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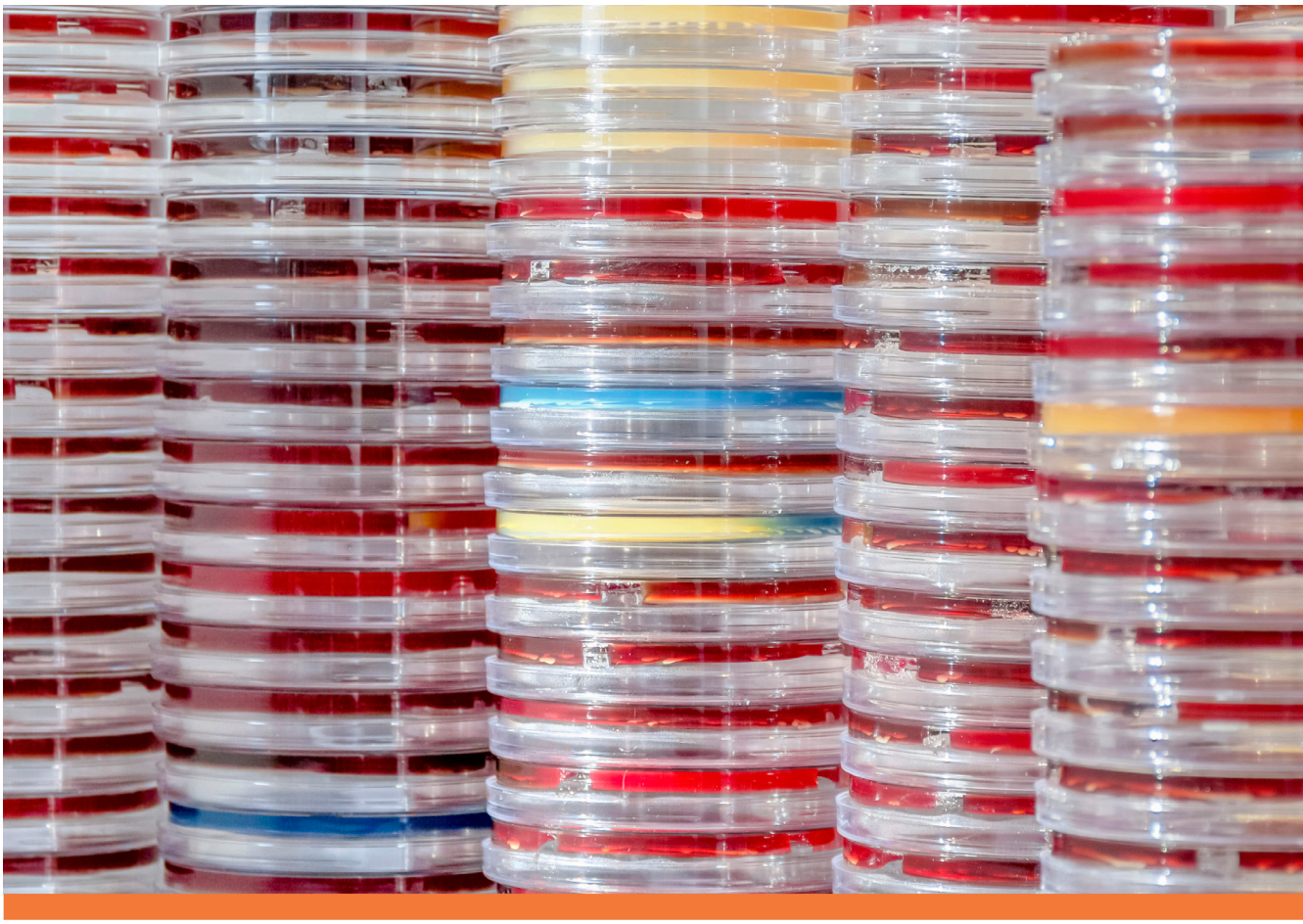

11th EQAsia External Quality Assessment trial:

Salmonella spp., Enterococcus spp., Campylobacter spp. and Neisseria gonorrhoeae – 2025

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1st edition, March 2026

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Photo:
DTU National Food Institute

Published by:
DTU National Food Institute
Henrik Dams Allé
2800 Kgs. Lyngby
Denmark

ISBN:
978-87-7586-077-7

food.dtu.dk

Index

Executive Summary

This report presents the results of the 11th External Quality Assessment (EQA) trial of the EQAsia programme, carried out between September and November 2025 as part of the Fleming Fund initiative to strengthen the provision of EQA services across the One Health sector among National Reference Laboratories and Centres of Excellence in South and Southeast Asia. The trial forms part of the final phase of EQAsia (2023–2025), during which the established EQA programme has continued to support Human Health (HH) and Animal Health (AH) laboratories. All activities are scheduled to conclude ahead of the Fleming Fund's closure in March 2026.

The EQA included bacterial identification and antimicrobial susceptibility testing (AST) of four WHO and FAO priority pathogen groups: *Salmonella* spp., *Enterococcus faecalis/faecium*, *Campylobacter coli/jejuni*, and *Neisseria gonorrhoeae*. Similarly to previous EQAsia EQAs, participating laboratories could choose one or more panels among the ones offered in the current EQA round. A total of 57 laboratories submitted results across the panels, representing 14 countries in the region (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam).

Participation was strongest in the *Salmonella* panel (54 labs), followed by the *Enterococcus* panel (29 labs) and the *Campylobacter* panel (18 labs), while the *N. gonorrhoeae* panel (15 labs) faced significant viability challenges. None of the laboratories were able to successfully revive all the *N. gonorrhoeae* isolates, and post-trial review identified preservation-related issues affecting strain stability. Work is ongoing to strengthen pre-distribution viability and shipment procedures for fastidious organisms.

Bacterial identification performance varied by panel. Identification of *Enterococcus* spp. showed more variability (correct ID rates ranging from 75% to 89%), and *Campylobacter* spp. posed the greatest challenges (0-100% correct), largely due to difficulties in reconstituting the strains and differences in laboratory familiarity with culturing fastidious organisms. These challenges contributed to lower identification scores and limited AST submissions in the *Campylobacter* panel.

AST performance displayed notable heterogeneity. In the *Salmonella* panel, levofloxacin 61.5%, colistin 60%, and cefoxitin 20% showed the highest deviation rates. In the *Enterococcus* panel, major deviations were observed for daptomycin 33.3%, chloramphenicol 26.2%, tigecycline 25.9%, ampicillin 25.3%, ciprofloxacin 23.3%, teicoplanin 20.4%, and gentamicin 18.4%, while erythromycin 2.5% and quinupristin/dalfopristin 0% were lowest. In the *Campylobacter* panel (limited submissions), deviation rates were tetracycline 40%, ciprofloxacin 40%, erythromycin 30%, and gentamicin 16.7%.

In the AH sector, disc diffusion was the predominant AST method. Performance in the *Salmonella* panel was generally strong, although deviations were again concentrated in antimicrobials such as levofloxacin (77.5%), colistin (48%), and ciprofloxacin (27.8%). AST performance in the *Enterococcus* and *Campylobacter* panels showed greater variability, mirroring the challenges observed in HH laboratories.

Quality control (QC) strain testing remained a major area of concern. Several laboratories did not submit QC results at all, particularly in the *Enterococcus* (14 labs missing) and *Campylobacter* (13 labs missing) panels. Among laboratories submitting QC data, performance varied widely. For *Enterococcus* QC, deviations ranged from 33.3% to 81.8%, while for *Campylobacter*, three laboratories achieved 100% concordance and one laboratory showed 50%. Inconsistent QC submission, combined

with high deviation rates among those who did perform QC, underscores the need for stronger routine QC implementation.

Overall AST performance also varied greatly between laboratories. In the *Salmonella* panel, laboratory deviations ranged from 0% to 22.9% among HH labs and 0% to 30.7% among AH labs. In the *Enterococcus* panel, HH laboratory deviations spanned 4.5–75.0%, and AH laboratories ranged 3.3–17.9%. The *Campylobacter* panel showed the greatest variability of all panels, with HH laboratory deviations 0–75% and AH laboratory deviations 0–50%.

Despite the challenges, many laboratories achieved strong overall performance. Cross-panel ranking of laboratories showed an average score of 93.5% (median 94.5%; range 70.8–100%; n = 57), demonstrating both high-quality performance among top laboratories and the presence of critical performance gaps requiring targeted support.

The findings of this final EQA round highlight both the progress made and areas requiring further improvement. Core competencies in culture and AST of *Salmonella* spp. are well established across the region; however, the identification and susceptibility testing of more demanding organisms, particularly *Campylobacter* spp. and *Enterococcus* spp., require further technical improvement. Inconsistent QC implementation remains a critical weakness and should be prioritized in future training and capacity-building initiatives.

As the EQAsia programme concludes under the Fleming Fund in 2026, sustaining and institutionalizing high-quality EQA systems will be crucial for maintaining progress. Continued national and regional support will be vital to ensure laboratories across the One Health sector can produce high-quality, reliable AMR surveillance data beyond the conclusion of the Fleming Fund-supported programme

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 entered its second phase, continuing to deliver the established EQA programme for both Human Health (HH) and Food and Animal Health (AH) laboratories across the region until the end of 2025. As this phase concludes, it encompasses the final EQA trial of the programme, with all remaining activities expected to wrap up ahead of the Fleming Fund's closure in March 2026.

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 ten 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 five 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 eleventh EQA trial of the EQAsia project (EQA11) carried out in September – November 2025. The trial included four EQA panels, each containing seven test strains. Of these, five were the organism in question (target organism, i.e., *Salmonella* spp.), whereas the other two test strains were different from the targeted species (reported as non-[organism], i.e., non-*Salmonella* 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 eleventh EQA trial includes identification and AST of *Salmonella* 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 disc diffusion and MIC determination are listed in the EQA11 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 EQA11 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 EQA11

A total of 57 laboratories participated in the 11th EQA trial of the EQAsia project: 33 laboratories belonging to the HH Sector and 24 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 EQA11, 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 *Salmonella*, 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 was tested and selected by University of New South Wales, Sydney, Australia (UNSW). The expected MIC values are available in the appendix of this report (**Appendix 2d**).

