ciprofloxacin		Tigecycline	
Colistin		Vancomycin	
Doripenem			
Ertapenem			
Gentamicin			
Imipenem			
Levofloxacin			
Meropenem			
Nalidixic acid			
Piperacillin/tazobactam			
Sulfamethoxazole			
Tetracycline			
Tigecycline			
Tobramycin			
Trimethoprim			
Trimethoprim/ sulfamethoxazole			

2.4 Distribution

The bacterial strains were dispatched either as lyophilized strains or on swabs in transport medium in October 2024 by CUVET to all participating laboratories. The shipments (UN3373, biological substances category B) were sent according to the International Air Transport Association (IATA) regulations. Participating laboratories received detailed information on how to open, revive and store these lyophilized cultures as part of the EQA9 protocol (**Appendix 1**).

2.5 Procedure

Protocols and all relevant information were sent to sites and were also 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 within the EQAsia programme and adapted 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 several 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).

3. Results – Human Health Laboratories

3.1 Overall participation

Among the 35 Human Health laboratories participating in the 9th EQA of the EQAsia project, only one did not submit results. Among these, 32, 20 and 8 laboratories submitted results for *Shigella spp.*, enterococci*, and Campylobacter spp.,* panels*,* respectively. Additionally, 15 HH laboratories enrolled in the *Neisseria gonorrhoeae* panel, reflecting strong interest in assessing diagnostic performance for this pathogen. No AH laboratories participated in this panel.

Despite the high level of engagement, *Neisseria gonorrhoeae* strains could not be successfully revived by any participating laboratory. A post-trial review identified preservation-related challenges that impacted strain viability, preventing bacterial identification, AST, and ATCC reference strain testing.

To ensure successful implementation in future trials, preservation and shipment protocols are being reassessed, and additional quality control measures are being introduced. These improvements aim to maintain strain viability and ensure that participating laboratories can effectively conduct *Neisseria gonorrhoeae* testing in upcoming EQA rounds.

The methodologies applied primarily by the laboratories for the panels varied and are summarized in **Figure 2**. The participants were invited to report inhibition zone diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each drug-bug combination. Only the categorisation was evaluated, whereas the inhibition zone diameters/MIC values were used as supplementary information. The majority of participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results (**Figure 3**).

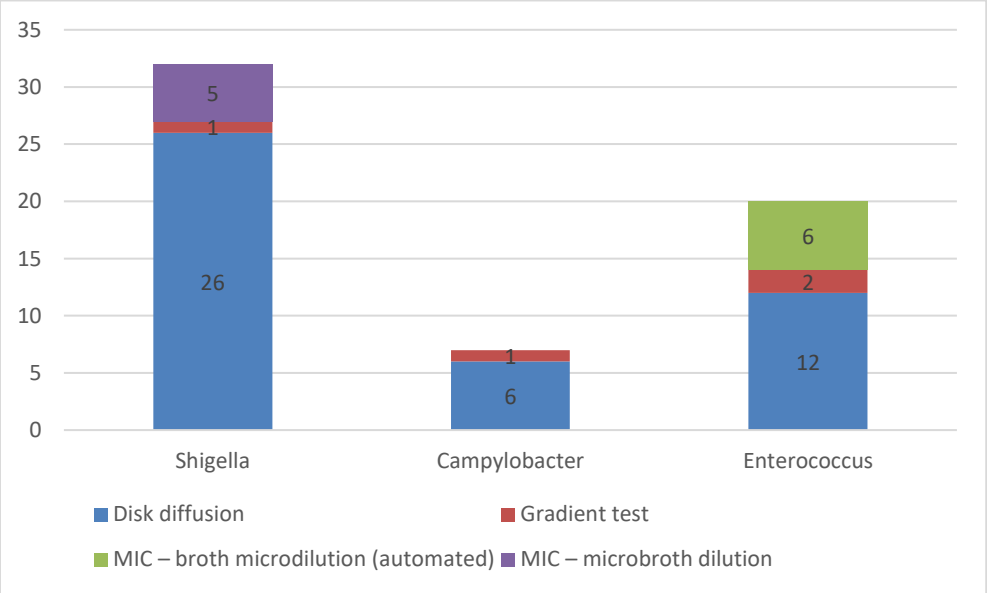

Figure 2. Methodologies primarily used by the laboratories for antimicrobial susceptibility testing in each of the panels.

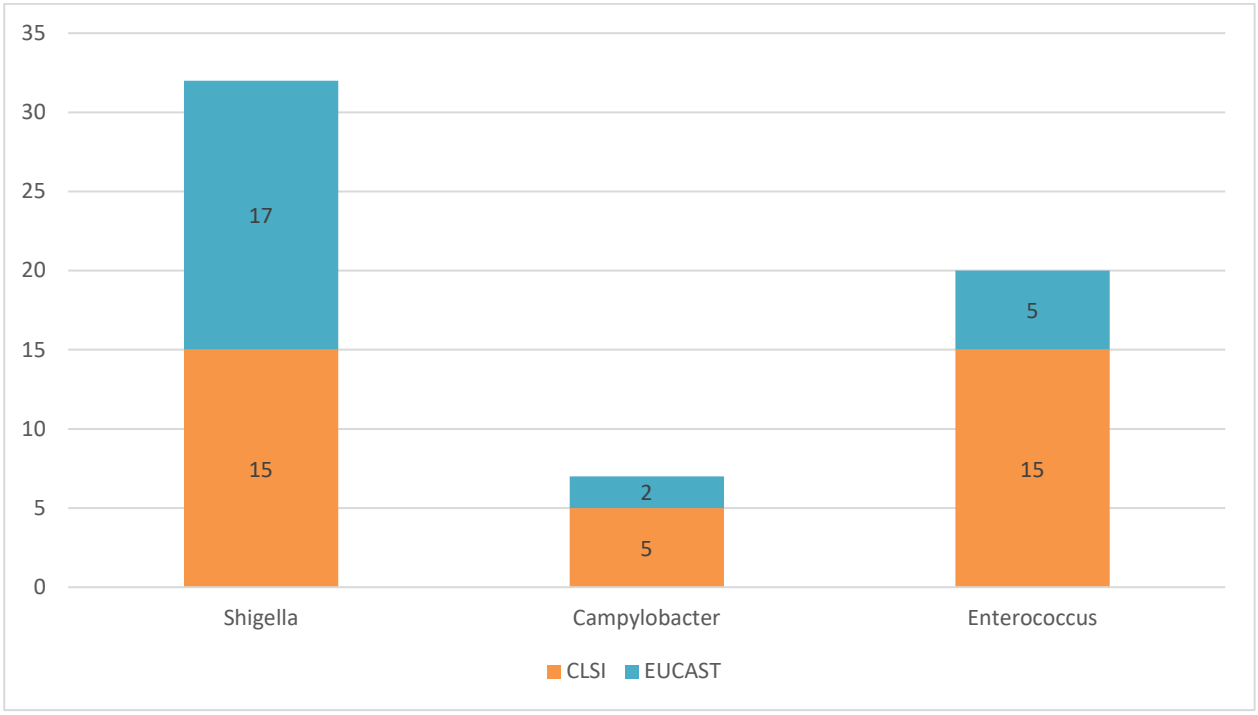

Figure 3. Use of international guidelines for interpretation of AST results by the participating laboratories.

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

The *Shigella* panel had the highest number of total AST results (n=1486) reported by 32 participating laboratories according to the recommended antimicrobials in EUCAST or CLSI (**Table 2**). One of the most frequently tested antibiotics were ciprofloxacin, ampicillin, chloramphenicol and meropenem. In the enterococci panel, participating laboratories tested and reported most frequently ampicillin, ciprofloxacin, erythromycin and trimethoprim/Sulfamethoxazole. Only four antibiotics, ciprofloxacin, erythromycin, gentamicin, and tetracycline were tested and reported for *Campylobacter* (**Table 2**).

Table 2. Total of ASTs performed for each antimicrobial and in total for each of the panels by HH laboratories.

	Shigella		***Enterococcus***		***Campylobacter***	
Amikacin	79	5.3%	--	--	--	--
Ampicillin	146	9.8%	85	13.2%	--	--
Azithromycin	26	1.7%	--	--	--	--
Cefepime	67	4.5%	--	--	--	--
Cefotaxime	52	3.5%	--	--	--	--
Cefoxitin	37	2.5%	--	--	--	--
Ceftazidime	110	7.4%	--	--	--	--
Chloramphenicol	128	8.6%	68	10.6%	--	--
Ciprofloxacin	159	10.6%	82	12.8%	8	32.0%
Colistin	30	2.0%	--	--	--	--
Daptomycin	--	--	11	1.7%	--	--
Ertapenem	59	3.9%	--	--	--	--

Erythromycin	--	--	70	10.9%	8	32.0%
Gentamicin	90	6.0%	32	5.0%	4	16.0%
Imipenem	93	6.2%	--	--	--	--
Levofloxacin	30	2.0%	--	--	--	--
Linezolid	--	--	68	10.6%	--	--
Meropenem	112	7.5%	--	--	--	--
Nalidixic acid	52	3.5%	--	--	--	--
Quinupristin and dalfopristin	--	--	5	--	--	--
Sulfamethoxazole	10	0.7%	--	--	--	--
Teicoplanin	--	--	46	7.2%	--	--
Tetracycline	87	5.8%	62	9.6%	5	20.0%
Tigecycline	24	1.6%	29	4.5%	--	--
Trimethoprim	5	0.3%	--	--	--	--
Trimethoprim/Sulfamethoxazole	100	6.7%	--	--	--	--
Total	**1496**		**643**		**25**	

Missing data or incomplete AST results entries were observed in two out of three EQA panels among the HH laboratories participating in EQA9. A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains.

Three out of 32 laboratories had partially incomplete results submitted for the *Shigella* panel (**Table 3**). The incomplete results in the *Shigella* panel was seen for laboratories #06, 60 and #61.

Two out of 20 laboratories that selected the enterococci panel did not submit complete results of their own available antimicrobial agents (**Table 4**). The incomplete results in this panel were seen for laboratories #06 and #08.

There were no missing data in the *Campylobacter* panel data set. However, very few laboratories (n=3) reported results in this part of the EQA9 trial.

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *Shigella* strains reported by HH laboratories (n=32) participating in the 9th EQA of the EQAsia project.

Lab ID No.	Shi EQAsia 24.1	Shi EQAsia 24.2	Shi EQAsia 24.5	Shi EQAsia 24.6	Shi EQAsia 24.7
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	FEP IMI NAL	MERO	--	--	--
#07	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#13	--	--	--	--	--
#14	--	--	--	--	--
#17	--	--	--	--	--
#32	--	--	--	--	--
#34	--	--	--	--	--

#35	--	--	--	--	--
#40	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#52	--	--	--	--	--
#60	GEN	GEN	--	GEN	GEN
#61	--	FOT	FOT	FOX	FOX
#62	--	--	--	--	--
#63	--	--	--	--	--
#64	--	--	--	--	--
#70	--	--	--	--	--
#71	--	--	--	--	--
#72	--	--	--	--	--
#73	--	--	--	--	--
#74	--	--	--	--	--
#75	--	--	--	--	--
#76	--	--	--	--	--
#77	--	--	--	--	--

Shi, *Shigella*

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *E. faecalis/E. faecium* strains reported by HH laboratories (n=20) participating in the 9th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 24.1	Ef EQAsia 24.2	Ef EQAsia 24.3	Ef EQAsia 24.4	Ef EQAsia 24.6
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#06	DAP	DAP TEI	--	--	DAP
#07	--	--	--	--	--
#08	--	AMP	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#14	--	--	--	--	--
#17	--	--	--	--	--
#32	--	--	--	--	--
#34	--	--	--	--	--
#35	--	--	--	--	--
#40	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#52	--	--	--	--	--
#61	--	--	--	--	--
#64	--	--	--	--	--

Ef, *E. faecalis/E. faecium*

3.2 *Shigella spp.* panel

32 laboratories from 14 countries uploaded results for the *Shigella spp.* panel.

3.2.1 Bacterial identification

32 laboratories submitted results for bacterial identification (**Table 5**). The five target *Shigella* strains were identified correctly by all laboratories.

Table 5. Bacterial identification of each of the 7 test strains provided in the *Shigella* panel. Number of correct results out of all HH participating laboratories.

Strain	Bacterial ID	No. correct
Shi EQASIA 24.1	*Shigella*	32/32
Shi EQASIA 24.2	*Shigella*	32/32
Shi EQASIA 24.3	Non-*Shigella*	32/32
Shi EQASIA 24.4	Non-*Shigella*	32/32
Shi EQASIA 24.5	*Shigella*	32/32
Shi EQASIA 24.6	*Shigella*	32/32
Shi EQASIA 24.7	*Shigella*	32/32

Shi, Shigella

3.2.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 77.9% (strain Shi EQASIA 24.1) to 94.1% (strain Shi EQASIA 24.7) (**Table 6**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were amikacin (25.3%), azithromycin (13.8%), cefepime (31.3%), cefotaxime (11.5%), ceftazidime (13.6%), ciprofloxacin (15.1%), colistin (50%), gentamicin (30%), levofloxacin (56.7%), sulfamethoxazole (30%) and Trimethoprim/Sulfamethoxazole (11.5%), whereas ertapenem, imipenem, meropenem, tigecycline and trimethoprim revealed no deviation from the expected results (**Figure 4**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed in 4 laboratories: #32, #48, #71 and #75 (**Figure 5**). In average, the deviation was 10.8% (ranging from 0.0% to 32.7%). As the acceptance level was set to 5% deviation, 23 laboratories (#49, #50, #13, #61, #14, #34, #40, #63, #01, #04, #12, #35, #62, #64, #72, #60, #17, #07, #74, #06, #02, #05, #11, #52, #70, #73, #76 and #77) did not perform within the expected range for the *Shigella* panel.

3.2.3 β-lactamase-producing Shigella

None of the ten participating laboratories uploaded results for this component of the *Shigella* trial.

Table 6. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results submitted by 32 HH laboratories for the *Shigella* panel.