Reference strains for the *Salmonella*, enterococci, and *Campylobacter* panels [*Escherichia coli* ATCC 25922/CCM 3954 (for disc 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 disc diffusion of the enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC)] were supplied during previous EQA rounds. The QC strains provided within EQA11 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 M100-35th 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 EQA11 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 disc diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 35th Ed. [3]. and VET06, 1st Ed. [5].) When not available, EUCAST clinical breakpoints (Tables v. 15.0, 2025) [6] or epidemiological cut off values [7] 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.

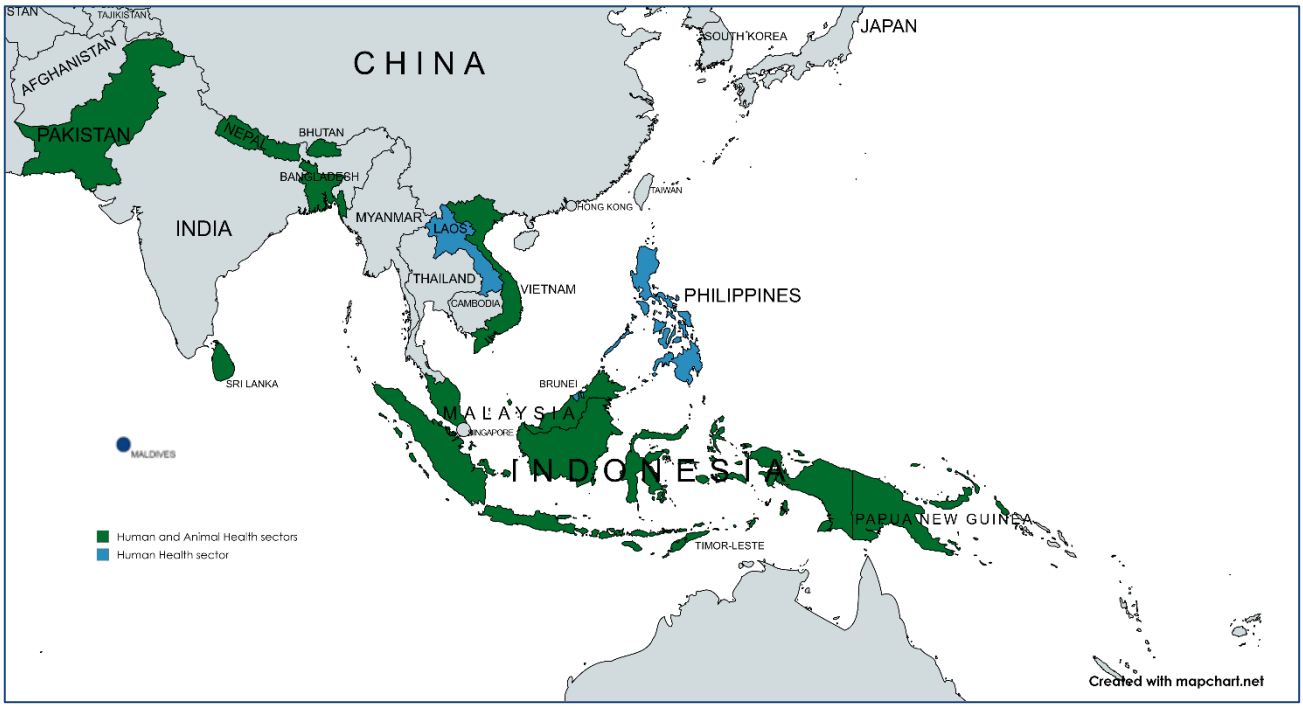

Figure 1. Countries participating in the 11th EQA of the EQAsia project. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA11 2025.

***Salmonella* 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
Cefoxitin	Tetracycline	Gentamicin	Penicillin
Ceftazidime		Linezolid	Tetracycline
Chloramphenicol		Quinupristin/ dalfopristin	
Ciprofloxacin		Teicoplanin	
Levofloxacin		Tetracycline	
Colistin		Tigecycline	
Ertapenem		Vancomycin	
Gentamicin			
Imipenem			
Meropenem			
Nalidixic acid			
Sulfamethoxazole			
Tetracycline			
Trimethoprim			
Trimethoprim/sulfamethoxazole			

2.4 Distribution

The bacterial strains were dispatched either as lyophilized strains or on swabs in transport medium in September 2025 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 EQA11 protocol (**Appendix 1**).

2.5 Procedure

Protocols were shared with participants all relevant information were available at the EQAsia website [8], to allow access to all the necessary information at any time. The participants were recommended to store the lyophilized strains in a dark, dry and cool place until performance of AST.

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

Laboratories used procedures such as disc 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 33 Human Health laboratories participating in the 11th EQA of the EQAsia project, 31, 20 and 11 laboratories submitted results for *Salmonella* spp., *Enterococcus* spp., *and Campylobacter* spp., panels, respectively. Additionally, 17 HH laboratories enrolled in the *Neisseria gonorrhoeae* panel. Out of these, 15 labs submitted the data and only 2 laboratories submitted the AST results for evaluation.

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

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. Most participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results (**Figure 3**).

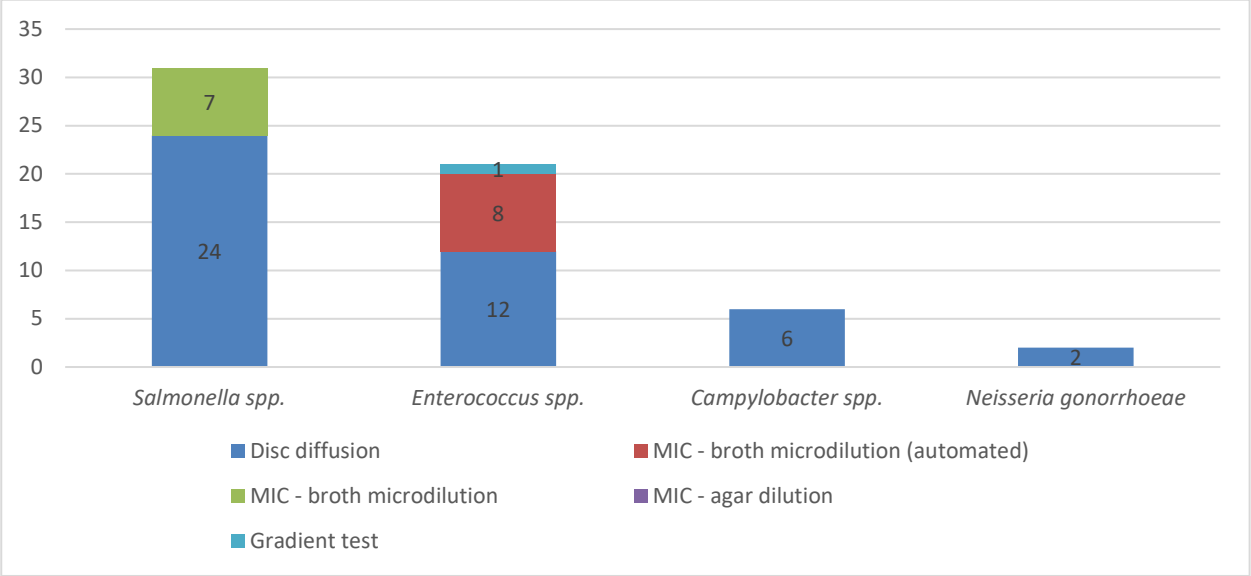

Figure 2. Methodologies primarily used by the laboratories for antimicrobial susceptibility testing in each of the panels.

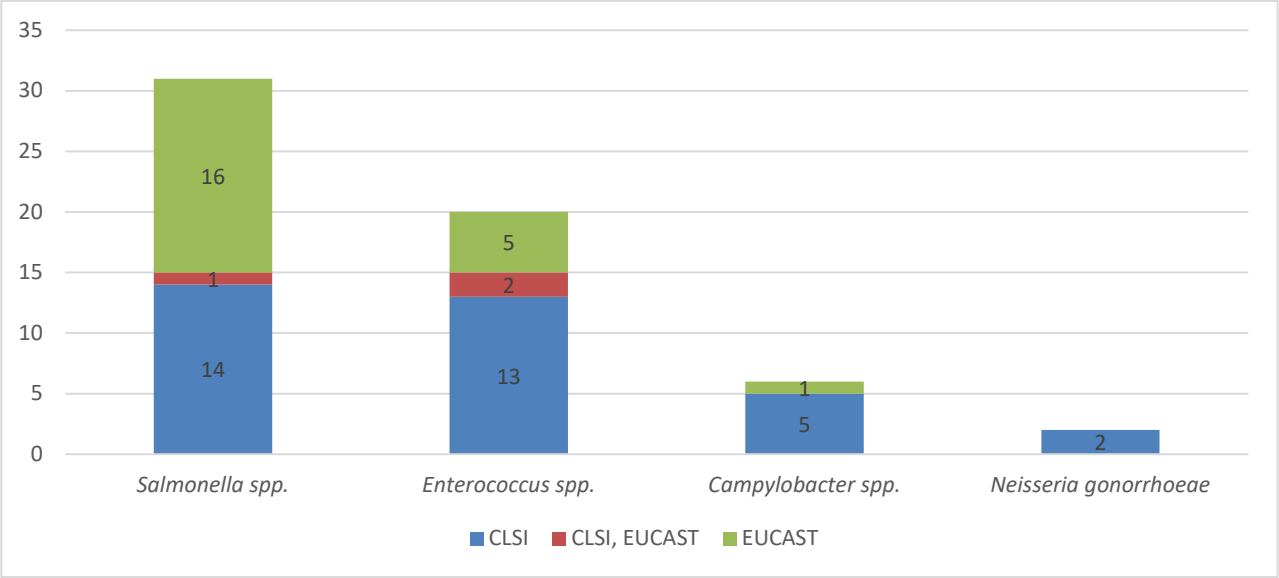

Figure 3. Use of international guidelines for interpretation of AST results by the participating laboratories.

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 *Salmonella* panel had the highest number of total AST results (n=1260) reported by 31 participating laboratories according to the recommended antimicrobials in EUCAST or CLSI (**Table 2**). In *Salmonella* spp. panel, the most frequently tested antibiotics were ampicillin, ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and meropenem. In the *Enterococcus* spp. panel, most tested antimicrobials were tested and reported most frequently ciprofloxacin, ampicillin, vancomycin and erythromycin. 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.

	***Salmonella* spp.**		***Enterococcus* spp.**		***Campylobacter* spp.**		***Neisseria gonorrhoeae***	
Amikacin	76	6,0%		--	--	--		--
Ampicillin	154	12,2%	87	12,83%	--	--		--
Azithromycin	35	2,8%	--	--	--	--	2	8,33%
Cefepime	63	5,0%	--	--	--	--		0,00%
Cefixime	--	--	--	--	--	--	2	8,33%
Cefotaxime	52	4,1%	--	--	--	--	--	--
Cefoxitin	40	3,2%	--	--	--	--	--	--
Ceftazidime	75	6,0%	--	--	--	--	--	--
Ceftriaxone	--	--	--	--	--	--	5	20,83%
Chloramphenicol	125	9,9%	61	9,00%	20	30,30%	--	--
Ciprofloxacin	141	11,2%	90	13,27%	20	30,30%	5	20,83%
Colistin	25	2,0%			--	--	--	--
Daptomycin	--	--	18	2,65%	--	--	--	--
Ertapenem	56	4,4%	--	--	--	--	--	--

Erythromycin	--	--	80	11,80%	--	--	--	
Gentamicin	74	5,9%	38	5,60%	6	9,09%	--	
Imipenem	54	4,3%	--		--		--	
Levofloxacin	26	2,1%	--		--		--	
Linezolid	--	--	58	8,55%	--		--	
Meropenem	84	6,7%	--		--		--	
Nalidixic acid	5	0,4%	--		--		--	
Penicillin	--		--		--		5	20,83%
Quinupristin and dalfopristin	--		4	0,59%	--		--	
Sulfamethoxazole	--		--	--	--		--	
Teicoplanin	--		49	7,23%	--		--	
Tetracycline	59	4,7%	80	11,80%	20	30,30%	5	20,83%
Tigecycline	11	0,9%	27	3,98%	--	--		--
Trimethoprim	--	--	--	--	--	--		--
Trimethoprim/Sulfamethoxazole	105	8,3%	--	--	--	--		--
Vancomycin	--	--	86	12,68%	--	--		--
Total	**1260**		**678**		**66**		**24**	

Missing data or incomplete AST results entries were observed in two out of three EQA panels among the HH laboratories participating in EQA11. A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains.

Seven out of 31 laboratories had incomplete results submitted for the *Salmonella* panel (**Table 3**). The incomplete results in the *Salmonella* panel was seen for laboratories #04, #07, #13, #17, #50, #51 and #74.

Five out of 20 laboratories that signed up 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, #08, #32, #49 and #66.

There were no missing data in the *Campylobacter* panel data set. However, very few laboratories (n=7) reported results in this part of the EQA11 trial.

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *Salmonella* strains reported by HH laboratories (n=32) participating in the 11th EQA of the EQAsia project.

Lab ID No.	Salm EQAsia 25.1	Salm EQAsia 25.2	Salm EQAsia 25.3	Salm EQAsia 25.4	Salm EQAsia 25.5
#04	CIP, IMI	--	--	--	--
#07	--	FOT	FOT, CIP	FOT	FEP, CIP
#13	--	--	--	--	FEP
#17	--	--	--	--	GEN
#50	MERO	--	--	--	--
#51	TET	--	--	--	--
#74	--	--	--	--	AMP

Salm, *Salmonella* spp.

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *Enterococcus faecalis/Enterococcus faecium* strains reported by HH laboratories (n=20) participating in the 11th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 25.2	Ef EQAsia 25.3	Ef EQAsia 25.4	Ef EQAsia 25.5	Ef EQAsia 25.7
#06	--	DAP	--	--	DAP
#08	--	--	--	VAN	--
#32	VAN		VAN	VAN	--
#49	--	TIG	--	--	TIG
#66	LIN, TEI	CHL	--	--	--

Ef, *Enterococcus faecalis/Enterococcus faecium*

3.2 *Salmonella* spp. panel

32 laboratories from 14 countries signed up for the *Salmonella* spp. panel.

3.2.1 Bacterial identification

Thirty-one laboratories submitted bacterial identification results **(Table 5)**. One laboratory was unable to revive all seven *Salmonella* strains. All *Salmonella* isolates were correctly identified by all participating laboratories.

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

Strain	Bacterial ID	No. correct
Salm EQAsia 25.1	*Salmonella*	31/31
Salm EQAsia 25.2	*Salmonella*	31/31
Salm EQAsia 25.3	*Salmonella*	31/31
Salm EQAsia 25.4	*Salmonella*	31/31
Salm EQAsia 25.5	*Salmonella*	31/31
Salm EQAsia 25.6	Non-*Salmonella*	31/31
Salm EQAsia 25.7	Non-*Salmonella*	31/31

Salm, *Salmonella*

3.2.2 AST performance

The *Salmonella* panel had n = 1260 AST results reported by 31 participating laboratories.

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 87.3% (strain Salm EQASIA 25.2) to 96.4% (strain Salm EQASIA 25.3) (**Table 6**).

Antimicrobial-based analysis

Deviations from the expected antimicrobial susceptibility results varied across the tested agents. Antimicrobials with deviations greater than 5% were levofloxacin (61.5%), colistin (60%), cefoxitin (20%), ciprofloxacin (14.9%), amikacin (13.2%), gentamicin (12.2%), imipenem (9.3%), ampicillin (9.1%), tigecycline (9.1%) and tetracycline (5.1%) **(Figure 4)**. Antimicrobials that showed no deviation from the expected results were azithromycin, ertapenem, meropenem and nalidixic acid. All remaining antimicrobials demonstrated deviations $\leqslant$5%, specifically cefotaxime (3.8%), ceftazidime (2.7%), trimethoprim/sulfamethoxazole (1.9%), cefepime (1.6%) and chloramphenicol (0.8%).

Laboratory-based analysis

A deviation below or equal to 5% in laboratory performance regarding the interpretation of results (R/I/S) was observed in 13 laboratories: #05, #14, #17, #34, #35, #49, #52, #62, #63. #64, #72, #73, #76. **(Figure 5).** On average, the deviation was 6.8%, ranging from 0.0% to 22.9%. As the acceptance level was set at a maximum deviation of 5%, 18 laboratories (#01, #02, #04, #06, #07, #11, #12, #13, #48, #50, #51, #60, #66, #70, #71, #74, #75, #77) did not perform within the expected range for the

Salmonella spp. panel.

Table 6. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results submitted by 31 HH laboratories for the *Salmonella* panel.

Strain	AST in total	% Correct
Salm EQAsia 25.1	252	90.5%
Salm EQAsia 25.2	252	87.3%
Salm EQAsia 25.3	253	96.4%
Salm EQAsia 25.4	252	92.9%
Salm EQAsia 25.5	251	89.2%

Salm, Salmonella

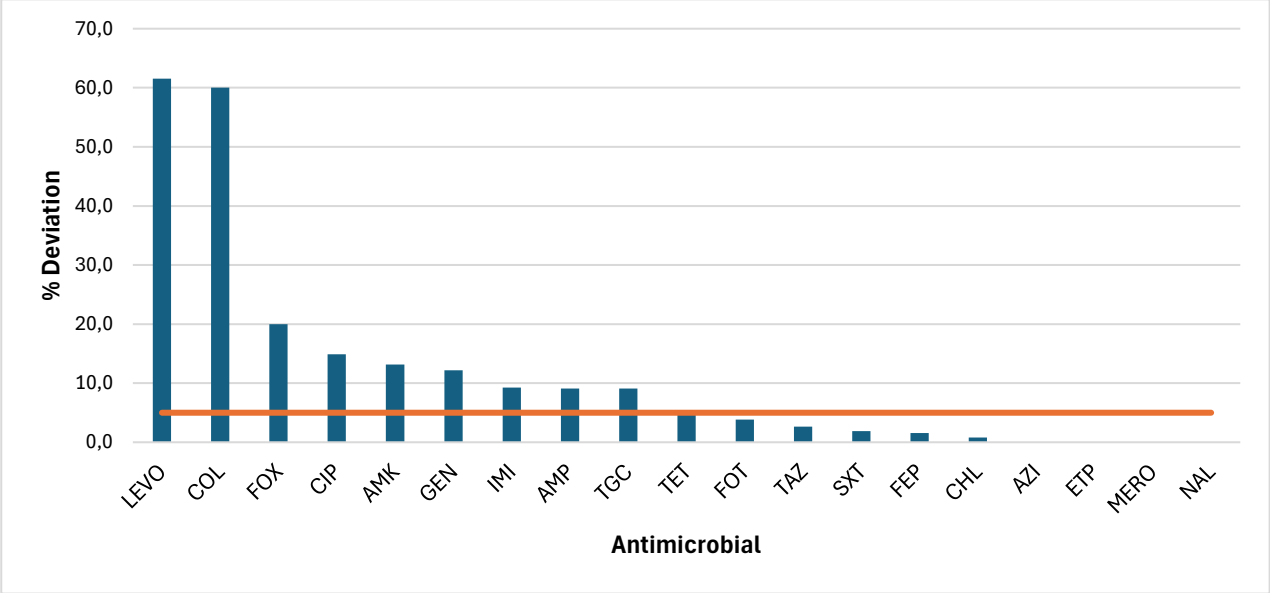

Figure 4. Percentage of deviation in the AST interpretation (R//I/S) among *Salmonella* strains by HH laboratories (n=31) participating in the 11th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