Strain	AST in total	% Correct
Shi EQASIA 24.1	294	77.9%
Shi EQASIA 24.2	307	91.2%
Shi EQASIA 24.5	308	89.9%
Shi EQASIA 24.6	308	87.0%
Shi EQASIA 24.7	306	94.1%

Shi, Shigella

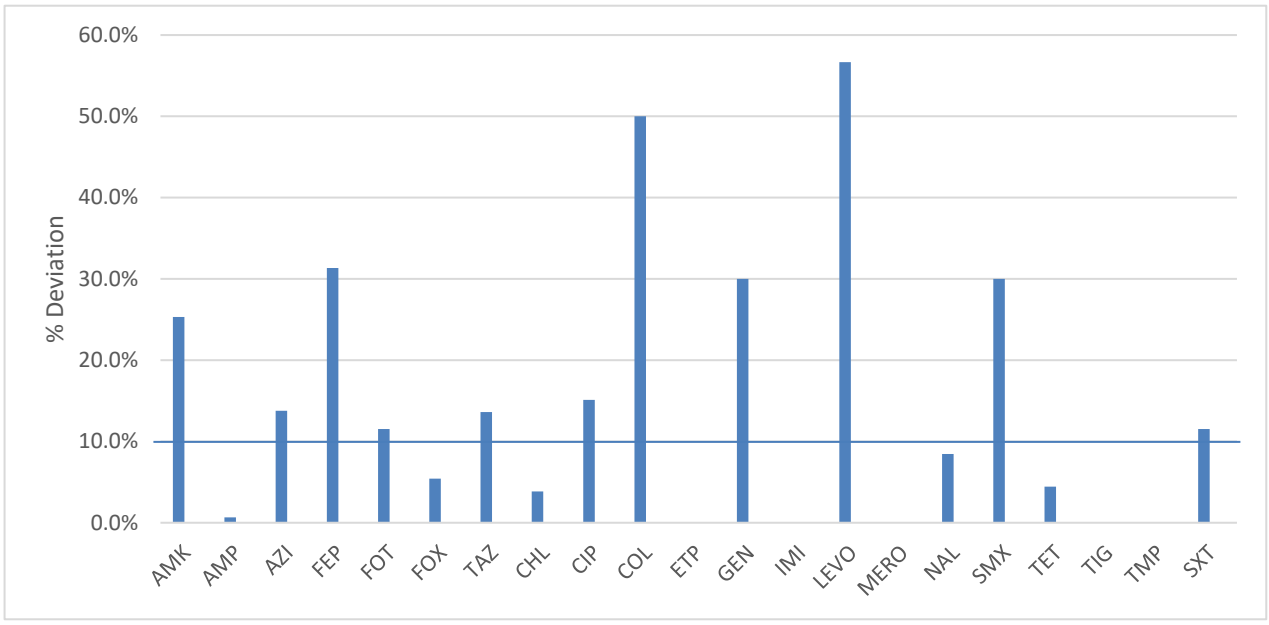

Figure 4. Percentage of deviation in the AST interpretation (R//I/S) among *Shigella* strains by HH laboratories (n=32) participating in the 9th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

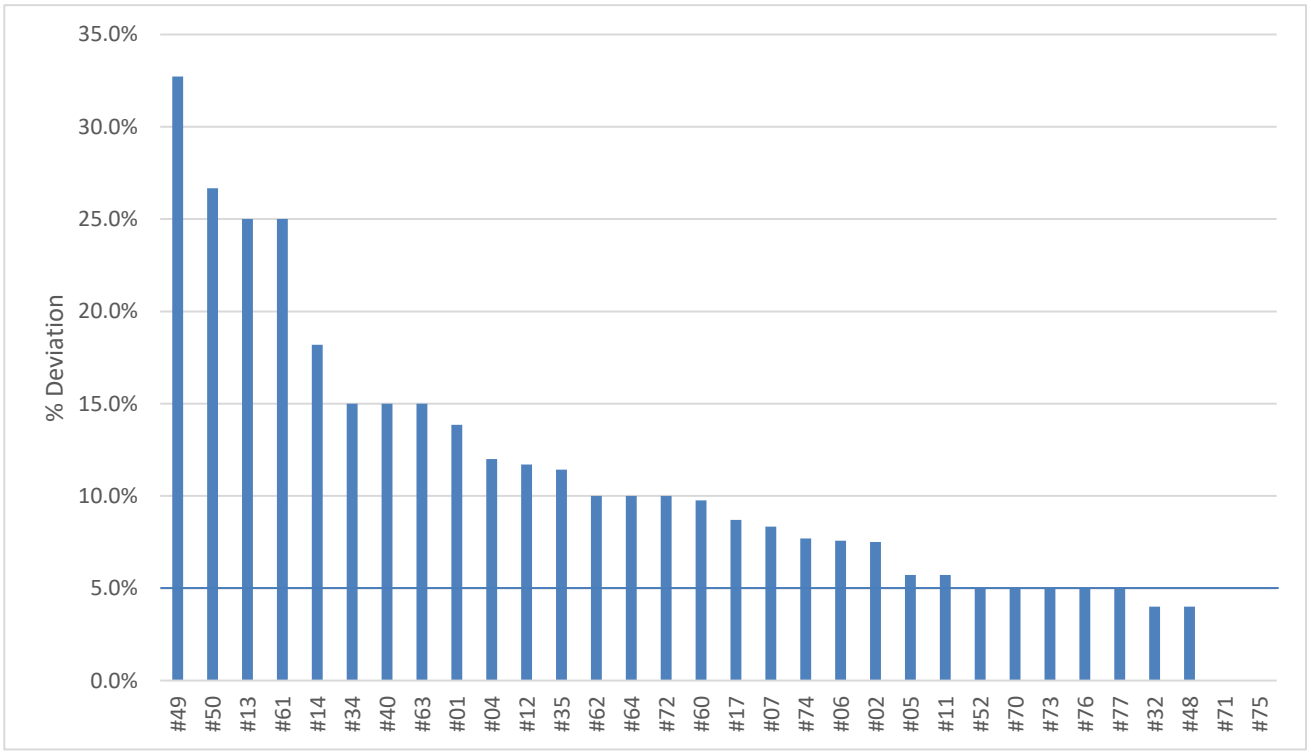

Figure 5. Percentage of deviation in the AST interpretation (R/I/S) among *Shigella* strains by HH laboratories (n=32) participating in the 9th EQA in the EQAsia project. Results are categorized by laboratory ID number.

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

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge to participating laboratories as part of previous EQAsia EQA trials to be used as reference strains for the *Shigella* panel.

26 out of 32 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only two performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test, or broth microdilution (**Table 7**). For testing *E. coli* NCTC 13846, MIC was determined by standard method by broth microdilution. The highest proportion of test results outside of the expected range was observed in sulfamethoxazole (6 out of 8) (**Table 7**).

Table 7. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *Shigella* panel. A proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diffusion	Gradient	MIC	**Total**
AMK	2/15	0/1	0/3	**2/19**
AMP	2/19	--	0/6	**2/25**
FEP	3/14	0/1	2/3	**5/18**
FOT	1/9	0/1	1/3	**2/11**
FOX	0/12	--	0/1	**0/13**
TAZ	5/18	0/1	2/4	**7/23**
CHL	2/18	0/1	--	**2/19**
CIP	2/21	--	5/5	**7/26**
COL	--	--	0/2	**0/2**
ETP	2/8	0/1	4/4	**6/13**
GEN	1/19	--	0/5	**1/24**
IMI	2/14	0/1	0/4	**2/19**
MERO	2/18	0/1	5/5	**7/24**
SMX	6/8	--	--	**6/8**
TET	3/15	0/1	--	**3/16**
TMP	1/4	--	0/1	**1/5**

Disk Diffusion – inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth micro or macrodilution.
*Gradient test and disk diffusion are not recommended for colistin testing

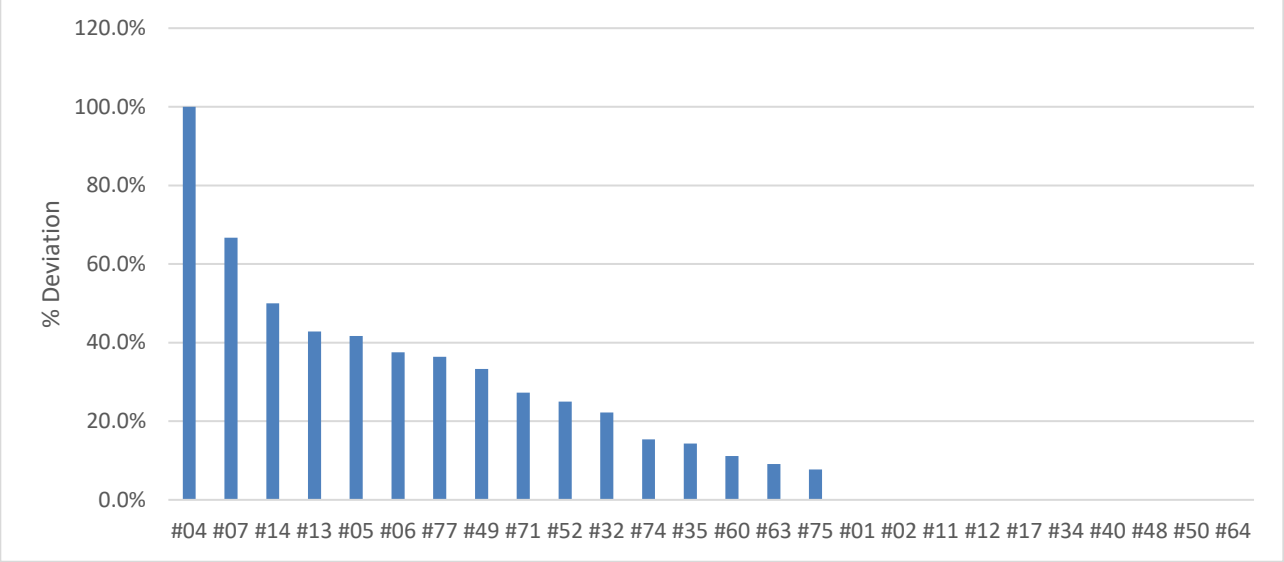

Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Shigella* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 6).** Laboratories #01, #02, #11, #12, #17, #34, #40, #48, #50 and #64 presented no deviation. I.e. laboratories #01, #02, #17, #34, #40, #48 and #50 used only disk diffusion, laboratory #11 and #48 applied disk diffusion and gradient test, while laboratory #12, #50 and #11 used all three methods (MIC broth microdilution, gradient test, and disk diffusion). All other laboratories presented deviations that ranged from 7.7% to 100% (**Figure 6**).

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

3.3 *Enterococcus faecium/ Enterococcus faecalis* panel

For *Enterococci* panel, 20 laboratories from 13 countries uploaded results.

3.3.1 Bacterial identification

20 participating laboratories submitted results for bacterial identification (**Table 10**). None of the laboratories could revive and identify correctly all seven strains of this panel. Strains Ef EQAsia 24.1, Ef EQAsia 24.3 and Ef EQAsia 24.4 were correctly identified by all the labs while strain Ef EQAsia 24.6 was correctly identified by only one lab (#17).

Table 10. Bacterial identification of each of the 7 test strains provided within the enterococci panel. The number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ef EQASIA 24.1	*Enterococcus faecium*	20/20
Ef EQASIA 24.2	*Enterococcus faecium*	18/20
Ef EQASIA 24.3	*Enterococcus faecalis*	20/20
Ef EQASIA 24.4	*Enterococcus faecalis*	20/20
Ef EQASIA 24.5	Non-*Enterococcus faecalis/faecium*	17/20
Ef EQASIA 24.6	*Enterococcus faecalis*	1/20
Ef EQASIA 24.7	Non-*Enterococcus faecalis/faecium*	16/20

Ef, *E. faecalis/ E. faecium*

3.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 41.5% (strain Ef EQASIA 24.3) to 95.1% (strain Ef EQASIA 24.2) (**Table 11**). 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 24.3 (58.5%) and was caused by several instances of results' misinterpretation of the obtained results mainly for tigecycline, linelozid, chloramphenicol, daptomycin and gentamycin. Strains Ef EQAsia 24.6 and Ef EQAsia 24.4 also presented quite high deviations (close to 52.3% and 25.2%, respectively) that resulted from several incorrect results reported mostly for erythromycin and tetracycline.

Table 11. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 20 HH laboratories for the enterococci panel.

Strain	AST in total	% Correct
Ef EQASIA 23.1	138	94.2%
Ef EQASIA 23.3	122	95.1%
Ef EQASIA 23.4	260	41.5%
Ef EQASIA 23.5	139	74.8%
Ef EQASIA 23.7	132	47.7%

Ef, E. faecalis/ E. faecium

Antimicrobial-based analysis

All antimicrobials showed deviations higher than 10% from the expected result ranging from 10.3% for ciprofloxacin and tigecycline, to 40% for Quinupristin and dalfopristin. Only teicoplanin showed a deviation less than 5% (2.1%) (**Figure 7**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was not observed in any laboratory (**Figure 8**). In average, the deviation was 23.3% (ranging from 11.1 to 50%). As the acceptance level was set to 5% deviation, all 20 laboratories did not perform within the expected range for the enterococci panel.

Laboratory #04 presented the highest deviation observed for this panel. The deviations in the results submitted by laboratory #04 were in the AST of Ef EQASIA 24.1, 24.2, 24.3 and 24.5, leading to the low performance score for this part

of the trial.

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

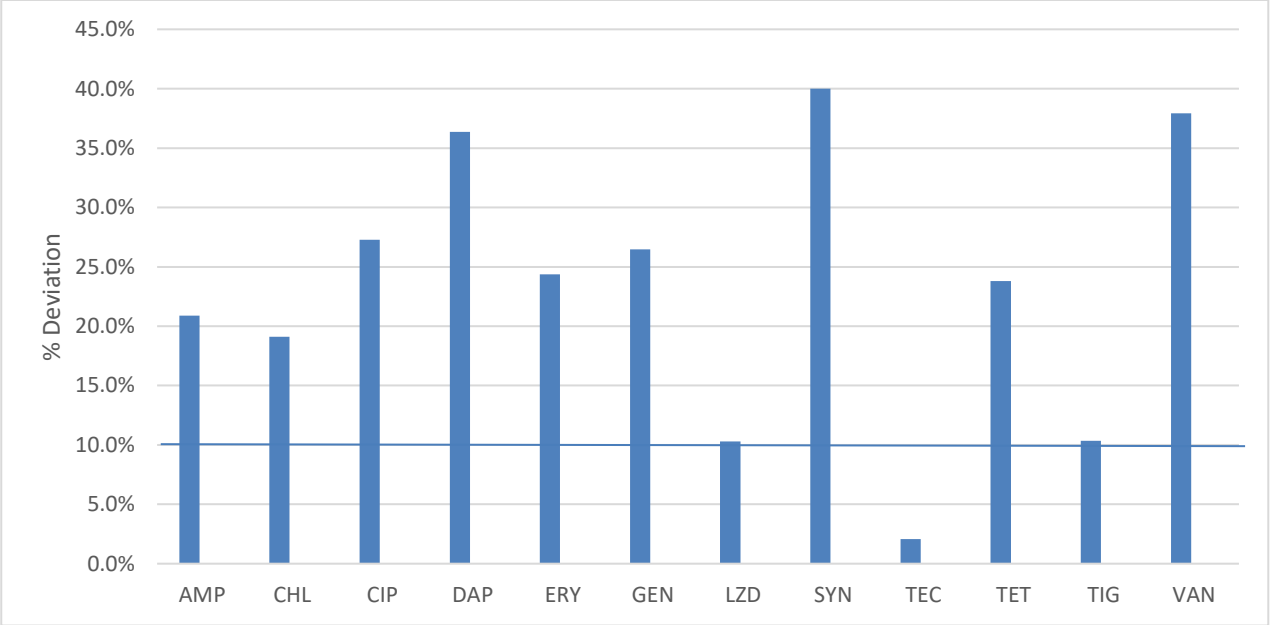

Figure 7. Percentage of deviation in the AST interpretation (R/S) among enterococci strains by HH laboratories (n=20) participating in the 9th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

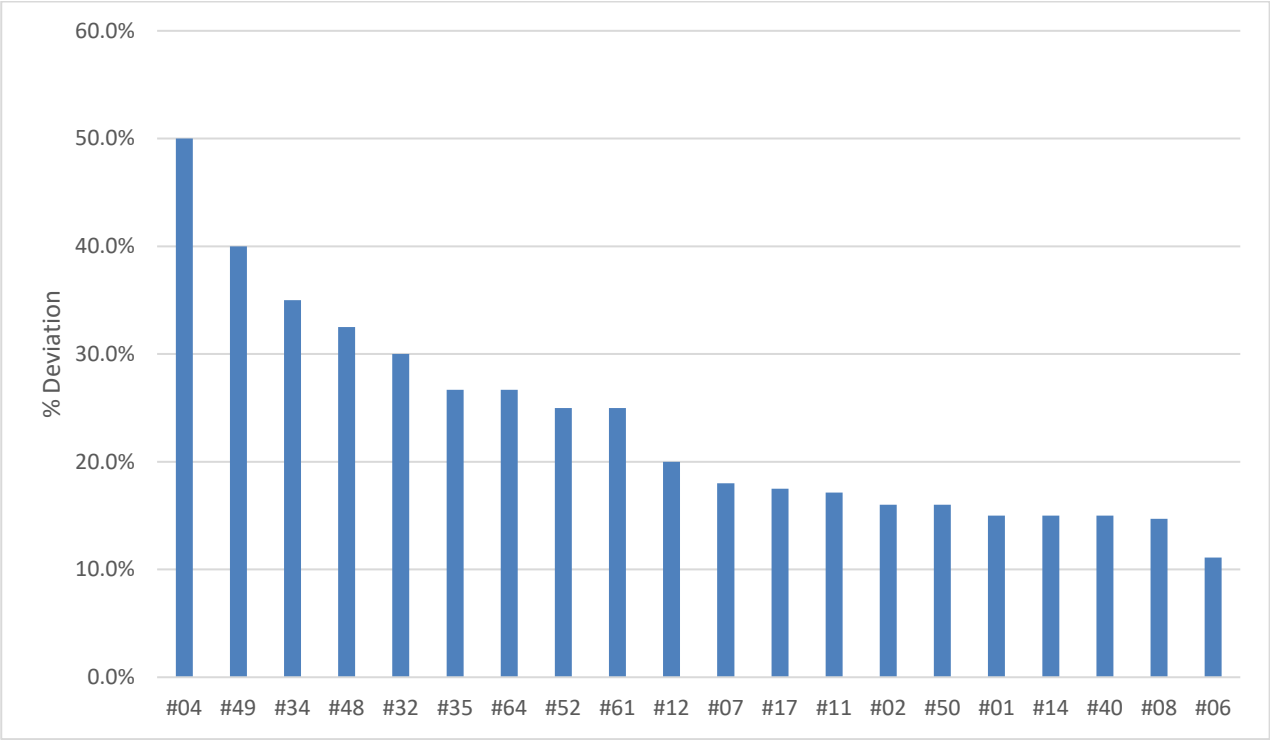

Figure 8. Percentage of deviation in the AST interpretation (R/S) among *E. faecalis/E. faecium* strains by HH laboratories (n=20) participating in the 9th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.3.3 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 previous trials) to participating laboratories to be used as reference strains for the *E. faecium/ E. faecalis* panel.

14 out of 20 participating laboratories submitted results for this part of the enterococci panel. Nine laboratories reported results for the reference strain *S. aureus* ATCC 25923. Six laboratories entered results also for *E. faecalis* ATCC 29212. Both disk diffusion and MIC test results were reported for both reference strains by some laboratories. However, it should be noted that the reference strain *S. aureus* ATCC 25923 could only be used to determine inhibition zone diameters by disk diffusion, while *E. faecalis* ATCC 29212 is recommended for MIC testing.

Highest proportion of test results outside of the expected range was observed in ampicillin (2 out of 14) (**Table 12**). Tetracycline and vancomycin have also showed deviations (1 out of 11 and 1 out of 12, respectively).

Table 12. 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. *	MIC **	Total
AMP	2/9	0/4	**2/14**
CHL	0/8	0/1	**2/11**
CIP	0/8	0/4	**0/14**
DAP	--	0/1	**0/1**
ERY	0/8	0/4	**0/13**
GEN	0/4	0/2	**0/16**
LZD	1/4	0/4	**1/10**
QND	--	0/1	**0/1**
TEI	0/4	0/2	**0/7**
TET	1/7	0/2	**1/11**
TGC	0/2	0/2	**0/4**
VAN	1/6	0/4	**1/12**

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

Only laboratories #35 and #52 presented deviations. They have used disk diffusion for testing, while the other 12 laboratories did use disk diffusion and/or MIC testing (**Figure 9**). Overall, the average deviation for this part of the panel was 4.4%.

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

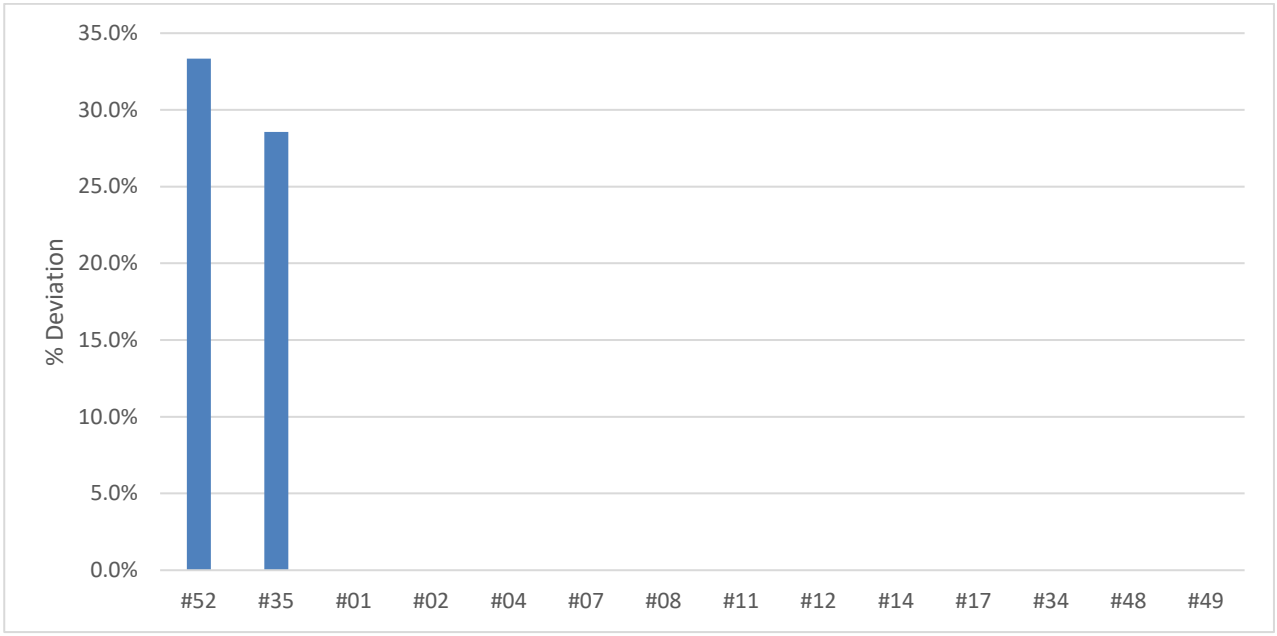

Figure 9. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 reference strains in the *E. faecium/ E. faecalis* panel by the HH laboratories.

3.4 *Campylobacter jejuni/coli* panel

Only 9 HH laboratories signed up for this part of the EQA9 panel. Overall, 3 laboratories submitted AST data. Four laboratories did not submit any data at all where three of them laboratories could not revive any of the panel strains.

3.4.1 Bacterial identification

Five participating laboratories submitted results for bacterial identification (**Table 13**). None of the laboratories correctly identified all seven strains in this panel. Among them, Laboratories #17 and #40 successfully revived all strains. Laboratory #17 correctly identified five isolates, while Laboratory #40 correctly identified four. Laboratories #34 and #35 accurately identified four out of the five strains they reported. Laboratory #04 identified one out of two reported isolates correctly.

Table 13. Bacterial identification of each of the seven test strains provided related to the *Campylobacter spp.* panel. Number of correct results out of the total of HH participating laboratories that submitted results for the respective strain is presented.

Strain	Bacterial ID	No. correct
Camp EQAsia 24.1	*Campylobacter jejuni*	2/5
Camp EQAsia 24.2	*Campylobacter coli*	4/5
Camp EQAsia 24.3	Non-*Campylobacter coli/jejuni*	3/5
Camp EQAsia 24.4	*Campylobacter jejuni*	3/5
Camp EQAsia 24.5	*Campylobacter coli*	3/5
Camp EQAsia 24.6	*Campylobacter jejuni*	3/5
Camp EQAsia 24.7	Non-*Campylobacter coli/jejuni*	3/5

Camp, C. jejuni/ C. coli

3.4.2 AST performance

In this subsection, the AST performance was analysed from a strain-, antimicrobial-, and laboratory-based perspective for a comprehensive overview. Only three laboratories submitted AST data for one or more of the expected target strains that could be analysed.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) for all five target strains reported was completely in line (100.0%) (**Table 14**).

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

Strain	AST in total	% Correct
Camp EQASIA 24.1	3	100.0%
Camp EQASIA 24.2	6	100.0%
Camp EQASIA 24.4	6	100.0%
Camp EQASIA 24.5	3	100.0%
Camp EQASIA 24.6	10	100.0%

Camp, C. jejuni/ C. coli

Antimicrobial-based analysis

The total number of antimicrobials tested was four (ciprofloxacin, erythromycin, gentamicin, and tetracycline). In total, there were only 25 available AST results to evaluate for the entire panel from the three labs that submitted AST data. No deviations were observed for any of the tested antimicrobials (**Figure 10**).

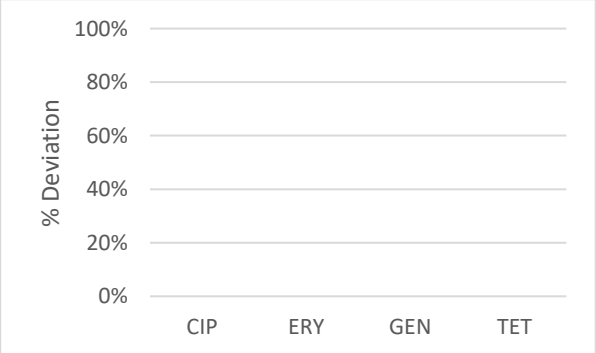

Figure 10. Percentage of deviation in the AST interpretation (R/S) among *C. jejuni/C. coli* strains by HH laboratories (n=3) participating in the 9th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

Laboratories #17, #35 and #40 who submitted

AST results for all the 4 antimicrobials did not show any deviations in their results (**Figure 11**).

Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among C. jejuni/ C. coli strains by HH laboratories (n=3) participating in the 9th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.4.3 Quality control strain *C. jejuni* ATCC 33560

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

The three participating laboratories (#17, #35 and #40) that submitted AST results used 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 3c**). Therefore, laboratories did not submit results for other antimicrobials (**Table 15**).

The three laboratories had no deviations in their expected results for the reference strain for these two antibiotics.

Table 15. 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 Diffusion	Total
CIP	0/3	0/3
ERY	0/3	0/3

Disk Diffusion – inhibition zone diameter determination by disk diffusion.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 16 Animal Health laboratories participating in the 9th EQA of the EQAsia Programme, 10 laboratories submitted results for the *Shigella* spp. trial, 10 for the *Enterococcus faecium/ E. faecalis* trial and 6 laboratories submitted results for the *Campylobacter jejuni/ C. coli* trial (Figure 12).

Applied AST methodologies for the three trials are presented in Figure 15. Disk diffusion as the sole method was the preferred choice for all the trials. Laboratory #18 was the only participant that used broth microdilution (automated). Laboratory #37 used a mixture of disk diffusion and broth microdilution. Laboratory #68 did not report AST results for *C. jejuni/ C. coli.*

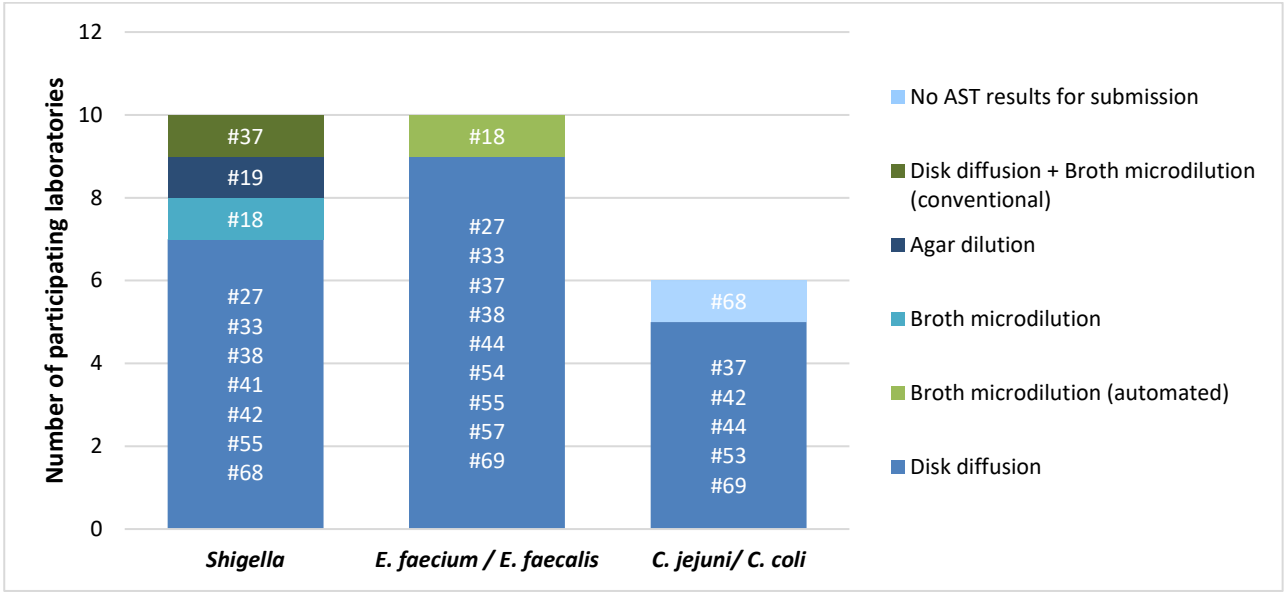

Figure 12. Methodologies applied by the AH laboratories participating for each of the panels.

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 majority of participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results (Figure 13).

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

For Gram negative bacteria Shigella trial (Table

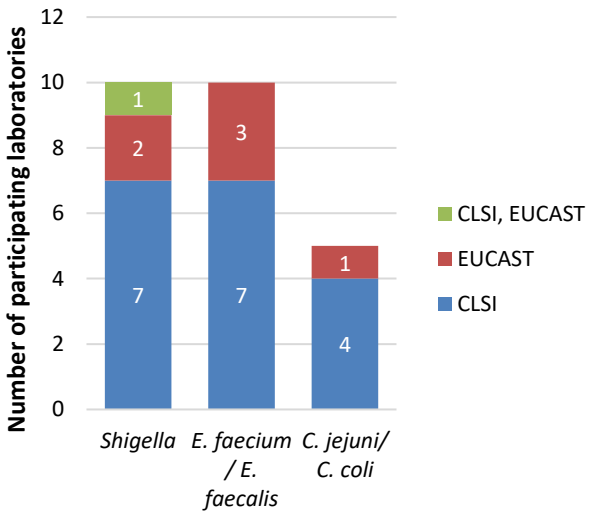

Figure 13. Use of international guidelines for interpretation of AST results by the participating laboratories.

16), ciprofloxacin and gentamicin were tested by most of the laboratories. In contrast, colistin and tigecycline were tested by less than half of the participating laboratories. For Gram-positive bacteria, ciprofloxacin, vancomycin and gentamicin were tested by most laboratories in the E. faecium/ E. faecalis panel, whereas daptomycin was tested by only one AH laboratory. Lastly, in the C. jejuni/ C. coli trial, ciprofloxacin, erythromycin, gentamicin and tetracycline were tested by all five participating laboratories, whereas chloramphenicol was tested by only two AH laboratories.

Table 16. Total of ASTs performed for each antimicrobial and in total for each of the panels by AH laboratories

	Shigella		***Enterococcus***		***Campylobacter***	
Amikacin	28	5.4%	-	-	-	
Ampicillin	42	8.0%	37	9.5%	-	-
Azithromycin	15	2.9%	-	-	-	-
Cefepime	19	3.6%	-	-	-	-
Cefotaxime	29	5.5%	-	-	-	-
Cefoxitin	23	4.4%	-	-	-	-
Ceftazidime	37	7.1%	-	-	-	-
Chloramphenicol	24	4.6%	40	10.3%	7	9.3%
Ciprofloxacin	48	9.2%	46	11.9%	17	22.7%
Colistin	10	1.9%	-	-	-	-
Daptomycin	-	-	2	0.5%	-	-
Ertapenem	18	3.4%	-	-	-	-
Erythromycin	-	-	40	10.3%	17	22.7%
Gentamicin	48	9.2%	42	10.8%	17	22.7%
Imipenem	24	4.6%	-	-	-	-
Levofloxacin	15	2.9%	-	-	-	
Linezolid	-	-	34	8.8%	-	-
Meropenem	33	6.3%	-	-	-	-
Nalidixic acid	29	5.5%	-	-	-	
Quinupristin/dalfopristin	-	-	10	2.6%	-	-
Teicoplanin	-	-	14	3.6%	-	-
Tetracycline	43	8.2%	41	10.6%	17	%
Tigecycline	10	1.9%	39	10.1%	-	-
Trimethoprim-Sulfamethoxazole	28	5.4%	-	-	-	-
Vancomycin	-	-	43	11.1%	-	-
Total	**523**		**388**		**75**	

Scattering of missing data or incomplete AST results entries were observed in the two trials (Tables 17, and 18). Four of the ten laboratories selecting *Shigella* did not submit complete results.