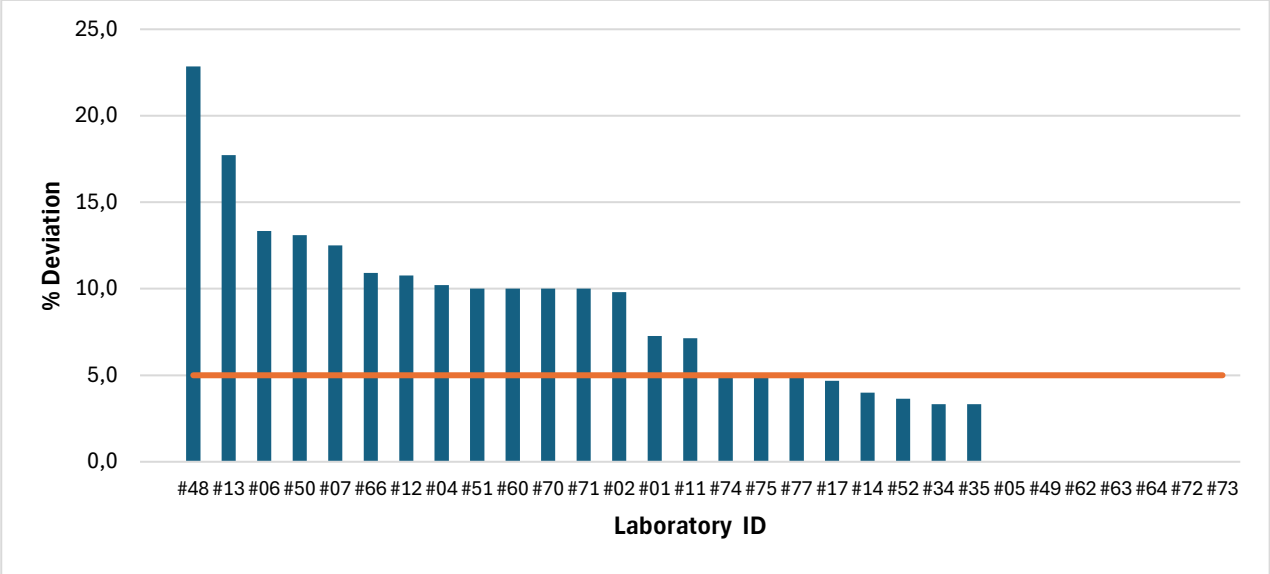

Figure 5. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by HH laboratories (n=31) participating in the 11th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.4 Quality control strain *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 provided free of charge to participating laboratories as part of previous EQAsia EQA trials to be used as reference strains for the *Salmonella* panel.

All 31 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only 3 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 disc diffusion, and MIC was determined by 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 ertapenem (5 out of 11), while no deviation was observed for imipenem (0 out of 14) (**Table 7**).

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

Antimicrobial	Proportion outside of range			
	Disc Diffusion	MIC-gradient	MIC-dilution	**Total**
Amikacin	0/15	--	2/7	2/22
Ampicillin	0/22	--	1/5	1/27
Cefepime	1/12	--	3/5	4/17
Cefotaxime	0/7	0/1	1/1	1/9
Cefoxitin	1/15	--	0/2	1/17
Ceftazidime	1/17	--	4/5	5/22
Chloramphenicol	2/23	--	--	2/23
Ciprofloxacin	0/23	0/1	6/6	6/30
Colistin	--	--	1/3	1/3
Ertapenem	0/6	--	5/5	5/11
Gentamicin	1/17	--	2/6	3/23
Imipenem	0/9	--	0/5	0/14
Meropenem	2/19	--	6/6	8/25
Nalidixic acid	2/2	--	--	2/2
Sulfamethoxazole	3/11	0/1	--	3/12
Tetracycline	0/15	--	2/7	2/22

Disc Diffusion – inhibition zone diameter determination by disc diffusion; MIC-Gradient – MIC determination by gradient test; MIC-dilution – MIC determination by broth micro or macrodilution.
*Gradient test and disc diffusion are not recommended for colistin testing. Azithromycin, Tigecycline and Trimethoprim were not tested by any lab.

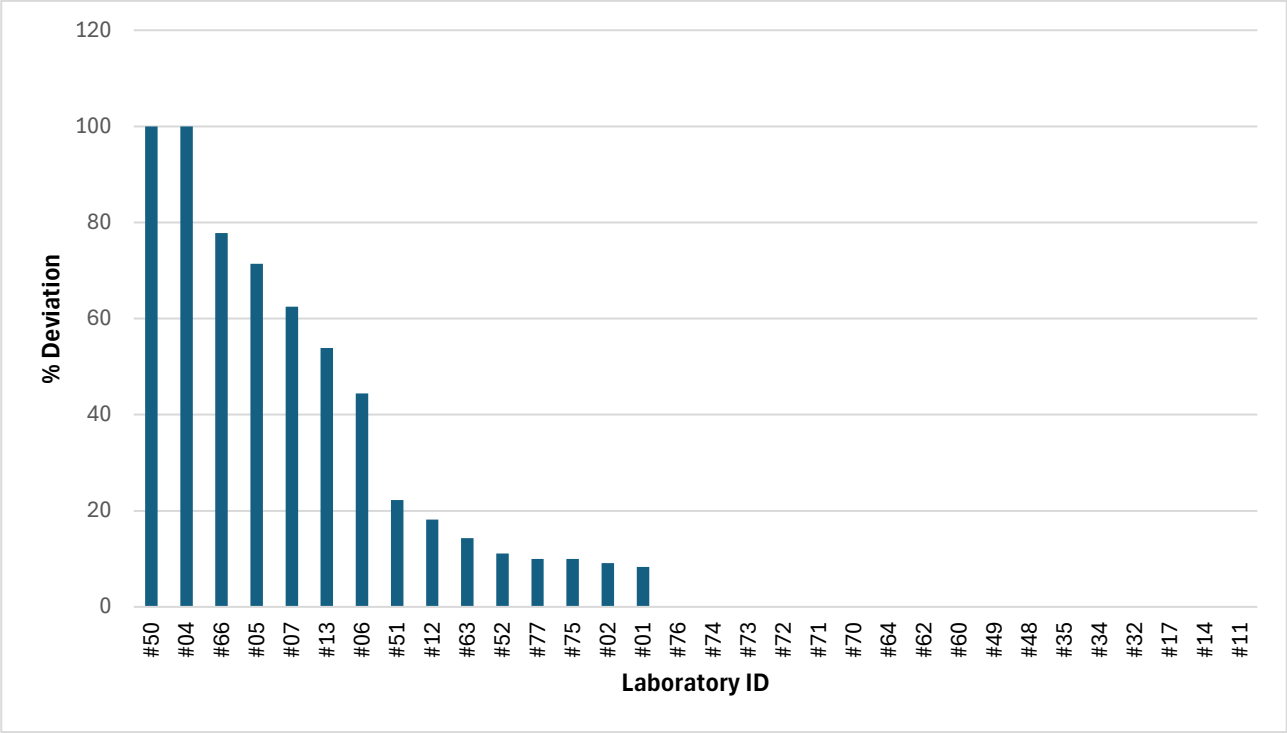

Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Salmonella* spp. panel by the HH laboratories.

Considering the deviations observed, the laboratories' performance appeared to be partly associated with the methodology applied for AST of the quality control strains **(Figure 6).** A large group of laboratories (n=17; #11, #14, #17, #32, #34, #35, 48, #49, #60, #62, #64, #70, #71, #72, #73, #74, and #76) presented no deviation across all tests performed. All these laboratories applied the disc diffusion method. Only one laboratory using MIC broth microdilution (#11) showed zero deviation.

In contrast, all laboratories employing MIC broth microdilution exclusively (#04, #05, #06, #07, #13, and #66) showed considerable deviations ranging from 44% to 100%, suggesting substantial methodological inconsistencies. Among disc diffusion users, deviations varied widely. Several laboratories demonstrated low deviation rates between 8% and 14% (e.g., #01, #02, #75, #77, #52, #63), whereas others recorded moderate to high deviation levels (18% to 22% for #12 and #51, and up to 100% for #50).

These deviations indicate that, although many laboratories using disc diffusion achieved excellent performance, others applying the same method encountered notable difficulties. Overall, the findings highlight substantial variability in laboratory performance, emphasizing the need for strengthened technical capacity, particularly for laboratories using MIC broth microdilution and for those disc diffusion laboratories exhibiting higher deviation rates.

3.3 *Enterococcus faecium/ Enterococcus faecalis* panel

For *Enterococcus* spp. panel, 20 laboratories from 13 countries submitted the results for assessment.

3.3.1 Bacterial identification

Twenty participating laboratories submitted results for bacterial identification **(Table 10).** None of the laboratories were able to correctly revive and identify all seven strains in the panel. Strains Ef EQAsia 25.1, Ef EQAsia 25.3, Ef EQAsia 25.4, and Ef EQAsia 25.5 showed the

highest correct identification rates, with 17/20, 18/20, 17/20, and 18/20 laboratories identifying them correctly, respectively. Strains Ef EQAsia 25.2, Ef EQAsia 25.6, and Ef EQAsia 25.7 were less consistently identified, with correct identification rates of 15/20, 16/20, and 16/20 laboratories, respectively.

Overall performance was better for the target strains *E. faecalis* and *E. faecium* than for the non-target strains, which showed slightly lower correct identification rates across laboratories.

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

Strain	Bacterial ID	No. correct
Ef EQAsia 25.1	Non-*Enterococcus faecalis/faecium*	17/20
Ef EQAsia 25.2	*Enterococcus faecalis*	15/20
Ef EQAsia 25.3	*Enterococcus faecium*	18/20
Ef EQAsia 25.4	*Enterococcus faecalis*	17/20
Ef EQAsia 25.5	*Enterococcus faecalis*	18/20
Ef EQAsia 25.6	Non-*Enterococcus faecalis/faecium*	16/20
Ef EQAsia 25.7	*Enterococcus faecium*	16/20

Ef, *Enteroccus faecalis/ Enterococcus 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 categories (R/I/S) for the *Enterococci* panel ranged from 75.4% (strains Ef EQASIA 25.3 and Ef EQASIA 25.5) to 89.5% (strains Ef EQASIA 25.4 and Ef EQASIA 25.7) **(Table 11).** All AST results submitted for the five *Enterococcus* strains were included in the evaluation, even when laboratories reported incorrect species identification between *E. faecium* and *E. faecalis*, as interpretive breakpoints for these species do not differ substantially.