Regarding the *E. faecium/ E. faecalis* trial, three out of the ten participating laboratories revealed incomplete results of their own available antimicrobial agents (Table 4). Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance.

Table 17. Distribution of incomplete or missing data of antimicrobial agents among *Shigella* strains reported by AH laboratories (n=10) participating in the 9th EQA of the EQAsia project.

Lab ID No.	Shi EQAsia 24.1	Shi EQAsia 24.2	Shi EQAsia 24.5	Shi EQAsia 24.6	Shi EQAsia 24.7
#18	FEP, IMI	-	-	-	-
#27	-	FOT	FOT	FOT	AZI, FOT, ETP, MERO
#33	-	-	-	TAZ	-
#55	AMP, AZI	AZI	AZI	-	-

Shi, *Shigella*

Table 18. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by AH laboratories (n=10) participating in the 9th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 24.1	Ef EQAsia 24.2	Ef EQAsia 24.3	Ef EQAsia 24.4	Ef EQAsia 24.6
#18	DAP	DAP	-	-	DAP
#27	-	-	ERY	-	-
#57	-	-	-	CHL	-

Ef, *E. faecium/ E. faecalis*

4.2 *Shigella* spp. panel

Ten laboratories from six countries uploaded results for the Shigella trial.

4.2.1 Bacterial identification

Ten participating laboratories submitted results for bacterial identification (Table 19). The complete panel of five target *Shigella* strains was identified correctly by eight laboratories. Two non-*Shigella* strains (strain Shi EQAsia 24.3 and Shi EQAsia 24.4) were misidentified as *Shigella* by laboratory #41 (table 19).

Table 19. Bacterial identification of each of the seven test strains provided related to the *Shigella* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Shi EQAsia 24.1	*Shigella*	10/10
Shi EQAsia 24.2	*Shigella*	10/10
Shi EQAsia 24.3	Non-*Shigella*	7/10
Shi EQAsia 24.4	Non-*Shigella*	7/10
Shi EQAsia 24.5	*Shigella*	10/10
Shi EQAsia 24.6	*Shigella*	10/10
Shi EQAsia 24.7	*Shigella*	8/10

Shi, *Shigella*

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

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 90.7% (strain Shi EQAsia 24.1) to 95.5% (strain Shi EQAsia 24.7) for each strain (Table

20).

Table 20. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from fifteen AH laboratories for the *Shigella* trial.

Strain	AST in total	% Correct
Shi EQAsia 24.1	432	90.7
Shi EQAsia 24.2	444	92.6
Shi EQAsia 24.5	440	92.5
Shi EQAsia 24.6	440	93.9
Shi EQAsia 24.7	336	95.5

Shi, *Shigella*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were cefepime (23.7%), followed by levofloxacin (20.0%). In reverse, cefoxitin, imipenem and tigecycline revealed no deviation from the expected results (Figure 14).

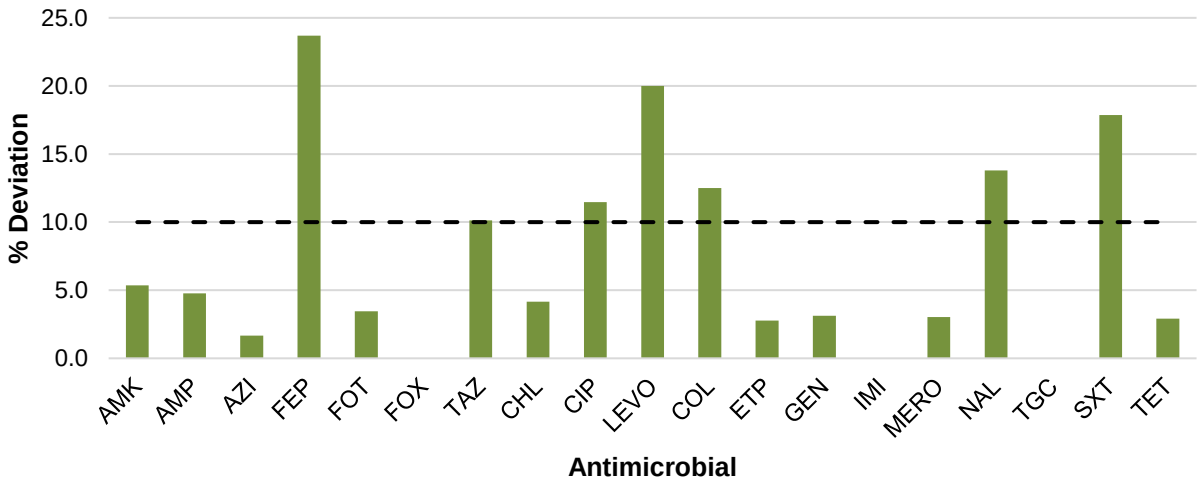

Figure 14. Percentage of deviation in the AST interpretation (R/I/S) among *Shigella* strains by AH laboratories (n=10) participating in the 9th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation equal or below to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for 5 out of the 10 participants (Figure 15). In average, the deviation was 7.3% (ranging from 1.8 to 18.6%). As the acceptance level was set to 5% deviation, 5 laboratories did not perform within the expected range for the trial.

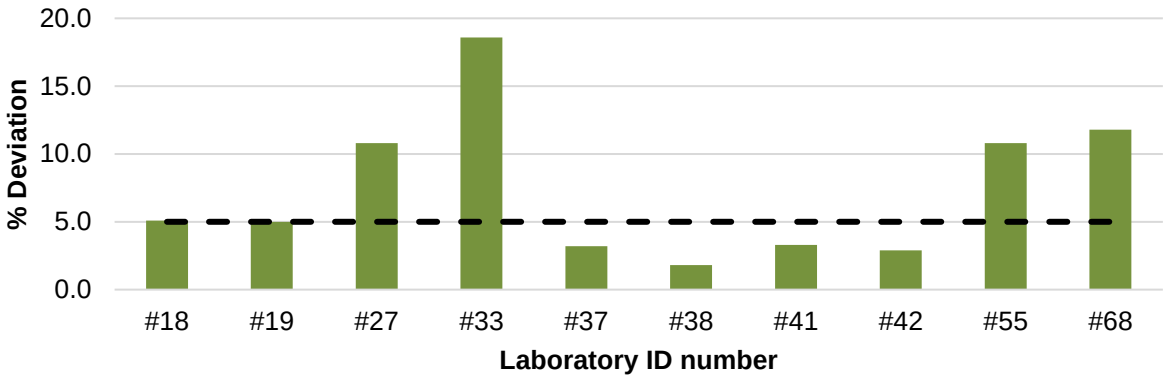

Figure 15. Percentage of deviation in the AST interpretation (R/I/S) among *Shigella* strains by AH laboratories (n=10) participating in the 9th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.2.4 β-lactamase-producing *Shigella*

None of the ten participating laboratories uploaded results for this component of the *Shigella* trial.

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

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in previous trials) to all participating laboratories to be used as reference strains for the *Shigella* trial.

Among the 10 participating laboratories, 9 submitted results for the reference strain *E. coli* ATCC 25922 and only one (#37) performed colistin testing and reported results for *E. coli* NCTC 13846.The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated and conventional) and agar dilution (Table 21). For testing *E. coli* NCTC 13846, MIC was determined by broth microdilution methods. The highest proportion of test results outside of the expected range was observed for ertapenem (2 out of 3), meropenem (3 out of 6) and cefepime (2 out of 4) (Table 21).

Regarding the laboratories' performance (Figure 16), laboratories #19 and #42 presented no deviation. While laboratories #19 applied agar dilution, laboratory #42 used disk diffusion. The remaining seven laboratories presented deviations that ranged from 10.0% to 38.5% (Figure 16). Overall, the average deviation for this part of the panel was 19.8%.

Table 21. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *Shigella* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMK	0/4	0/1	**0/5**
AMP	2/7	0/1	**2/8**
FEP	2/3	0/1	**2/4**
FOT	1/4	-	**1/4**
FOX	0/5	-	**0/5**

TAZ	2/5	0/1	**2/6**
CHL	1/4	-	**1/4**
CIP	1/7	1/2	**2/9**
COL	-	0/1	**0/1**
ETP	2/3	-	**2/3**
GEN	1/7	0/2	**1/9**
IMI	0/3	0/1	**0/4**
MERO	2/5	1/1	**3/6**
SMX	-	0/1	**0/1**
TET	3/7	0/1	**3/8**
TMP	0/1	-	**0/1**

Disk Diffusion – Inhibition Zone Diameter determination by Disk Diffusion;
MIC – MIC determination by broth macro- or microdilution, or by agar dilution.

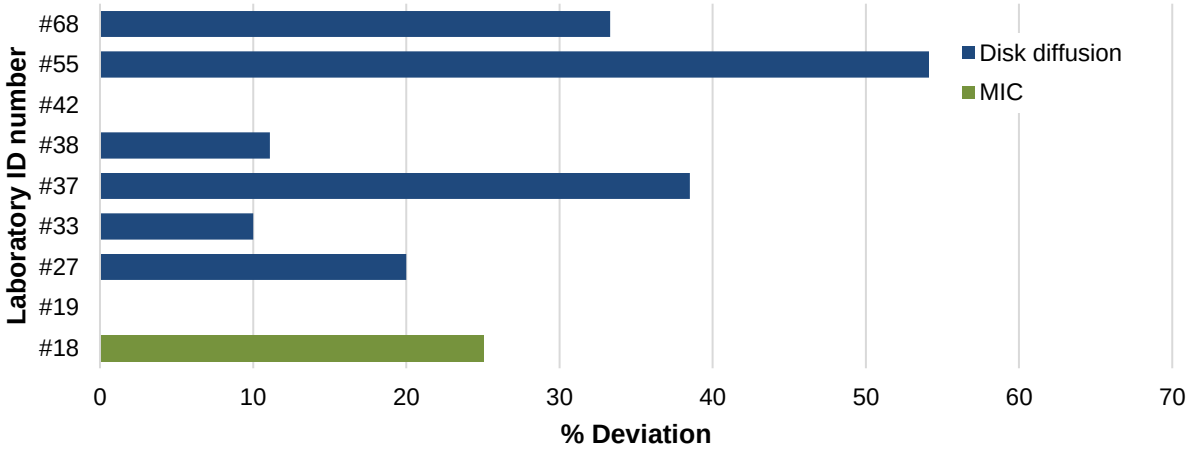

Figure 16. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Shigella* trial by the AH laboratories.

4.3 *Enterococcus faecium/ Enterococcus faecalis* panel

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

4.3.1 Bacterial identification

All ten participating laboratories submitted results for bacterial identification (Table 22). None of the laboratories could identify correctly all seven strains of this panel. Six out of eight laboratories misidentified Strain Ef EQAsia 24.6.

Table 22. 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 24.1	*Enterococcus faecium*	8/10
Ef EQAsia 24.2	*Enterococcus faecium*	7/9
Ef EQAsia 24.3	*Enterococcus faecalis*	9/10
Ef EQAsia 24.4	*Enterococcus faecalis*	8/10

Ef EQAsia 24.5	Non-*Enterococcus faecalis/faecium*	6/9
Ef EQAsia 24.6	*Enterococcus faecalis*	2/8
Ef EQAsia 24.7	Non-*Enterococcus faecalis/faecium*	5/6

Ef, *Enterococcus*

4.3.2 AST performance

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

Table 23. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 10 AH laboratories for the *E. faecium/ E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQAsia 24.1	332	90.1
Ef EQAsia 24.2	304	87.2
Ef EQAsia 24.3	308	87.3
Ef EQAsia 24.4	308	84.7
Ef EQAsia 24.6	300	74.3

Ef, *Enterococcus*

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 74.3% (strain Ef EQASIA 24.6) to 90.1% (strain Ef EQASIA 24.1) for each strain (Table 23).

Antimicrobial-based analysis

Antimicrobials with the highest deviations from the expected results were quinupristin and dalfopristin (37.5%) and ampicillin (25.7%), whereas chloramphenicol, erythromycin, and gentamicin showed deviations of less than 10% from the expected results (Figure 17).

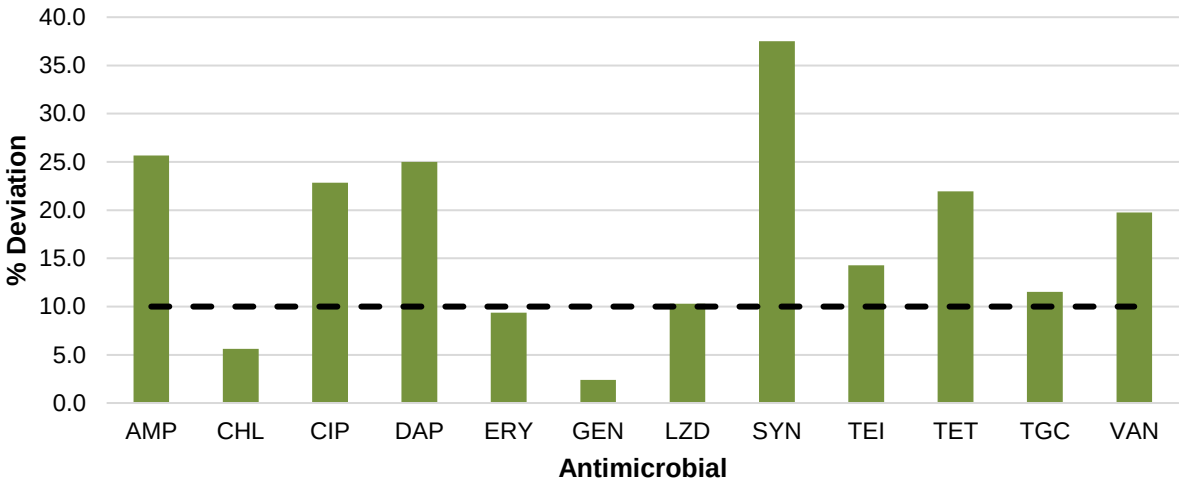

Figure 17. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=10) participating in the 9th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

All the laboratories had a deviation above 5% in their performance in terms of interpretation of the results (R/S) (Figure 18). On average, the deviation was 15.2% (ranging from 6.5% to 32.2%).

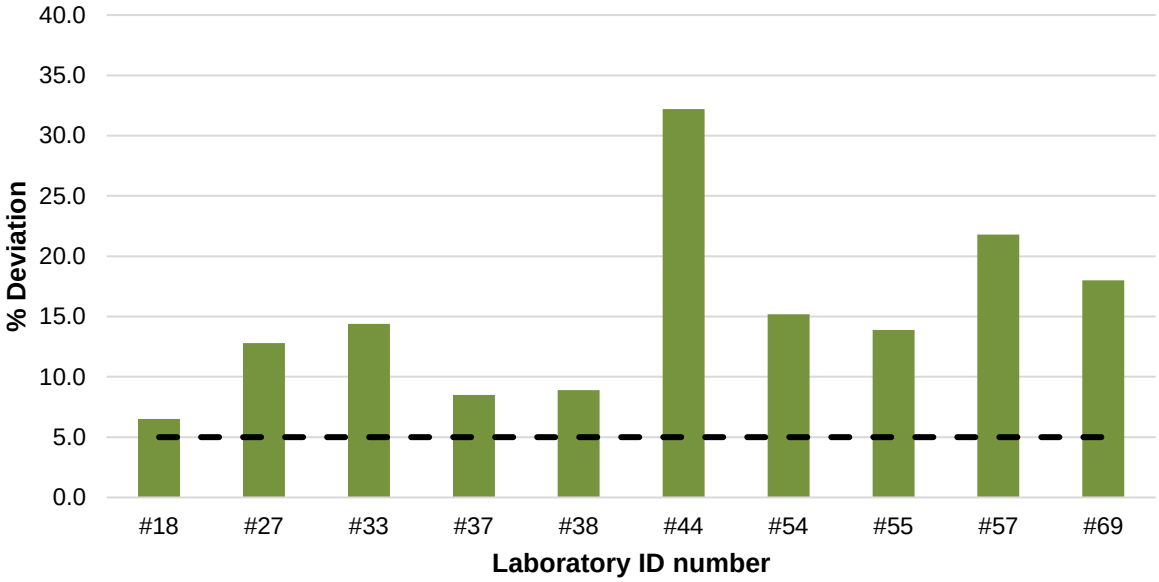

Figure 18. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=10) participating in the 9th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 previous trials) to all participating laboratories to be used as reference strains for the *E. faecium/ E. faecalis* trial.

Among the ten participating laboratories, seven submitted results for the reference strain. Different methodologies for testing the reference strain *E. faecalis ATCC 29212* were applied: MIC was determined by broth microdilution (Table 24, **). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine Inhibition Zone Diameters by disk diffusion (Table 24, *).

The highest proportion of test results outside of the expected range was observed for vancomycin (3 out of 4), linezolid (1 out of 3) and tigecycline (1 out of 3) (Table 24).

Regarding the laboratories' performance (Figure 21), laboratories #18 and #44 presented no deviation. The other five laboratories had deviations ranging from 12.5% to 30.0% (Figure 19). In this panel, all the reported deviations were above the acceptance interval.

Table 24. 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.

Antimicrobial	Proportion outside of range		
	Disk Diff. *	MIC **	Total
AMP	2/6	--	**2/6**
CHL	0/5	--	**0/5**
CIP	0/5	0/1	**0/6**
DAP	--	0/1	**0/1**
ERY	0/5	0/1	**0/6**
GEN	1/6	--	**1/6**
LZD	1/2	0/1	**1/3**
SYN	1/1	--	**1/1**
TET	0/4	0/1	**0/5**
TGC	1/2	0/1	**1/3**
VAN	3/3	0/1	**3/4**

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

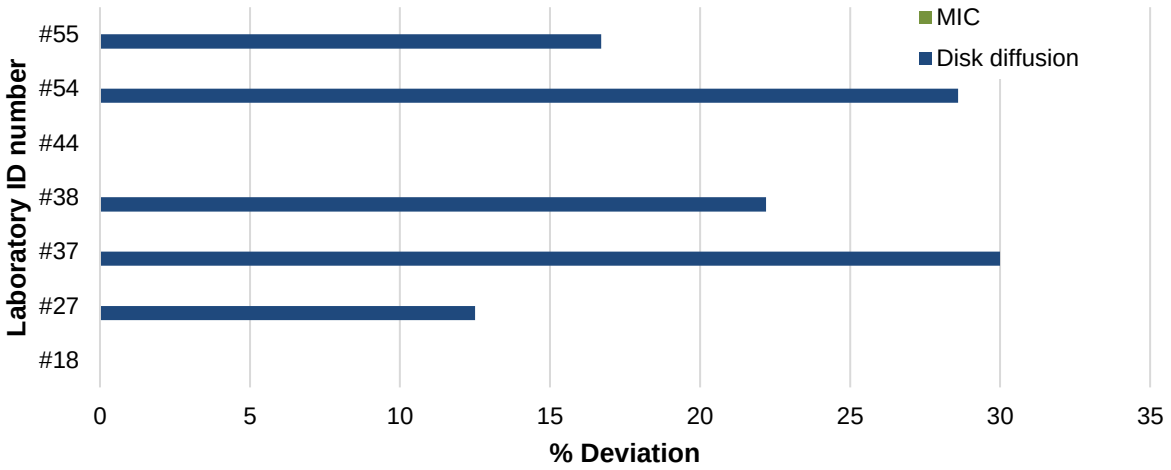

Figure 19. Percentage of deviation in the AST *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* trial by the AH laboratories.

4.4 *Campylobacter jejuni/coli* panel

Six laboratories from five countries uploaded results for the *C. jejuni/ C. coli* trial.

4.4.1 Bacterial identification

Six participating laboratories submitted results for bacterial identification (Table 25). None of the laboratories identify correctly all seven strains of this panel. All of laboratories misidentified Strain Camp EQAsia 24.7.

Table 25. Bacterial identification of each of the six 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
Camp EQAsia 24.1	*Campylobacter jejuni*	2/6
Camp EQAsia 24.2	*Campylobacter coli*	4/6
Camp EQAsia 24.3	Non-*Campylobacter coli/jejuni*	5/6
Camp EQAsia 24.4	*Campylobacter jejuni*	2/4
Camp EQAsia 24.5	*Campylobacter coli*	4/6
Camp EQAsia 24.6	*Campylobacter jejuni*	3/5
Camp EQAsia 24.7	Non-*Campylobacter coli/jejuni*	0/4

Camp, *C. jejuni/ C. Coli*

4.4.2 AST performance

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

Strain-based analysis

Laboratory #68 did not submit the results for AST. The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 51.9% (strain Camp EQAsia 24.4) to 87.5% (strain Camp EQAsia 24.5) for each strain (Table 26).

Table 26. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 5 AH laboratories for the *C. jejuni/ C. coli* trial.

Strain	AST in total	% Correct
Camp EQAsia 24.1	36	75.0
Camp EQAsia 24.2	88	80.7
Camp EQAsia 24.4	52	51.9
Camp EQAsia 24.5	88	87.5
Camp EQAsia 24.6	36	83.3

Camp, *C. jejuni/ C. coli*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were ciprofloxacin (30.9%), erythromycin (23.5%) and tetracycline (23.5%) (Figure 20). Only chloramphenicol revealed no deviation from the expected results.

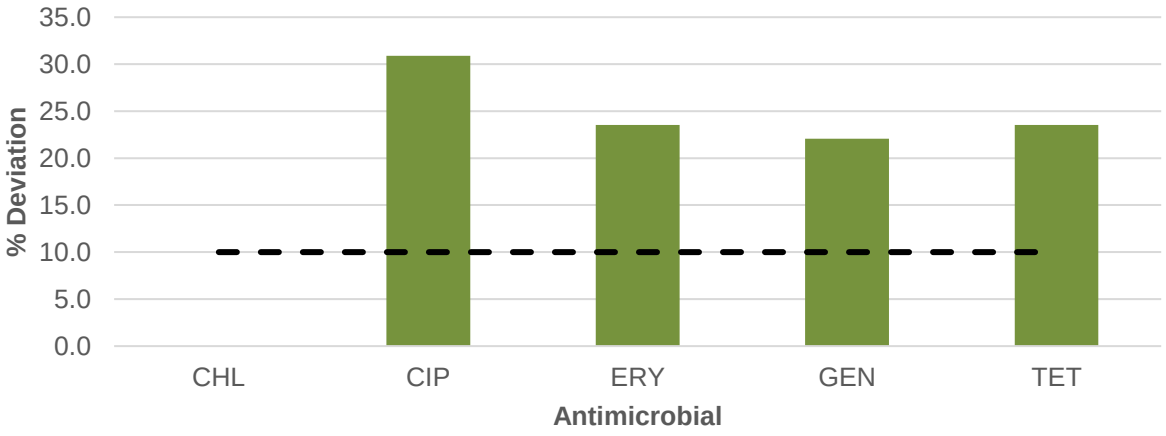

Figure 20. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=5) participating in the 9th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for two out of the five participants (Figure 21). Laboratory #37 presented the highest deviation.

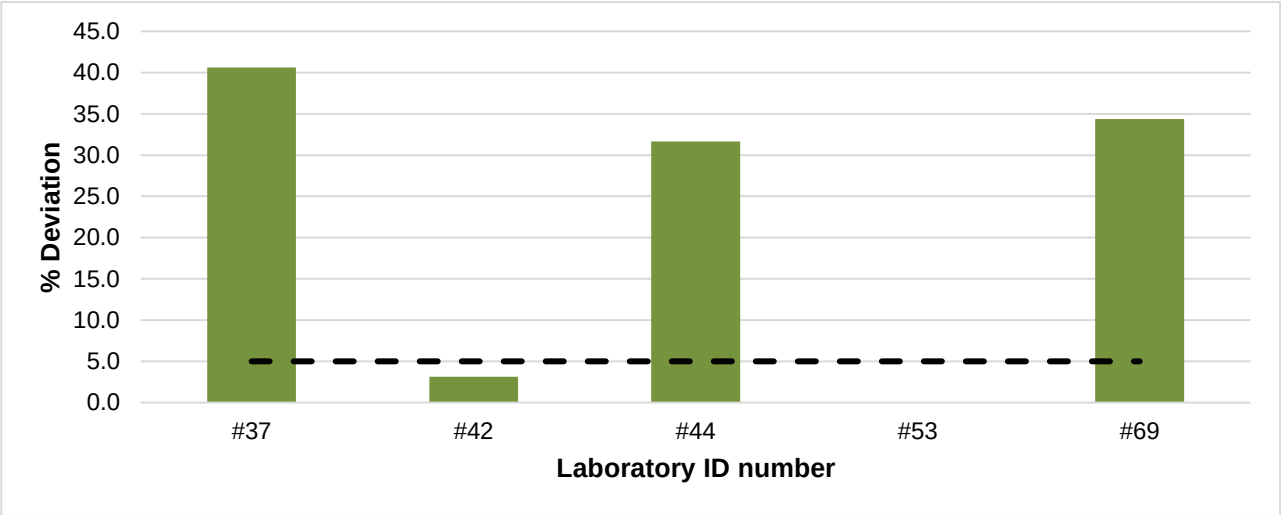

Figure 21. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=5) participating in the 9th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strain *C. jejuni* ATCC 33560

The quality control strain *C. jejun*i ATCC 33560 was sent to all participating laboratories free of charge to be used as a reference strain for the *C. jejuni/ C. coli* trial. Among the four participating laboratories, three submitted results for the reference strain *C. jejun*i ATCC 33560. There were no deviations in the QC results reported by the participating laboratories (Table 26).

In terms of performance, laboratories #53 and #69 showed no deviations for the two antimicrobials tested (Figure 21).

Table 26. AST of the reference strain *C. jejun*i 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	0/2	**0/2**

Disk Diffusion – inhibition zone diameter determination by disk diffusion

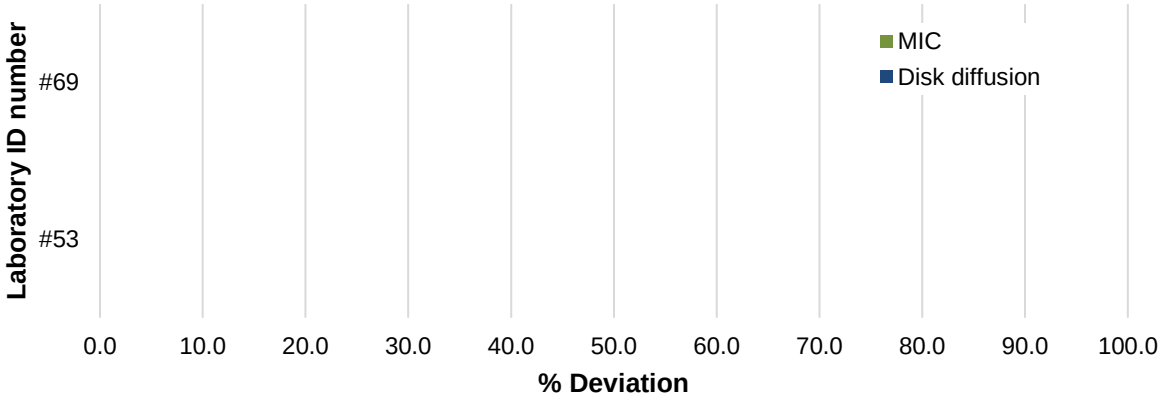

Figure 21. Percentage of deviation in the AST of *C. jejun*i ATCC 33560 in the *C. jejuni/ C. coli* panel by the AH laboratories.

5. Results – Overall

5.1 Bacterial identification

A total of 34 HH and 16 AH laboratories participated in this EQA trial. The participating laboratories were from 14 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam). In total, data were submitted by 42 laboratories for the *Shigella spp.* panel, 30 laboratories for the *E. faecalis/E. faecium* panel, and 10 for *Campylobacter spp.*.

Considering the test strains tested by each laboratory in each of the trials, it was possible to calculate the percentage of incorrectly identified isolates. **Figure 22** shows the distribution of laboratories that had a deviation for each of the panels.

Minor deviations were observed in the submitted data by very few laboratories for the bacterial identification component of the target strains in the *Shigella spp.* panel. To the contrary, laboratories were divided in the data they reported for the *E. faecalis/E. faecium*, and *Campylobacter spp.*. For Enterococci, none of the laboratory correctly identified reported strains. The difficulty to revive several *Campylobacter* have led to skewed results in addition, to the challenge faced by several laboratories to identify the target strains correctly.

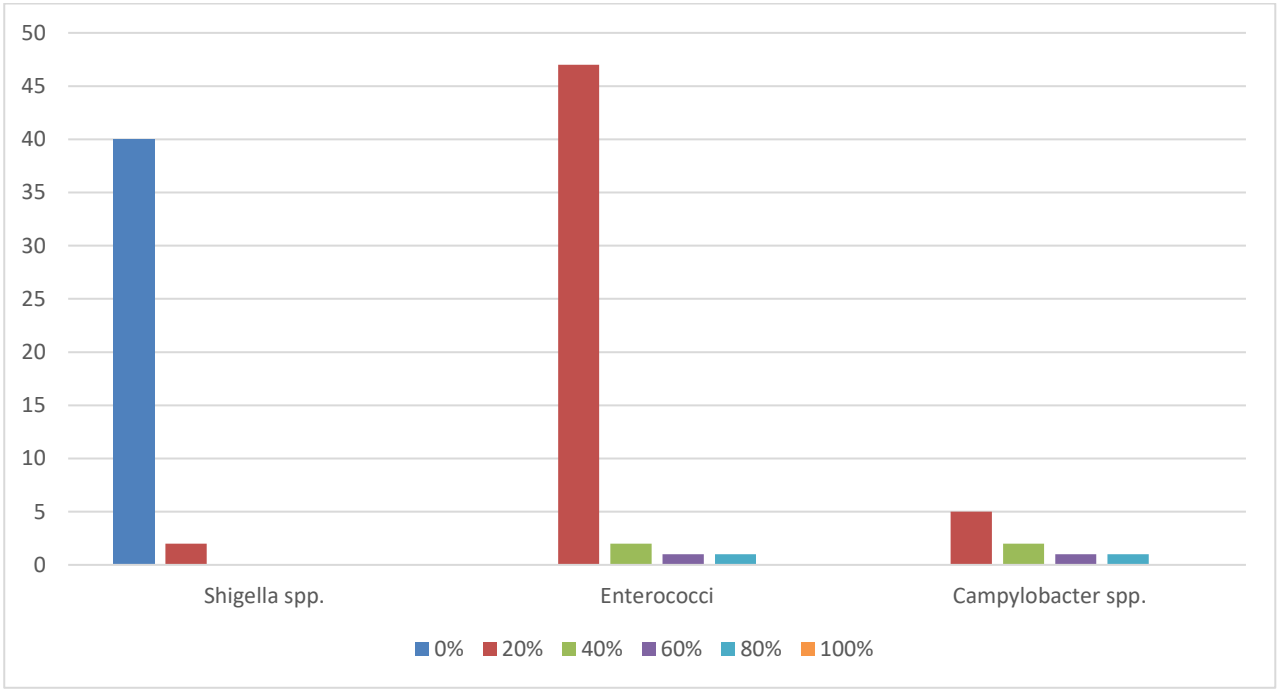

Figure 22. Percentage of deviation in the bacterial identification of target strains in the *Shigella spp.*, *E. faecalis/E. faecium* and *Campylobacter spp.,* panels by the participating laboratories.

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 panels, the antimicrobials were tested by a varying number of laboratories.

There were several deviations from the expected results in the *Shigella spp.* panel mainly attributed to levofloxacin and colistin (57.8% and 50.0%, respectively). The recent update in the CLSI guidelines reflecting new breakpoints for aminoglycosides for Shigella might partially explain this deviation (**Figure 23**). All other antimicrobials showed deviations below 40%.