The greatest deviation from expected interpretive results was observed for strain Ef EQASIA 25.3 and Ef EQAsia 25.5, for which 24.6% of the reported results were incorrect, suggesting recurrent issues in the interpretation of specific antimicrobial agents. Strains Ef EQASIA 25.2 and Ef EQASIA 25.4 showed deviations of 22.8% and 10.5%, respectively, whereas Ef EQASIA 25.7 exhibited the highest concordance (89.5%) among the tested isolates.

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 25.2	127	77.2
Ef EQASIA 25.3	142	75.4
Ef EQASIA 25.4	143	89.5
Ef EQASIA 25.5	142	75.4
Ef EQASIA 25.7	124	89.5

Ef, *Enteroccus faecalis/ Enterococcus faecium*

Antimicrobial-based analysis

The deviation rates from the expected interpretative results varied substantially across the different antimicrobials tested, ranging from 0% for quinupristin–dalfopristin to 33.3% for daptomycin **(Figure 7)**. Most antimicrobials exceeded the 5% deviation threshold. Only erythromycin (2.5%) and quinupristin–dalfopristin (0%) fell below this cutoff. All remaining antimicrobials showed deviation rates between 13.8% and 33.3%, with chloramphenicol (26.2%), tigecycline (25.9%), ampicillin (25.3%), and ciprofloxacin (23.3%) among those with notably high deviation rates.

Laboratory-based analysis

A deviation at or below the 5% threshold was achieved by only one laboratory, while the majority exceeded this threshold (Figure 8). Overall deviations ranged widely, from 4.5% to 75.0%, indicating substantial variation in laboratory performance across the *Enterococcus* spp. panel.

Laboratory #32 showed the highest deviation (75.0%), followed by #34 (64.0%). Several other

laboratories demonstrated markedly elevated deviations, including #52 (33.3%), #12 (30.0%), and #50 (30.0%). Laboratories #48 (22.0%), #51 (21.4%), #66 (21.4%), and #35 (20.8%) also exhibited deviations above 20%, indicating widespread challenges in this testing round.

Moderate deviations between 12–16% were observed for laboratories #49 (15.8%), #01 (15.0%), #02 (13.3%), #05 (13.3%), #04 (12.5%), #11 (12.5%), and #06 (12.1%), all still exceeding the acceptable deviation limit.

Lower but still above-threshold deviations were recorded for #17 (6.7%), #07 (6.3%), and #14 (5.0%). Only one laboratory achieved a deviation below 5%: #08 (4.5%), indicating a consistent and reliable performance relative to the rest of the panel.

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

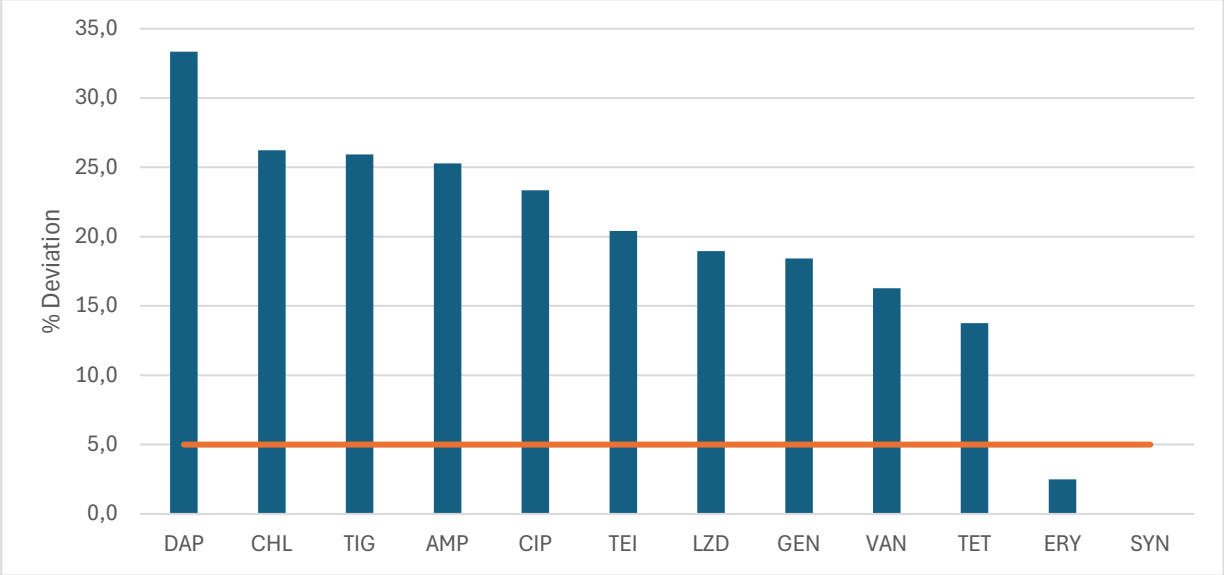

Figure 7. Percentage of deviation in the AST interpretation (R/S) among *Enterococcus faecalis/Enterococcus faecium* panel by HH laboratories (n=20) participating in the 11th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

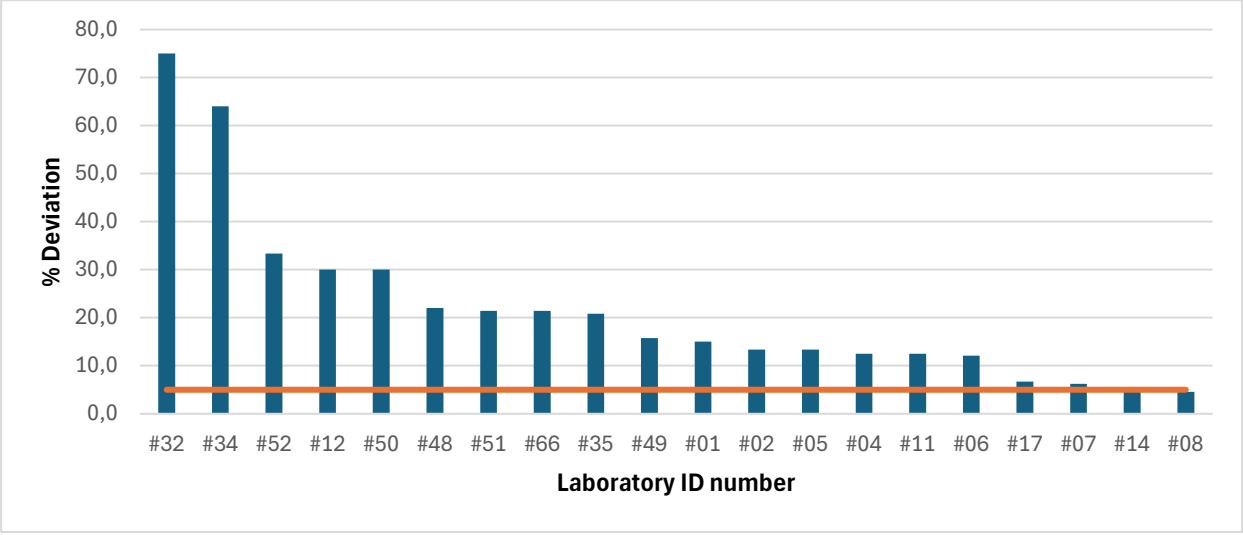

Figure 8. Percentage of deviation in the AST interpretation (R/S) among *Enterococcus faecalis/Enterococcus faecium* strains by HH laboratories (n=20) participating in the 11th EQA in the EQAsia project. Results are categorized by laboratory ID numbers.

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 disc 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.

15 out of 20 participating laboratories submitted results for this part of the *Enterococci* spp. panel. Ten laboratories reported results for the reference strain *S. aureus* ATCC 25923, while seven laboratories submitted the results also for *E. faecalis* ATCC 29212. Both disc diffusion and MIC test results were reported for both reference strains by two laboratories. However, as indicated in the EQA11 protocol, it should be noted that the reference strain *S. aureus* ATCC 25923 could only be used to determine inhibition zone diameters by disc diffusion, while *E. faecalis* ATCC 29212 is recommended for MIC testing.

The highest proportion of test results outside the expected range was observed for tetracycline (3 out of 13) and ampicillin (3 out of 14), followed by vancomycin (2 out of 13) and tigecycline (2 out of 4) **(Table 12)**. Single deviations were recorded for chloramphenicol (1 out of 12), ciprofloxacin (1 out of 15), erythromycin (1 out of 14), gentamicin (1 out of 11), and linezolid (1 out of 10). No deviations were observed for daptomycin (0 out of 2), quinupristin–dalfopristin (0 out of 1), or teicoplanin (0 out of 8). Overall, the average deviation for this part of the panel was 12.8%.

These overall deviations indicate the need to strengthen laboratory performance. To ensure consistent and reliable AST outcomes, attention should be given to agents with higher out-of-range proportions, most notably tetracycline and ampicillin.

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.

Antimicrobial	Proportion of tests outside of range			
	Disc Diffusion*	MIC-Gradient**	MIC- Dilution**	Total
Ampicillin	3/10	0/1	0/3	3/14
Chloramphenicol	1/9	0/1	0/2	1/12
Ciprofloxacin	1/9	0/2	0/4	1/15
Daptomycin	--	--	0/2	0/2
Erythromycin	0/8	0/1	1/5	1/14
Gentamicin	0/7	0/1	1/3	1/11
Linezolid	0/5	1/1	0/4	1/10
Quinupristin and dalfopristin	--	--	0/1	0/1
Teicoplanin	0/4	0/1	0/3	0/8
Tetracycline	1/8	0/2	2/3	3/13
Tigecycline	0/1	1/1	1/2	2/4
Vancomycin	2/7	0/1	0/5	2/13

Disc Diff. – inhibition zone diameter determination by disc diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution

**S. aureus* ATCC 25923 for disc diffusion

***E. faecalis* ATCC 29212 for MIC

The highest proportions of test results outside the expected range were observed in laboratories #52 (66.7%) and #05 (60.0%), followed by laboratories #51 (28.6%), #48 (20.0%), #50 (20.0%), and #66 (18.2%) **(Figure 9)**. All remaining laboratories (n = 9) showed no deviation. Overall, the average deviation for this part of the panel was 13.2%.