The results submitted for the enterococci panel showed most deviations for quinupristin and dalfopristin and daptomycin (53.5% and 46.2%, respectively) mainly because of the low number of tests performed (**Figure 24)**. Other antimicrobials with high percentage of deviations were vancomycin (41.5%%) and ciprofloxacin (32.1%).

Figures 23-25 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).

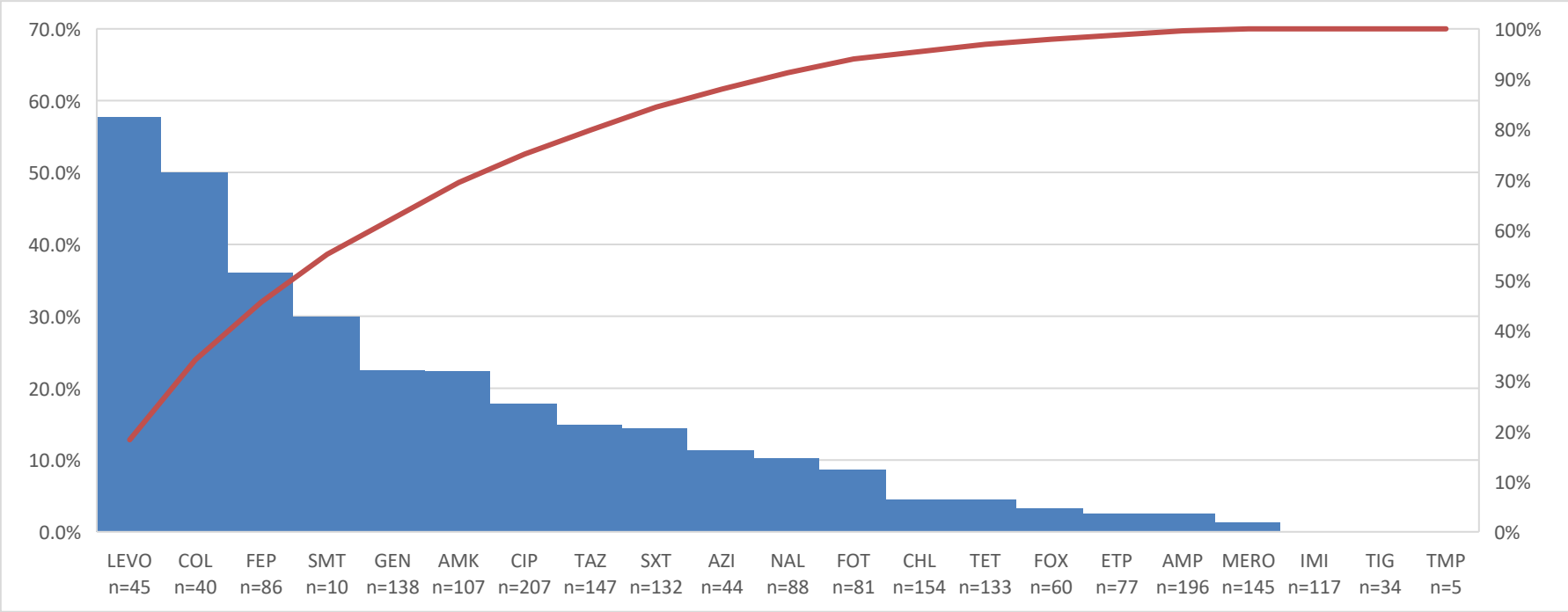

Figure 23. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Shigella spp.* strains by the participating laboratories (n=42) in the 9th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

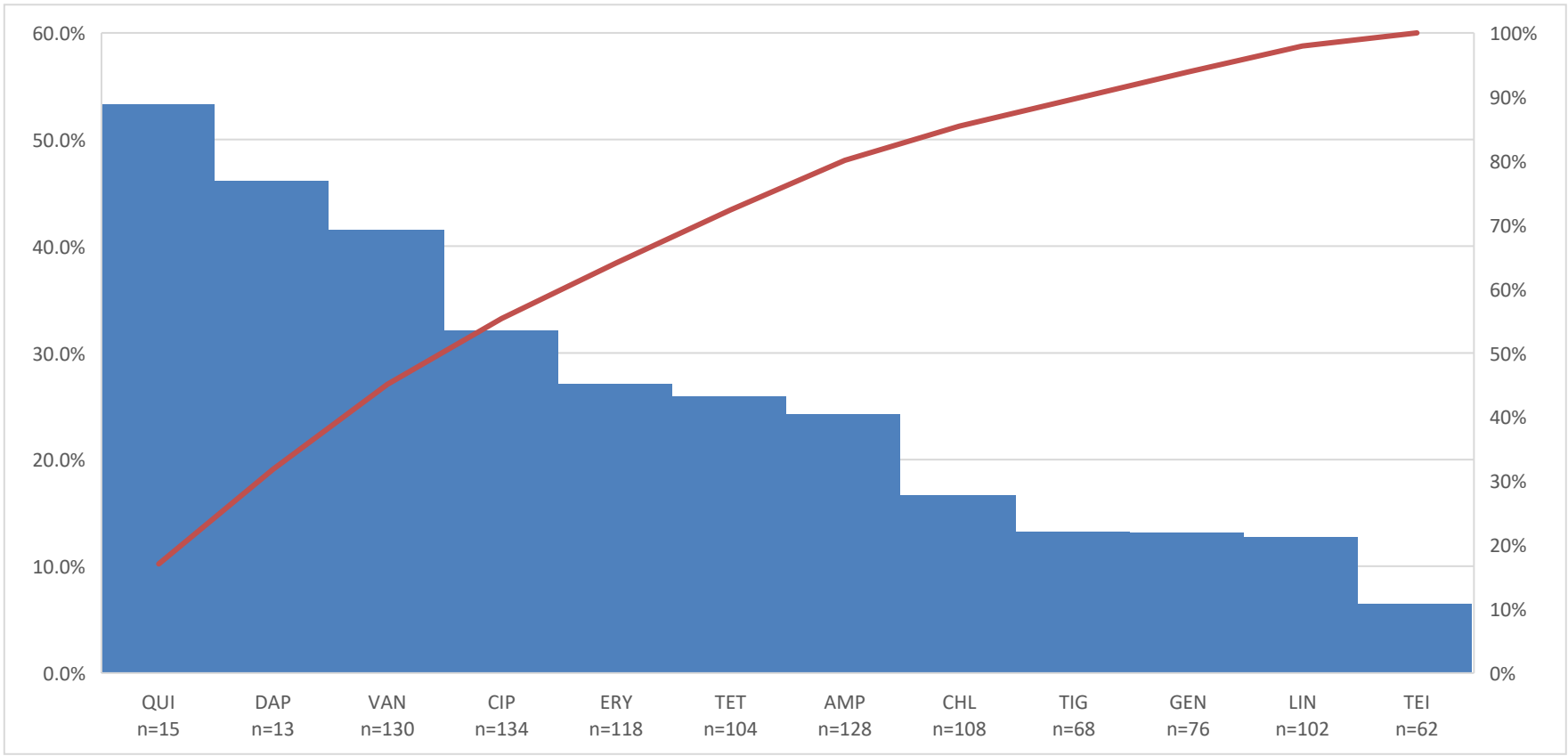

Figure 24. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. faecalis/E. faecium* strains by the participating laboratories (n=30) in the 9th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

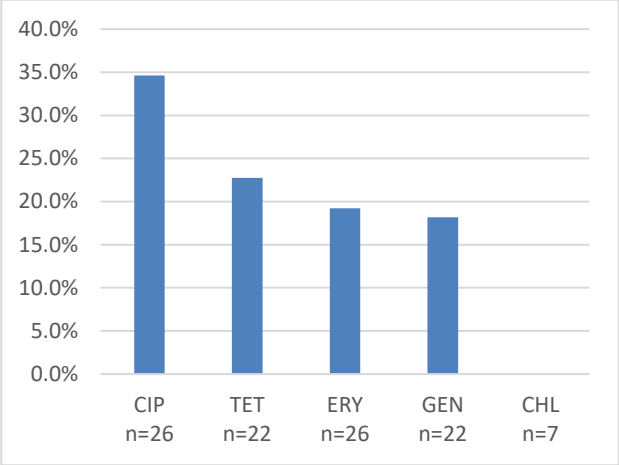

Figure 25. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Campylobacter spp*. strains by the participating laboratories (n=10) in the 9th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation.

There were only 58 AST results that were submitted and scored in the *Campylobacter spp.* panel. The low overall number of results is partially the reason for high percentage of deviations, mostly for and ciprofloxacin (34.6%) (**Figure 25)**. All other results showed deviations of less than 25% while chloramphenicol shows no deviation.

5.2.2 Laboratories performance

In each of the panels, the overall performance of laboratories varied according to their performance score. There was more heterogeneity between the laboratories in the *Campylobacter spp.* panel (**Figure 26**).

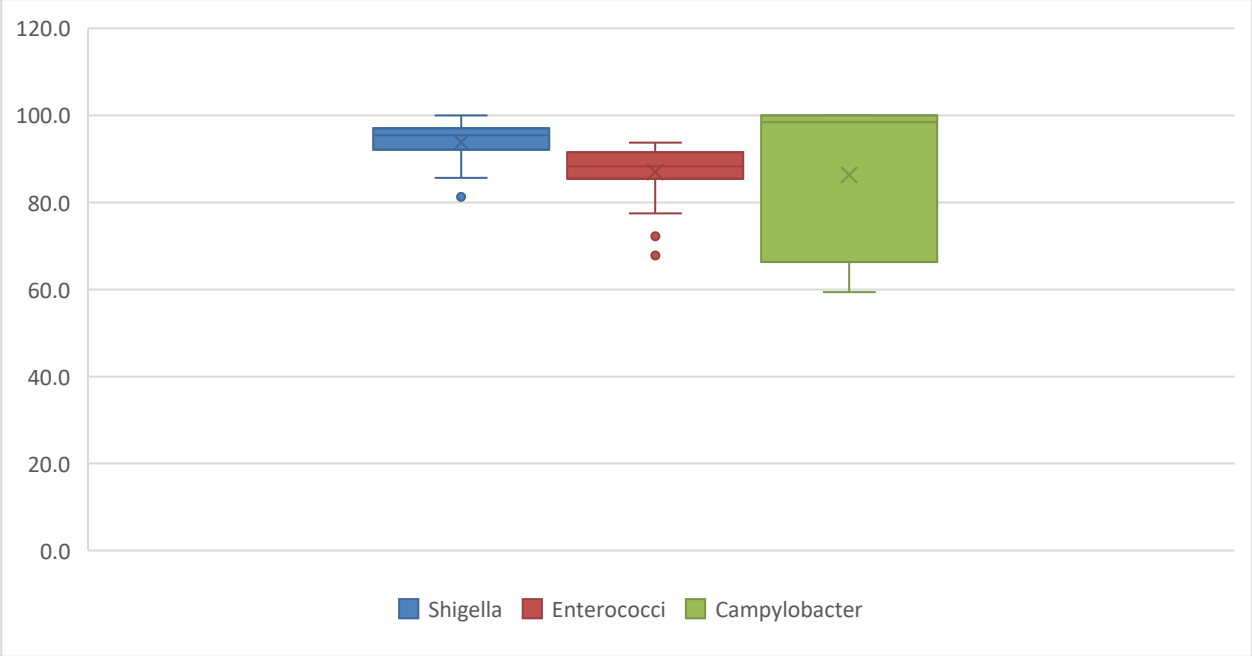

Figure 26. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 9th EQA of the EQAsia project.

Out of the three panels included in this trial, the obtained results were the best for the *Shigella spp.* panel (average score 93.8%). The labs with minimum score in this panel had a performance rate of 81.4%. The lowest performance score in the *Campylobacter spp.* panel was 59.4%, while for the enterococci panel – 67.8%.

Laboratories were ranked (#1 to #36) based on their average score across the panels in which they participated and submitted results for. The average score varied between 68.1% (rank #36) and 100% (rank #1). The total average score among all participating laboratories that submitted results was 93.4%, while the median was 93.3%.

Overall, a large heterogeneity was observed in this EQA trial which suggests once again that the level of proficiency varies greatly among the participating laboratories.

5.3 Quality control strains

Relevant quality control strains were tested for each of the panels: *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were used as reference strains for the *Shigella spp.* panel, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disk diffusion) and *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) – for the enterococci panel, *Campylobacter jejuni* ATCC 33560/ CCM 6214 for the *Campylobacter spp.* panel, and *Neisseria gonorrhoeae* ATCC49226, WHO G, WHO L, WHO O and WHO P for the *N. gonorrhoeae* panel.

As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (6 in the *Shigella spp.* panel, 4 in the enterococci panel and 6 in the *Campylobacter spp.* panel) did not submit results from reference strain testing at all. For the *Shigella spp.* EQA round, there were 13

laboratories (10 HH and 4 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=23) presented deviations between 7.7% and 100%. 14 laboratories (12 HH and 2 AH) showed no deviations in the reference strain testing in the enterococci panel. The remaining 7 laboratories submitted results that deviated between 12.5% and 30%. To the contrary, all the results submitted in the reference strain testing in the *Campylobacter* panel were according to the expected ranges.

Compared to the submitted AST results of the target strains, the results from the testing of the quality control strains were more heterogeneous and led to a much lower performance score in this component of the EQA trial. The greatest heterogeneity was observed in the *Shigella spp.* panel and partly also in the enterococci panel (**Figure 27**). The minimum score in the *Shigella spp.* panel was 0%, while in the enterococci panel it was 66.7%. The laboratories participating in the *Campylobacter* panel submitted a set of results that was within the expected values .

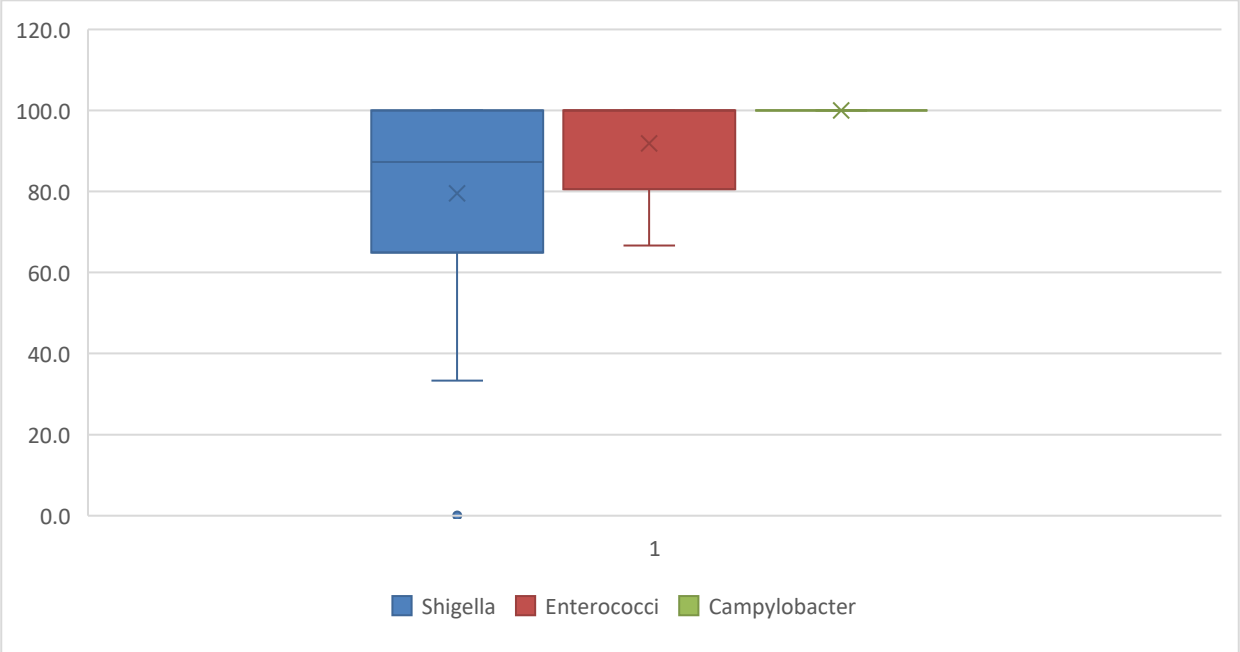

Figure 27. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 9th EQA of the EQAsia project.

6. Discussion

6.1 Human Health Laboratories

Overall, 34 Human Health laboratories participated in the 9th EQA of the EQAsia project and submitted EQA results for one or more EQA panels. Disk diffusion was chosen most frequently as a methodology for testing the recommended antimicrobials in each of the panels. Several laboratories performed MIC determination methods or a combination of disk diffusion and MIC testing by either gradient test or broth microdilution.

All laboratories that performed bacterial identification in the *Shigella spp.* and enterococci panels have also submitted AST results. However, this was not the case in the *Campylobacter spp*. Several isolates in these panels could not be revived by some of the laboratories or the reported identification of the revived isolates did not always match the baseline results. Attention should be paid to the use of appropriate media and following the protocol to reconstitute lyophilized bacteria, as these could be some of the main reasons why several laboratories were not able to cultivate isolates from the *Campylobacter spp.* panels.

Incomplete AST results' entries were observed in all panels, except *Campylobacter spp.* where only 3 HH laboratories participated. Two out of 20 HH laboratories that selected the enterococci panel 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.

The EQA participants showed high proficiency in correctly identifying the isolates in the *Shigella spp.* panel. In the other two panels, the bacterial identification success rate varied. The identification and differentiation between *E. faecium, E. faecalis* and other *Enterococcus* species appeared to be challenging for all of 20 participating laboratories whose results did not match the baseline for this panel. This underlines the need for targeted training on this particular species and the importance of the correct identification also related with antimicrobial susceptibility testing and possible resistance mechanisms.

The antimicrobial susceptibility testing performance was assessed from different angles to better identify deviations from the expected results.

For the *Shigella spp.* panel, some antimicrobials presented a high deviation from the expected results, such as: ampikacin (25.3%), azithromycin (13.8%), cefepime (31.3%), cefotaxime (11.5%), ceftazidime (13.6%), ciprofloxacin (15.1%), colistin (50%), gentamicin (30%), levofloxacin (56.7%), sulfamethoxazole (30%) and Trimethoprim/Sulfamethoxazole (11.5%). The AST results in the enterococci panel also showed deviations from the baseline expected results for all antimicrobials ranging from 10.3% for ciprofloxacin and tigecycline, to 40% for Quinupristin and dalfopristin. Only teicoplanin showed a deviation less than 5% (2.1%). The total number of tested antimicrobials in the *Campylobacter spp.* panels was relatively low but the performance was 100%.

On average, the AST performance of participating laboratories was the best in the *Campylobacter spp.* (100%), followed by *Shigella spp.* panel (94.1%) and enterococci (88%).

Detection and confirmation of presumptive beta-lactamase producing *Shigella spp.* was an optional component of this EQA and laboratories opted out and did not submit data for it.

Among all HH laboratories, there were a few that did not submit antimicrobial susceptibility testing results for the quality control strains across all three panels. According to the CLSI recommendations, 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.

The inability to recover *Neisseria gonorrhoeae* strains in this trial was not due to the lack of capacity of the participants but rather due to the shipment procedure. This highlights the critical importance of optimizing preservation and shipment processes to maintain strain viability. The strong participation in this panel, with 15 HH laboratories enrolling, underscores the need for continued efforts to ensure reliable distribution of strains for external quality assessment.

This challenge has provided valuable insights for refining preservation methods, quality control measures, and shipment strategies. Efforts are currently underway to validate alternative approaches and introduce pre-distribution viability testing to prevent similar issues in future rounds. By implementing these improvements, upcoming EQA trials will better support laboratories in assessing their performance in *Neisseria gonorrhoeae* identification and AST, ultimately strengthening diagnostic capacity in the region.

6.2 Animal Health Laboratories

For the Animal Health sector, 16 laboratories participated in the 9th EQA of the EQAsia project. The participating laboratories mostly applied disk diffusion alone for determining Inhibition Zone Diameters, others opted for agar dilution, broth microdilution or a mixture of the two methodologies.

The participants were asked to firstly perform bacterial identification and then proceed with AST of the target strains. Incomplete AST results' entries were observed in all panels, except the *C. jejuni/ C. coli* panel. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance. Although laboratory #68 performed bacterial identification, they did not submit AST results for the *C. jejuni/ C. coli* trial.

As mentioned above, bacterial identification was the first component in each of the panels. There were no major issues with bacterial identification of the five target strains among the seven isolates provided for *Shigella* panel. 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, suggesting that advice and training on the subject may be required among the AH laboratories. Similarly, all six laboratories that participated and submitted results to the *C. jejuni/ C. coli* trial demonstrated limitations on differentiation between *C. jejuni*, *C. coli* and other *Campylobacter* species.

For the antimicrobial susceptibility testing performance, cefepime presented quite high deviations in the *Shigella* panels (23.7%). In 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. faecalis* strains identified as *E. faecium*, and vice-versa), since the interpretation criteria is not substantially different for these two species; here, the highest deviations (quinupristin/dalfopristin and daptomycin) can be explained by the fact that these antimicrobials were tested by few laboratories. The AST deviations observed in the *C. jejuni/C. coli* trial were quite high for four of the five tested drugs (ciprofloxacin, erythromycin, gentamicin and tetracycline).
Regarding laboratories' performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. A deviation equal or below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for five out of the ten participants in the *Shigella* panel and for two participants in the *C. jejuni/C. coli* trial. All laboratories showed a deviation greater than 5% in the *E. faecium/E.*

faecalis trial.
None out of the ten participating laboratories in the *Shigella* panel submitted results for the detection and confirmation of presumptive beta-lactamase producing bacteria.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the panels. Nine out of ten participating laboratories submitted results for the reference strains in the *Shigella* panels. Three laboratories did not submit results for the *S. aureus* ATCC 25923 or *E. faecalis* ATCC 29212 reference strain in *E. faecium/ E. faecalis* trial. Two out of five participating laboratories submitted results for *C. jejuni* ATCC 33560. 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, which suggests that handling of reference strains needs to be strengthened to ensure the laboratories' good performance.

7. Conclusions

This report presents the results of the EQAsia 9th EQA trial, which was carried out in October – November 2024 and included bacterial identification and antimicrobial susceptibility testing (AST) of several prominent WHO and FAO priority pathogens: *Shigella spp., Enterococcus faecalis/ Enterococcus faecium, Campylobacter coli/ Campylobacter jejuni,* and *Neisseria gonorrhoeae.*

An ultimate goal of EQAsia is to enable EQA participation to both Human and Food and Animal Health laboratories and to assist them along their way to performing accurate bacterial identification and antimicrobial susceptibility testing of the offered pathogens. As in previous EQAsia EQAs, any result deviation level below 5% was tackled on an individual laboratory level and underperformance was addressed by providing additional support, feedback and 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 building further capacity in the reference laboratories in the South and Southeast Asian region.

In terms of bacterial identification, the pathogens included in this trial presented a lower degree of difficulty compared to previous panels. Identification remained particularly challenging in the *Campylobacter spp.* panel. Notably, all participating laboratories successfully revived *Enterococcus spp.*, marking an improvement from previous EQA trials.

The *Neisseria gonorrhoeae* panel was introduced for the second time since the start of the EQAsia project. While laboratories have previously faced challenges in handling this pathogen, this trial was particularly impacted by strain viability issues, preventing successful revival and identification

For this trial, the submitted data, incl. 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, there were several misinterpretations of the MIC/ inhibition zone diameter values in the reported results, especially in the enterococci and *Campylobacter spp.* panels, demonstrating lower level of proficiency of some of the participating laboratories. This EQA exercise also revealed

the need to place a special emphasis on detecting and identifying fastidious microorganisms. Capacity building is further needed in this direction since several laboratories were unable to reconstitute and isolate a number of strains from the *Campylobacter spp.* panel. It is also a requirement that all participating laboratories follow the same protocol and interpretation criteria to allow for comparison of results.

Antimicrobial susceptibility testing of the reference strains is also highly important and, therefore, largely recommended. Relevant reference strains have been sent to the participating laboratories during previous EQA rounds free of charge to be used not only in the EQAsia EQAs, but also in the routine work. Several reference strains for the microbiology diagnostics of gonococci were sent to participating laboratories for the first time within this EQA round. Laboratories need to make sure they have all necessary quality control strains that should be tested on a regular basis. 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. Furthermore, action needs to be taken every time the results from the quality control testing deviate from the ranges set in the methodological standards used. EQAsia has also prioritized quality control of AST as a training topic and is offering continuous support on this matter.

Overall, the results from this EQAsia EQA flag once more the need to focus on both basic and more advance methodologies within a training curriculum for the participating laboratories. Quality control testing and the use of the appropriate reference strains, as well as the translation of the QC results into action by laboratories is of utmost importance to ensure a decent level of quality in a microbiology laboratory. Providing and maintaining a standardized level of credible diagnostic services would allow laboratories to generate reliable results that would ultimately feed into a pool of reliable data for surveillance of AMR.

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.

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

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[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 13.0, 2023. http://www.eucast.org.

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

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

9. Appendices

Appendix 1: EQA9 Protocol

EQAsia EQA9 trial

Protocol

Identification and antimicrobial susceptibility testing (AST) of *Shigella spp., Enterococcus spp., Campylobacter spp.* and *Neisseria gonorrhoeae* test strains

1. INTRODUCTION

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

The **EQAsia EQA9 trial** includes four EQA panels each composed of seven test strains – *Shigella spp.*, *Enterococcus spp. (Enterococcus faecalis* and *Enterococcus faecium), Campylobacter spp. (Campylobacter coli* and *Campylobacter jejuni),* and *Neisseria gonorrhoeae*, respectively. Each of the four panels includes five strains of the targeted species and two non-target strains. Participating laboratories are asked to perform identification of all seven test strains from the panels they signed up for, as well as antimicrobial susceptibility testing (AST) only on the five target strains in each panel.

Additionally, AST of the relevant reference strains for quality control (QC) is also part of each EQA trial round. The QC reference strains supplied during previous EQA rounds are *Escherichia coli* ATCC 25922/CCM 3954 (for disk diffusion of Salmonella strains), *E. coli* NCTC 13846/CCM 8874 (for testing colistin), *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).

The QC strains provided within EQA9 include *Neisseria gonorrhoeae* ATCC49226, WHO G, WHO L, WHO O and WHO P and will be sent along with the *N. gonorrhoea*e test strains to all the laboratories that requested to participate in this panel.

All of the 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.

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 *Shigella spp.*, *Enterococcus spp. (Enterococcus faecalis* and *Enterococcus faecium), Campylobacter spp. (Campylobacter coli* and *Campylobacter jejuni),* and *Neisseria gonorrhoeae*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3. EQA9 OUTLINE

3.1.Shipping and receipt of strains

Your laboratory is one of the 56 human health and animal health laboratories from South and Southeast Asia participating in EQA9. In October 2024, you are expected to receive a parcel containing one or more of the following panels:

- ***Salmonella* panel** - seven test strains of which five are *Shigella spp.* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) reference strains have been provided in previous EQA rounds.

- ***Enterococcus* panel** - seven test strains of which five are *E. faecium* or *E. faecalis* and two are non-target species. The *Staphylococcus aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) reference strains have been provided in previous EQA rounds.

- ***Campylobacter* panel** - seven test strains of which five are *C. coli* or *C. jejuni* and two are non-target species. The *Campylobacter jejuni* ATCC 33560/ CCM 6214 reference strain has been provided in a previous EQA round.

- ***Neisseria gonorrhoeae* panel** - seven test strains of which five are *N. gonorrhoeae* and two are non-target species. The *Neisseria gonorrhoeae* ATCC49226, WHO G, WHO L, WHO O and WHO P reference strains are provided within this EQA round.

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

N.B.!!! The Campylobacter and N. gonorrhoeae panel strains are shipped lyophilized. The Shigella and Enterococcus strains are shipped on media in transport tubes (swabs).

3.2.Reviving and storing the strains

The **lyophilized strains** must be stored in a dark, cool place. The strains must be sub-cultured and prepared for storage in your strain collection (e.g., in a -80°C freezer). The stored test strains 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 also serve as reference material available at a later stage, when needed.

- **Reviving Enterococcus and Campylobacter lyophilised cultures**

Aseptic technique must be applied throughout. All testing should be performed in a BSL2 level laboratory or in a biosafety cabinet class II.

Needed material:

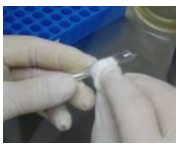
- An ampoule cutter or a file
- Sterile Luria Bertani (LB) broth
- LB agar plates (5 to 6 plates per one strain)
- Columbia broth for Campylobacter
- mCCDA agar plates (5 to 6 plates per one strain) for Campylobacter
- Autopipette with tips or Pasture pipettes
- Inoculating loop

1. Carefully take the ampoule out of the wrap.

   Note: To maintain the vacuum condition, **do not break the tip of the ampoule**. Otherwise, the air will enter the ampoule and the cotton wool plug will be pushed down and in contact with dried bacterial culture. If it happens, please simply remove the cotton plug with forceps.

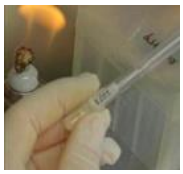
   Note: The ampoule can be cut in the middle or below the cotton wool plug.

2. Wipe the ampoule neck with 70% alcohol-dampened cotton wool.



3. Make a deep score on the around the circumference of the ampoule near the middle of the plug using ampoule cutter or a file. The ampoule should be cut in the middle or below the cotton wool plug.

4. Wrap thick cotton wool around the ampoule and break at the marked area.
5. Remove the pointed end of the ampoule and cotton into a biohazard container. Pipette 0.5 ml of sterile LB or Columbia broth into the dried cells. Mix gently and carefully to avoid creating aerosols.



6. Transfer one drop of each strain onto one LB agar plate for enterococci mCCDA agar plate for Campylobacter using autopipette or Pasteur pipette. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.

7. For enterococci, incubate the inoculated plates and the suspension tubes at 37^0C overnight and observe the bacterial growth. For Campylobacter, incubate the plates and the suspension tubes at 42^0C, 48 hours.

- **Reviving *N. gonorrhoeae* lyophilised cultures**

Needed material:

- Sterile nutrient broth (i.e. Tryptic Soy Broth)
- Sterile needles and syringes
- Chocolate agar plates
- Inoculating loop

The lyophilized (freeze-dried) specimens with which you are provided must be rehydrated. When reconstituting them, exercise extreme caution not to create aerosols or spills which could cause infection. Please follow standard safety procedures and exercise all the usual precautions when dealing with this material. It is recommended that freeze dried specimens be stored out of direct light and refrigerated until the reconstitution process commences.

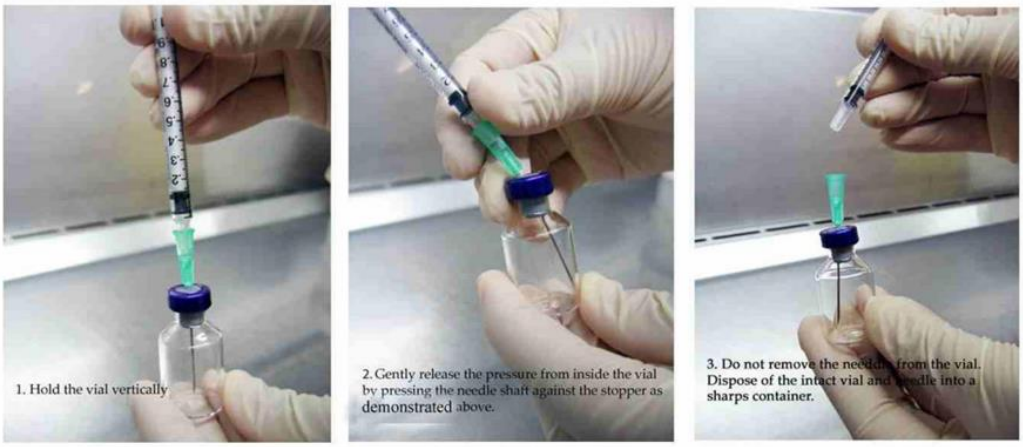
Do not mouth pipette and do not reconstitute the specimens until you are ready to plate them out.