These deviations indicate that only a subset of laboratories contributed to the observed errors, whereas the majority achieved full concordance. Nevertheless, the relatively high deviation rates identified in a few laboratories highlight the need to strengthen their performance, particularly

regarding the applied susceptibility testing procedures.

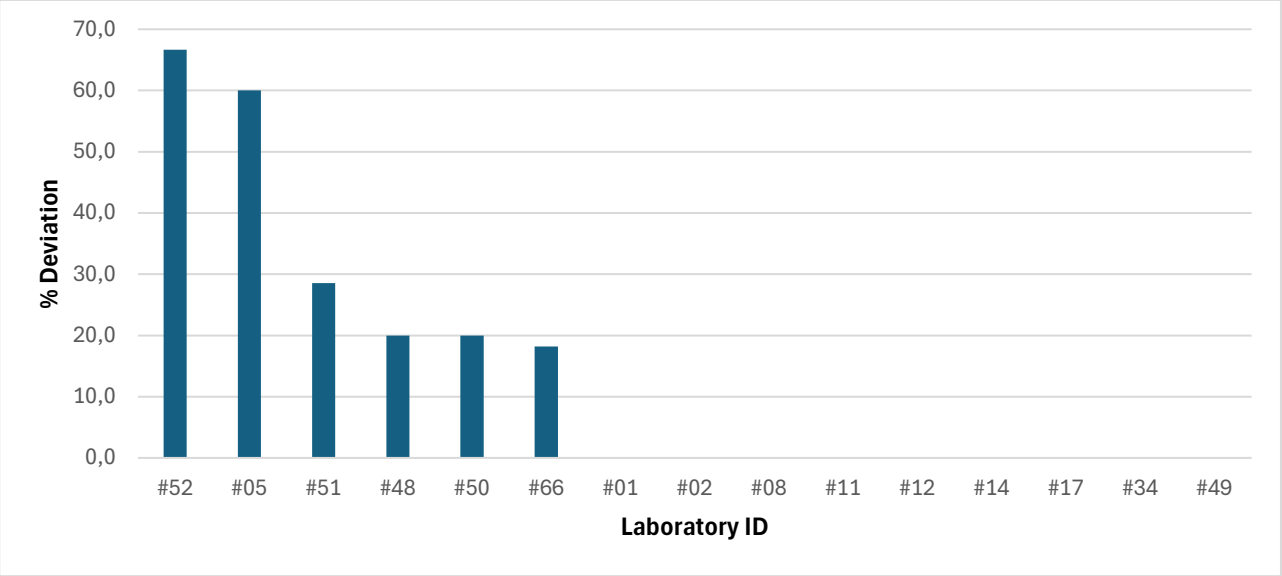

Figure 9. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 reference strains in the *E. faecium/ E. faecalis* panel by the HH laboratories.

3.4 *Campylobacter jejuni/coli* panel

Seventeen HH laboratories signed up for the *Campylobacter* spp. panel in EQA11. Overall, 11 laboratories submitted results for evaluation. One laboratory was unable to revive all seven test strains. Only four laboratories were able to revive all seven test strains.

3.4.1 Bacterial identification

Eleven participating laboratories submitted results for bacterial identification (**Table 13**). None of the laboratories correctly identified all seven strains in this panel. Among them, Laboratories #04, #07, and #35 successfully revived all strains. Laboratory #04 and #07 correctly identified 6 isolates, while Laboratory #35 correctly identified 5 strains.

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 25.1	*Campylobacter coli*	8/8
Camp EQAsia 25.2	*Campylobacter jejuni*	0/9
Camp EQAsia 25.3	Non-*Campylobacter coli/jejuni*	5/7
Camp EQAsia 25.4	*Campylobacter coli*	6/7
Camp EQAsia 25.5	Non-*Campylobacter coli/jejuni*	4/4
Camp EQAsia 25.6	*Campylobacter jejuni*	6/7
Camp EQAsia 25.7	*Campylobacter coli*	6/6

Camp, Campylobacter jejuni/ Campylobacter 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 seven laboratories submitted AST data for one or more of the expected target strains that could be analysed.

Strain-based analysis

Across the seven participating HH laboratories, the proportion of AST results in agreement with the expected interpretive categories (R/I/S) varied substantially between strains. The highest level of concordance was observed for Camp EQASIA 25.6, with 87.5% correct results (14/16), followed by Camp EQASIA 25.1 (80%) and Camp EQASIA 25.4 (76.9%). In contrast, markedly lower agreement was recorded for Camp EQASIA 25.2 (25%) and Camp EQASIA 25.7 (15.4%). These findings indicate considerable variability in laboratory performance across strains, with particularly poor concordance for two isolates in the Campylobacter jejuni/coli panel. (**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 seven HH laboratories for the *Campylobacter* spp. panel.

Strain	AST in total	% Correct
Camp EQASIA 25.1	20	80%
Camp EQASIA 25.2	4	25%
Camp EQASIA 25.4	13	76.9%
Camp EQASIA 25.6	16	87.5%
Camp EQASIA 25.7	13	15.4%

Camp, Campylobacter jejuni/ Campylobacter coli

Antimicrobial-based analysis

The total number of antimicrobials tested was four (ciprofloxacin, erythromycin, gentamicin, and tetracycline). In total, there were only 66 available AST results to evaluate for the entire panel from the seven HH labs that submitted AST data. Most deviations were observed for tetracycline and ciprofloxacin (40%), followed by erythromycin (30%) and gentamicin (16.7%). (**Figure 10**).

Laboratory-based analysis

Across the participating laboratories, deviation rates varied markedly. The highest proportions of results outside the expected range were observed for laboratory #35 (75%) and laboratory #51 (75%) **(Figure 11)**. Four additional laboratories, #66 (33%), #04 (25%), #17 (25%), #01 (11%) also reported deviations above 5% threshold. The #32 was the only laboratory that demonstrated 0% deviation, with all AST results in full agreement with expected results. (**Figure 11**). However, these results should be carefully interpreted since the total number of AST results submitted by the laboratories were very low.

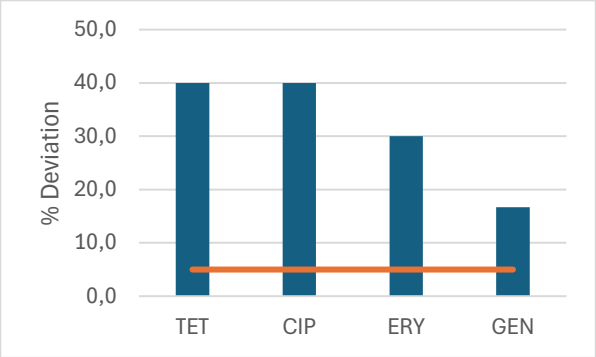

Figure 10. Percentage of deviation in the AST interpretation (R/S) among *Campylobacter jejuni/Campylobacter coli* strains by HH laboratories (n=7) participating in the 11th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

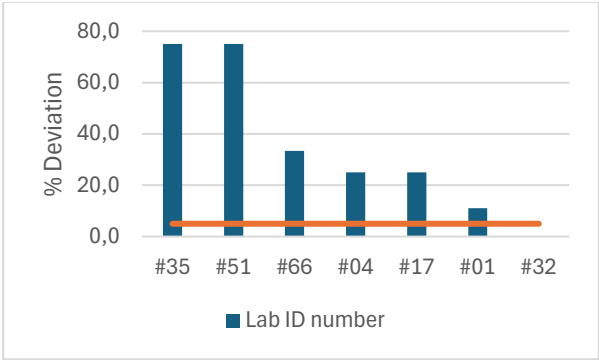

Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among *Campylobacter jejuni/ Campylobacter coli* strains by HH laboratories (n=7) participating in the 11th EQA in the EQAsia project. Results are categorized by laboratory ID numbers.

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.

Only one laboratory (#17) submitted the AST results for the QC strain for *Campylobacter* spp. and used disc diffusion results for *C. jejuni* ATCC

33560 when grown at 42°C for 24h; for these conditions, acceptance intervals for disc diffusion are only available for ciprofloxacin and erythromycin (**Appendix 3b**). Therefore, the laboratory did not submit results for other antimicrobials (**Table 15**).

The laboratory had no deviations the reference strain for these two antibiotics. **(Table 15).**

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 of tests outside of range	
	Disc Diffusion	Total
CIP	0/1	0/1
ERY	0/1	0/1

Disc Diffusion – inhibition zone diameter determination by disc diffusion.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 24 Animal Health laboratories participating in the 11th EQA of the EQAsia Programme, 23 laboratories submitted results for the *Salmonella* trial, 9 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 1. Disc diffusion as the sole method was the preferred choice for all the trials. Laboratories #28, #36, and #53 applied a combination of disc diffusion and broth microdilution methods, while Laboratory #38 used a combination of disc diffusion and automated broth microdilution.