1. Do **not** remove the whole cap - lift only the pre-cut section.
2. Sterilize the rubber stopper with a disinfectant swab as for inoculating a blood culture.
3. Add 1 ml of sterile Tryptic Soy Broth (or suitable substitute) to the vial with a needle and syringe.
4. Gently swirl the vial; allow 5 - 10 minutes for the dry material to rehydrate completely.
5. Gently release pressure inside the vial by pressing the needle shaft against the stopper.
6. Transfer an aliquot of the reconstituted specimen to the appropriate culture media using the syringe only.

   DO NOT REMOVE THE NEEDLE FROM THE VIAL. DISPOSE OF THE INTACT VIAL AND NEEDLE INTO A SHARPS CONTAINER

7. Hold the vial vertically.
8. Gently release the pressure from inside the vial by pressing the needle shaft against the stopper.
9. Draw the fluid up into the needle slowly.
10. Separate the needle tip from the syringe carefully.
11. Dispose of the intact vial and needle into a sharps container.
12. Plate one drop on a chocolate agar plate and spread.

13. Incubate for 16–18 hours at 36 ± 1°C in a 5 ± 1% CO_2-enriched humid atmosphere.

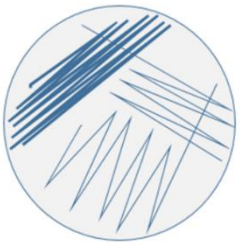


- **Reviving Shigella and Enterococcus isolates**

The **transport media swabs** must be stored in a dark place at 5°C to 25°C until microbiological analysis. We suggest that you subculture and process the strains within 48 hours from receipt of the parcel. Subculture the test strains onto non-selective media, e.g., a nutrient agar plate or blood agar plate, as illustrated below:

1. Inoculate it on one side of the agar plate using the swab to apply material gently and densely.

2. Turn the plate and use a sterile loop to streak once through the area first inoculated and allow further streaks to separate the culture aiming to obtain single colonies.



3. Turn the plate and use a sterile loop to streak once through the second area inoculated and allow further streaks to separate the culture aiming to obtain single colonies.

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

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

3.3. Identification of *Shigella spp., Enterococci, Campylobacter spp.* and *Neisseria gonorrhoeae* test strains

Each of the four panels in this EQA round contains five target species. i.e. five *Neisseria gonorrhoeae* isolates in the *N. gonorrhoeae* panel. The remaining two isolates in each panel are non-target species – their identification is different from the five target species.

Please follow the routinely used methods in your own laboratory for **identification** of all panel strains.

3.4. Antimicrobial susceptibility testing of *Shigella spp., Enterococci, Campylobacter spp.* and *Neisseria gonorrhoeae* test strains, and of the reference strains

The strains identified as *Shigella spp.*, *Enterococcus faecium, Enterococcus faecalis, Campylobacter coli, Campylobacter jejuni* and *Neisseria gonorrhoeae* (five isolates from each panel), as well as the appropriate reference strains, should be tested for susceptibility towards as many antimicrobials as possible indicated in the test form and in **Tables 1-4**. 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 range 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, 34th Ed.). When not available, EUCAST clinical breakpoints (Tables v. 13.1, 2023) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Shigella spp., Enterococci, Campylobacter spp.* and *Neisseria gonorrhoeae* can be found in **Tables 1-4**, respectively. **Please make sure to use the correct table for the interpretation**.

Table 1. Breakpoints for interpretation of MICs and zone diameters for *Shigella*

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

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 4	8	≥ 16	≥ 20	17-19	≤ 16
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 8	16	≥ 32	≥ 16	11-15	≤ 10
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.06	0.12-0.5	≥ 1	≥ 31	21-30	≤ 20
Levofloxacin	≤ 0.5	1	≥ 2	≥ 21	17-20	≤ 16
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Gentamicin, GEN	≤ 2	4	≥ 8	≥ 18	15-17	≤ 14
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Nalidixic acid, NAL	≤ 16	-	≥ 32	≥ 19	14-18	≤ 13
Trimethoprim-Sulfamethoxazole	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11

Reference values are based on Enterobacterales breakpoints from CLSI M100, 34th Ed.

Table 2. Breakpoints for interpretation of MICs and zone diameters for *E. faecium / E. faecalis*

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, 34th 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 13.1, 2023. http://www.eucast.org."

Table 3. Breakpoints for interpretation of MICs and zone diameters for *C. jejuni / C. coli*

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/ in August 2023.

**Reference values are based on EFSA (European Food Safety Authority) recommendation.

Table 4. Breakpoints for interpretation of MICs and zone diameters for *N. gonorrhoeae*

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Azithromycin, AZI	≤ 1	-	-	≥ 30	-	-
Cefixime, CFM	≤ 0.25	-	-	≥ 30	-	-
Ceftriaxone, CRO	≤ 0.25	-	-	≥ 35	-	-
Ciprofloxacin, CIP	≤ 0.06	0.12-0.5	≥ 1	≥ 41	28-40	≤ 27
Penicillin, PEN	≤ 0.06	0.12-1	≥ 2	≥ 47	27-46	≤ 26
Tetracycline, TET	≤ 0.25	0.5-1	≥ 2	≥ 38	31-37	≤ 30

Reference values are based on *N. gonorrhoeae* breakpoints from CLSI M100, 34th Ed.

N.B. For the interpretation of the AST results for *N. gonorrhoeae* quality control strains provided with this EQA panel (ATCC49226, WHO G, WHO L, WHO O and WHO P) please refer to Table 4B and 5C (Disk diffusion and MIC QC ranges for ATC49226) in CLSI M100, 34th Ed, as well as Table 1 in the publication by Unemo M et al.. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. *J Antimicrob Chemother.* 2016 Nov;71(11):3096-3108. doi: 10.1093/jac/dkw288. PMID: 27432602; PMCID: PMC5079299.

4. SUBMISSION OF RESULTS VIA THE INFORMATICS MODULE

We recommend that you write down your results in the enclosed test forms as it will help you when transferring results onto the online platform.

N.B. For all susceptibility testing results for which there are no breakpoints identified, please enter the susceptibility category that you interpret, i.e. if a N. gonorrhoeae isolate has an MIC > 1 µg/mL or zone inhibition diameter < 30mm for azithromycin, interpret either as resistant (R) or decreased susceptibility (DS).

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

Login to the Informatics Module:

Access the Informatics Module (incognito window) via the following link https://eqasia-pt.dtu.dk/

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 eqasia@food.dtu.dk

When you submit your results, remember to have by your side the completed test forms (template available for download from the EQAsia website). If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens.

Results must be submitted no later than November 25th, 2024.

If you have troubles entering your results or if you experience technical problems with the informatics module, please contact the DTU team directly at eqasia@food.dtu.dk, explaining the issues that you encountered.

Before submitting your final 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 further data entry.

After submission, the Informatics Module will allow you to view and print a report with your submitted results.

5. EVALUATION OF RESULTS

The scores for the submitted results will be released after the submission deadline has passed. 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).

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

Once the results have been evaluated, you will be able to access your certificate via the EQAsia Informatics Module. You will be notified by email when the certificate is available. The certificate will contain score for identification and for susceptibility testing for each of the panels for which you submitted results. Performance rate for each panel will also be shown on the certificate.

The EQAsia project team would like to thank you once again for your participation in this EQA round!

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

Appendix 2a: Reference values (MIC values and interpretation) – *Shigella spp.*

	Amikacin (AMK)		Ampicillin (AMP)		Azithromycin (AZI)		Cefepime (FEP)		Cefotaxime (FOT)		Cefoxitin (FOX)	
Shi EQAsia 24.1	≤4	S	>32	R	4	S	4	S	>4	R	4	S
ShiEQAsia 24.2	≤4	S	>32	R	64	R	2	S	>4	R	2	S
Shi EQAsia 24.5	≤4	S	2	S	≤2	S	≤0.06	S	≤0.25	S	4	S
Shi EQAsia 24.6	≤4	S	>32	R	>64	R	4	I	>4	R	8	S
Shi EQAsia 24.7	≤4	S	>32	R	4	S	0.25	S	≤0.25	S	2	S

R, Resistant; I, Intermediate; S, Susceptible

	Ceftazidime (TAZ)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)		Ertapenem (ETP)		Gentamicin (GEN)	
Shi EQAsia 24.1	2	S	≤8	S	0.25	S	≤0.25	I	≤0.25	S	≤1	S
ShiEQAsia 24.2	≤0.25	S	≤8	S	4	R	≤0.25	I	≤0.25	S	≤1	S
Shi EQAsia 24.5	≤0.25	S	≤8	S	0.12	S	≤0.25	I	≤0.25	S	≤1	S
Shi EQAsia 24.6	≤0.25	S	64	R	8	R	≤0.25	I	≤0.25	S	≤1	S
Shi EQAsia 24.7	≤0.25	S	64	R	8	R	≤0.25	I	≤0.25	S	≤1	S

R, Resistant; I, Intermediate; S, Susceptible

	Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Nalidixic acid (NAL)		Sulfamethoxazole (SMX)		Tetracycline (TET)	
Shi EQAsia 24.1	≤1	S	≤1	I	≤0.03	S	≤ 4	S	32	S	≤2	S
ShiEQAsia 24.2	≤1	S	4	R	≤0.03	S	>64	R	>512	R	>32	R
Shi EQAsia 24.5	≤1	S	≤1	I	≤0.03	S	64	R	≤8	S	≤2	S
Shi EQAsia 24.6	≤1	S	4	R	≤0.03	S	>64	R	≤8	S	>32	R
Shi EQAsia 24.7	≤1	S	4	R	≤0.03	S	>64	R	>512	R	>32	R

R, Resistant; I, Intermediate; S, Susceptible

	Tigecycline (TGC)		Trimethoprim (TMP)		Trime/Sulfa (SXT)	
Shi EQAsia 24.1	≤0.25	S	>16	R	≤0.5	S
ShiEQAsia 24.2	≤0.25	S	>16	R	>4	R
Shi EQAsia 24.5	≤0.25	S	>16	R	≤0.5	S
Shi EQAsia 24.6	≤0.25	S	>16	R	≤0.5	S
Shi EQAsia 24.7	≤0.25	S	>16	R	>4	R

R, Resistant; I, Intermediate; S, Susceptible

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

	Ampicillin (AMP)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Daptomycin (DAP)		Erythromycin (ERY)		Gentamicin (GEN)	
Ef EQAsia 24.1	>64	R	≤4	S	>16	R	2	S	>128	R	512	R
Ef EQAsia 24.2	>64	R	8	S	>16	R	8	R	>128	R	≤8	S
Ef EQAsia 24.3	≤0.5	S	8	S	1	S	1	S	>128	R	≤8	S
Ef EQAsia 24.4	1	S	8	S	1	S	1	S	2	S	≤8	S
Ef EQAsia 24.6	≤0.5	S	8	S	1	S	1	S	>128	R	≤8	S

R, Resistant; I, Intermediate; S, Susceptible

	Linezolid (LZD)		Quinu/Dalfo (SYN)		Teicoplanin (TEI)		Tetracycline (TET)		Tigecycline (TGC)		Vancomycin (VAN)	
Ef EQAsia 24.1	2	S	1	S	64	R	64	R	0.25	S	>128	R
Ef EQAsia 24.2	2	S	2	I	1	S	64	R	0.25	S	>128	R
Ef EQAsia 24.3	1	S	16	R	≤0.5	S	64	R	0.25	S	16	I
Ef EQAsia 24.4	2	S	8	R	≤0.5	S	32	R	0.25	S	≤1	S
Ef EQAsia 24.6	2	S	16	R	≤0.5	S	64	R	0.25	S	8	I

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2c: Reference values (MIC values and interpretation) – *Campylobacter spp.*

	Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Ertapenem (ETP)		Erythromycin (ERY)		Gentamicin (GEN)		Tetracycline (TET)	
Camp EQAsia 24.1	≤2	S	<=0.12	S	≤0.12	S	≤1	S	1	S	≤0.5	S
Camp EQAsia 24.2	4	S	0.25	S	0.25	S	≤1	S	1	S	≤0.5	S
Camp EQAsia 24.4	4	S	16	R	0.5	S	>512	R	>16	R	>64	R
Camp EQAsia 24.5	4	S	32	R	4	R	≤1	S	0.5	S	≤0.5	S
Camp EQAsia 24.6	4	S	≤0.12	S	0.25	S	≤1	S	0.5	S	≤0.5	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Azithromycin (AZI)		Ceftriaxone (CRO)		Cefixime (CFM)		Ciprofloxacin (CIP)		Penicillin (PEN)		Tetracycline (TET)	
NG EQAsia 24.1	>256	R	<0.016	S	0.064	S	>32	R	>32 (PPNG)	R	4	R
NG EQAsia 24.3	4		<0.016	S	0.002	S	0.004	S	0.125	I	1	I
NG EQAsia 24.4	1	S	0.125	S	0.25	DS	>32	R	2	R	2	R
NG EQAsia 24.6	1	S	2	DS	0.5	DS	>32	R	2	R	4	R
NG EQAsia 24.7	0.5	S	0.008/ 0.016	S	0.008	S	0.002/ 0.004	S	0.5	I	0.5	I

R, Resistant; I, Intermediate; S, Susceptible; PPNG, Penicillinase-producing *Neisseria gonorrhoeae*

Appendix 3: Quality control ranges for the reference strains

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

E. coli ATCC 25922		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amikacin, AMK	0.5-4	19-26
Ampicillin, AMP	2-8	15-22
Azithromycin, AZI	--	--
Cefepime, FEP	0.016-0.12	31-37
Cefotaxime, FOT	0.03-0.12	29-35
Cefotaxime and clavulanic acid, F/C	--	--
Cefoxitin, FOX	2-8	23-29
Ceftazidime, TAZ	0.06-0.5	25-32
Ceftazidime and clavulanic acid, T/C	--	--
Chloramphenicol, CHL	2-8	21-27
Ciprofloxacin, CIP	0.004-0.016	29-38
Doripenem, DOR	0.016-0.06	27-35
Ertapenem, ETP	0.004-0.016	29-36
Gentamicin, GEN	0.25-1	19-26
Imipenem, IMI	0.06-0.5	26-32
Levofloxacin, LEVO	0.008-0.06	29-37
Meropenem, MERO	0.008-0.06	28-35
Nalidixic acid, NAL	1-4	22-28
Piperacillin and tazobactam, P/T4	1-4	24-30
Sulfamethoxazole, SMX	8-32	15-23
Tetracycline, TET	0.5-2	18-25
Tigecycline, TGC	0.03-0.25	20-27
Tobramycin, TOB	0.25-1	18-26
Trimethoprim, TMP	0.5-2	21-28
Trimethoprim and sulfamethoxazole, SXT	≤ 0.5	23-29

MIC ranges and disk diffusion ranges are according to CLSI M100 34th edition, Tables 4A-1 and 5A-1

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

MIC range in accordance to "The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 13.0, 2023. http://www.eucast.org."

Appendix 3b: Quality control ranges for *Campylobacter 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 and disk diffusion ranges are according to CLSI M100 34th edition, Tables 4A-1 and 5A-1

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 3c: 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 34th edition, Tables 4A-2 and 5A-1

Appendix 3d: Quality control ranges for *Neisseria gonorrhoeae* ATCC 49226

Neisseria gonorrhoeae ATCC 49226		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Azithromycin, AZI	0.25-1	30-38
Cefepime, FEP	0.016-0.06	37-46
Cefixime, CFM	0.004-0.03	37-45
Cefotaxime, FOT	0.016-0.06	38-48
Cefoxitin, FOX	0.5-2	33-41
Ceftazidime, TAZ	0.03-0.12	35-43
Ceftriaxone, CRO	0.004-0.016	39-51
Ciprofloxacin, CIP	0.001-0.008	48-58
Gentamicin, GEN	4-16	15-20
Penicillin, PEN	0.25-1	26-34
Tetracycline, TET	0.25-1	30-42

MIC ranges and disk diffusion ranges are according to CLSI M100 34th edition, Tables 4B and 5C

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