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).**

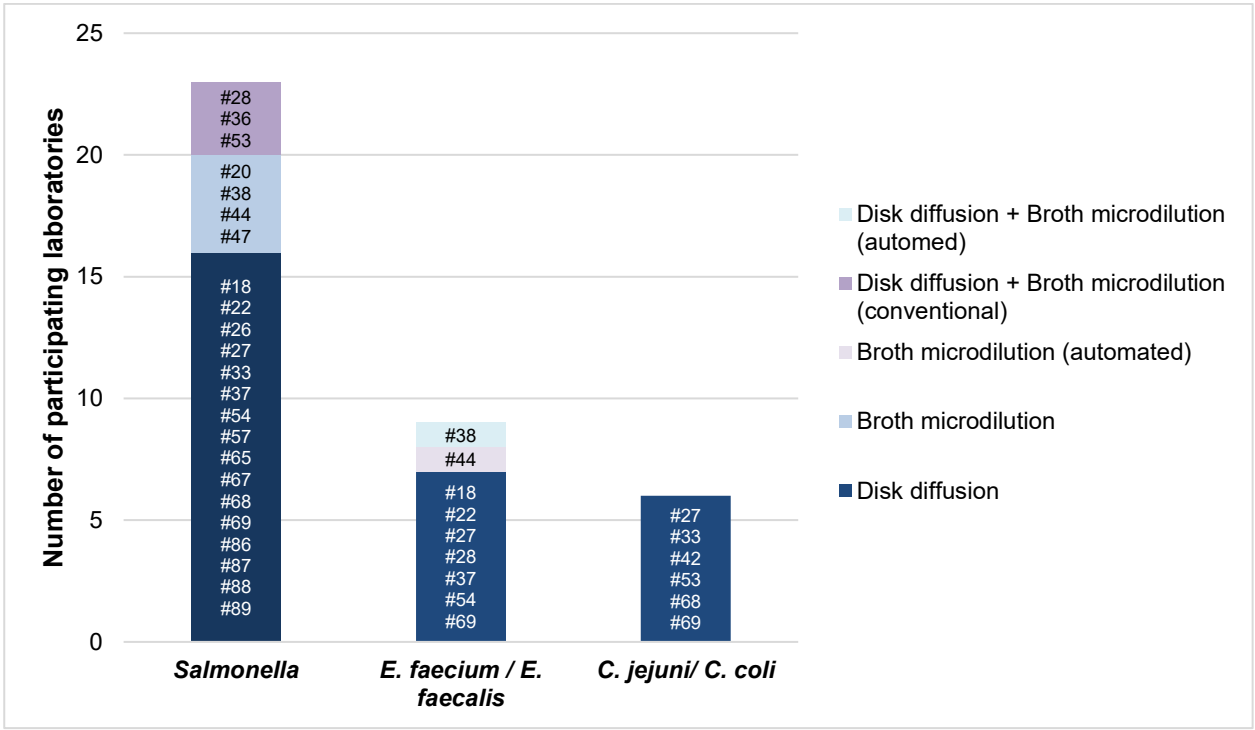

Figure 12. Methodologies applied by the AH laboratories participating for antimicrobial susceptibility testing in each of the panels.

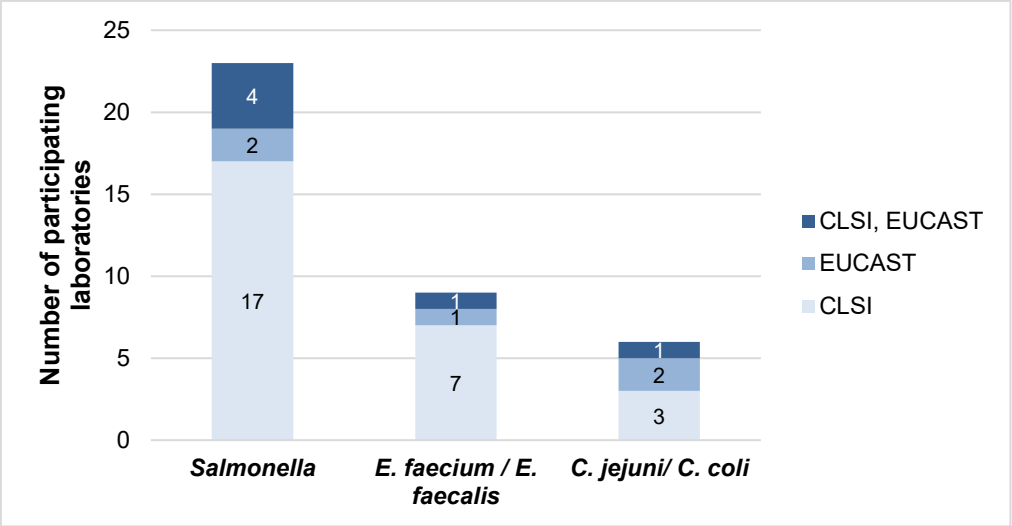

Figure 13. Use of international guidelines for interpretation of AST results by the participating laboratories.

The EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested antimicrobials **(Table 16).**

The *Salmonella* panel had the highest number of total AST results (n=1270) reported by 23 participating laboratories according to the recommended antimicrobials in EUCAST or CLSI **(Table 16)**. The most frequently tested antibiotics were gentamicin, ampicillin and ciprofloxacin. In the *Enterococcus* panel, participating laboratories tested and reported most frequently ciprofloxacin and tetracycline. Lastly, in the *C. jejuni/C. coli* trial, ciprofloxacin, erythromycin, gentamicin, and tetracycline were tested by all six participating laboratories. In contrast, chloramphenicol was tested by only two Animal Health laboratories, while ertapenem was tested by only one laboratory.

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

	Salmonella		*Enterococcus*		*Campylobacter*	
Amikacin	56	4.4%	-	-	-	
Ampicillin	103	8.1%	37	10.6%	-	-
Azithromycin	51	4.0%	-	-	-	-
Cefepime	60	4.7%	-	-	-	-
Cefotaxime	80	6.3%	-	-	-	-
Cefoxitin	59	4.6%	-	-	-	-
Ceftazidime	75	5.9%	-	-	-	-
Chloramphenicol	94	7.4%	37	10.6%	4	5.3%
Ciprofloxacin	100	7.9%	42	12.0%	18	24.0%
Colistin	25	2.0%	-	-	-	-
Daptomycin	-	-	3	0.9%	-	-
Ertapenem	34	2.7%	-	-	1	1.3%
Erythromycin	-	-	38	10.9%	17	22.7%
Gentamicin	108	8.5%	32	9.2%	18	24.0%
Imipenem	65	5.1%	-	-	-	-
Levofloxacin	40	3.1%	-	-	-	-
Linezolid	-	-	33	9.5%	-	-
Meropenem	80	6.3%	-	-	-	-
Nalidixic acid	10	0.8%	-	-	-	
Quinupristin/dalfopristin	-	-	10	2.9%	-	-
Sulfamethoxazole	10	0.8%	-	-	-	-
Teicoplanin	-	-	19	5.4%	-	-

Tetracycline	99	7.8%	42	12.0%	17	22.7%
Tigecycline	16	1.3%	28	8.0%	-	-
Trimethoprim	10	0.8%	-	-	-	-
Trimethoprim-Sulfamethoxazole	95	7.5%	-	-	-	-
Vancomycin	-	-	28	8.0%	-	-
Total	**1270**		**349**		**75**	

Scattering of missing data or incomplete AST results entries were observed in the two trials **(Tables 17, and 18).** Seven of the 23 laboratories selecting *Salmonella* did not submit complete results.
Regarding the *E. faecium/ E. faecalis* trial, only one out of the nine participating laboratories revealed incomplete results of their own available antimicrobial agents **(Table 18)**.
Participants need to carefully enter 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 *Salmonella* strains reported by AH laboratories (n=23) participating in the 11th EQA of the EQAsia project.

Lab ID No.	Salm EQAsia 25.1	Salm EQAsia 25.2	Salm EQAsia 25.3	Salm EQAsia 25.4	Salm EQAsia 25.5
#22	GEN, AZI	AMP, AZI	-	AZI	AZI
#33	TGC	TGC	TGC	TET	TGC
#36	AMK	AMK	AMK	AMK	AMP
#53	FOX	ERT	-	-	-
#54	-	-	-	CHL	-
#68	-	-	-	-	GEN
#88	FOT	FOT	FOX	FOT	FOT

Sal, *Salmonella*

Table 18. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by AH laboratories (n=9) participating in the 11th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 25.2	Ef EQAsia 25.3	Ef EQAsia 25.4	Ef EQAsia 25.5	Ef EQAsia 25.7
#38	-	DAP	-	-	DAP

Ef, *E. faecium/ E. faecalis*

4.2 *Salmonella* spp. panel

Twenty-three laboratories from nine countries uploaded results for the *Salmonella* spp. panel.

4.2.1 Bacterial identification

Twenty-three participating laboratories submitted results for bacterial identification **(Table 19).**

The complete panel of five target *Salmonella* strains was identified correctly by all 23 laboratories. One non-*Salmonella* strain (strain Salm EQAsia 25.6) was misidentified as *Salmonella* by laboratory #65 **(table 19).**

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

Strain	Bacterial ID	No. correct
Salm EQAsia 25.1	*Salmonella*	23/23
Salm EQAsia 25.2	*Salmonella*	23/23
Salm EQAsia 25.3	*Salmonella*	23/23
Salm EQAsia 25.4	*Salmonella*	23/23
Salm EQAsia 25.5	*Salmonella*	23/23
Salm EQAsia 25.6	Non-*Salmonella*	21/22
Salm EQAsia 25.7	Non-*Salmonella*	21/21

Salm, *Salmonella*

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 panel. Twenty out of 23 participating laboratories submitted AST results of *Salmonella* spp panel.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 82.6% (strain Salm EQAsia 25.2) to 88.7% (strain Salm EQAsia 25.1) **(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 20 AH laboratories for the *Salmonella* spp. panel.

Strain	AST in total	% Correct
Salm EQAsia 25.1	230	88.7
Salm EQAsia 25.2	230	82.6
Salm EQAsia 25.3	232	89.2
Salm EQAsia 25.4	231	86.1
Salm EQAsia 25.5	232	84.5

Salm, *Salmonella*

Antimicrobial-based analysis

Fifteen antimicrobials had deviations equal to or higher than 5% from the expected results. The antimicrobials showing the highest deviations from the expected results were levofloxacin (77.5%), followed by colistin (48.0%). Only six antimicrobials showed deviations of less than 5% (ceftazidime, sulfamethoxazole/trimethoprim, ampicillin, chloramphenicol, nalidixic acid and trimethoprim). **(Figure 14).**

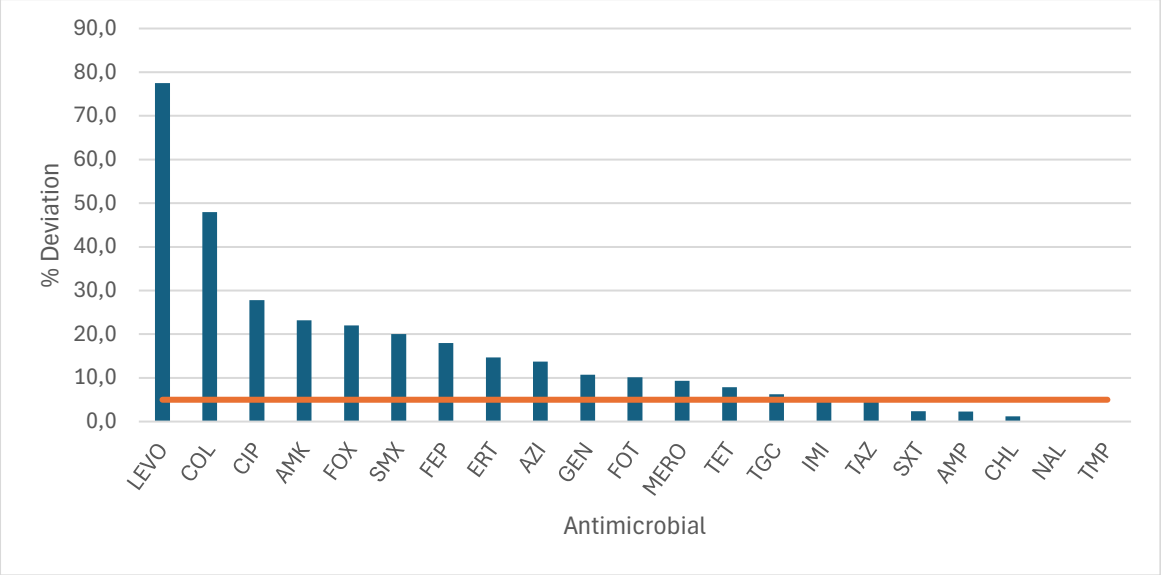

Figure 14. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=20) participating in the 11th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

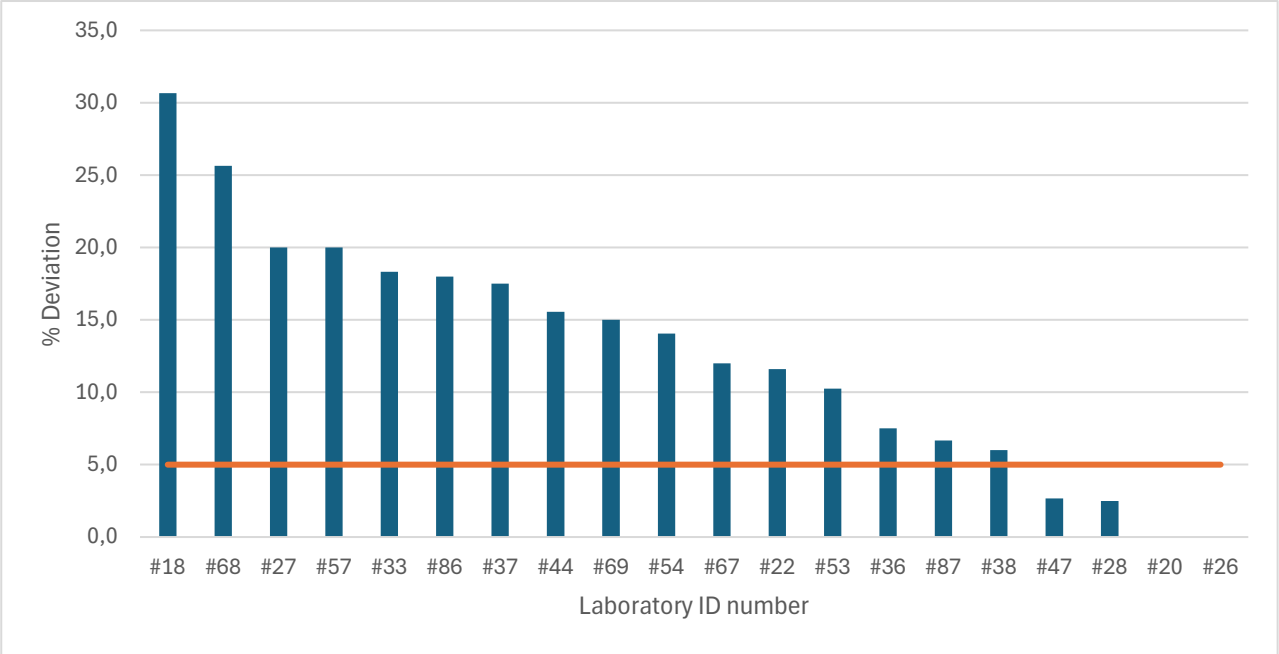

Figure 15. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=20) participating in the 11th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 4 out of the 20 participants **(Figure 15)**. In average, the deviation was 12.7% (ranging from 0.0 to 30.7%). As the acceptance level was set to 5% deviation, 16 laboratories (18, 22, 22, 26, 27, #33, #36, #37, #38, #44, #53, #54, #57, #67, #68, #69, #86 and #8) did not perform within the expected range for the *Salmonella* panel.

4.2.4 β-lactamase-producing *Salmonella*

None of the twenty-three participating laboratories uploaded results for this component of the *Salmonella* trial.

4.2.5 Quality control strains *E. coli* ATCC 25922

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 *Salmonella* trial.

Among the 23 participating laboratories, 21 submitted results for the reference strain *E. coli* ATCC 25922, while none of the laboratories 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 disc diffusion, and MIC was determined by broth microdilution **(Table 21)**. The highest proportion of test results outside of the expected range was observed for sulfamethoxazole (3 out of 5), ertapenem (2 out of 6), trimethoprim (2 out of 7), meropenem (4 out of 15) and cefotaxime (4 out of 15) **(Table 21).**

Regarding the laboratories' performance **(Figure 16)**, laboratories #20, # 22, 36, 53, #57 and #65 presented no deviation. The remaining fifteen laboratories presented deviations that ranged from 7.7% to 42.9%**.** Overall, the average deviation for this part of the panel was 16.0%.

Table 21. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *Salmonella* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disc Diffusion	MIC	Total
AMK	1/7	0/3	**1/10**
AMP	1/17	0/2	**1/19**
FEP	2/9	0/2	**2/11**
FOT	3/14	1/1	**4/15**
FOX	0/9	-	**0/9**
TAZ	2/11	0/2	**2/13**
CHL	4/16	0/1	**4/17**
CIP	1/15	2/3	**3/18**
COL	-	-	**-**
ETP	1/5	1/1	**2/6**
GEN	1/16	0/4	**1/20**
IMI	1/10	0/3	**1/13**
MERO	2/12	2/3	**4/15**
SMX	3/5		**3/5**
TET	1/15	0/2	**2/17**
TMP	2/6	0/1	**2/7**

Disc Diff. – Inhibition Zone Diameter determination by Disc Diffusion.
MIC – MIC determination by broth macro or microdilution, or by agar dilution.

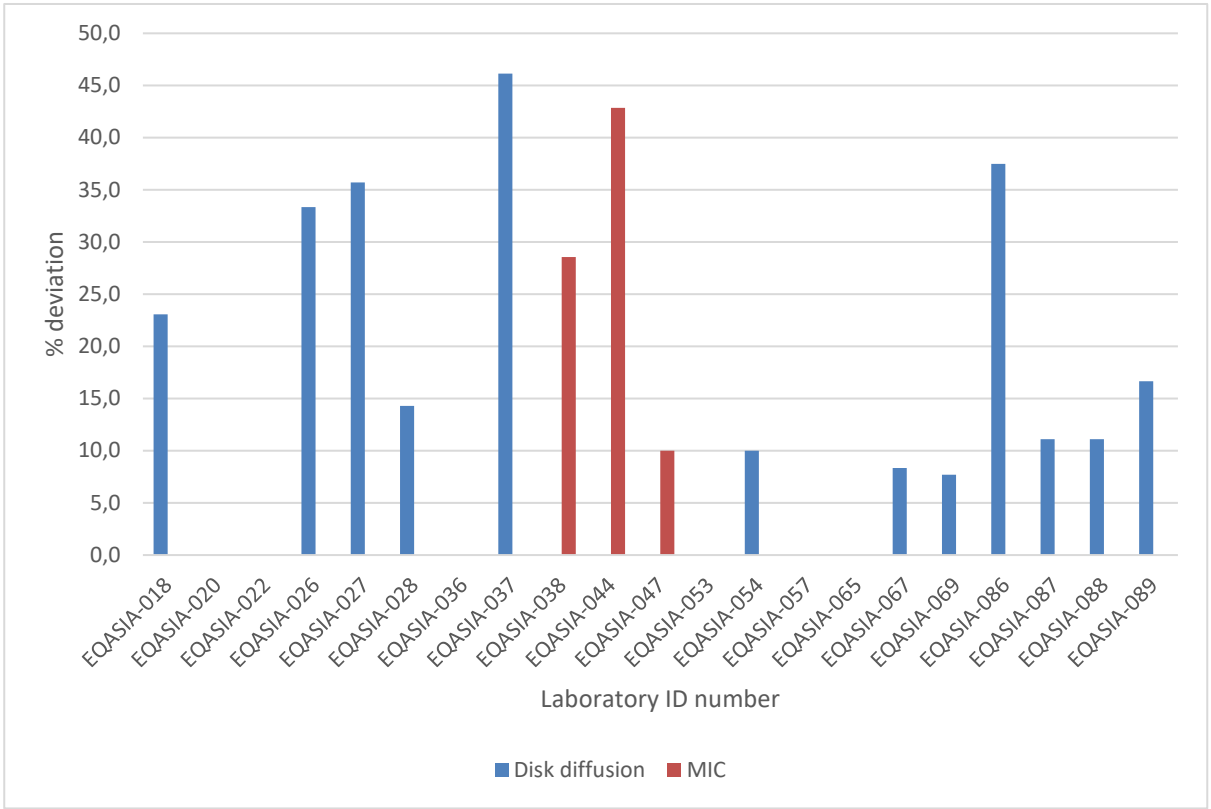

Figure 16. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Salmonella* trial by the AH laboratories.

4.3 *E. faecium/ E. faecalis* panel

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

4.3.1 Bacterial identification

All nine participating laboratories submitted results for bacterial identification **(Table 22)**. The complete panel of five target *E. faecalis* and *E. faecium* strains and two non-target strains was identified correctly by five laboratories. Strains Ef EQAsia 25.4 and Ef EQAsia 25.5 were correctly identified by all the labs.

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 25.1	Non-*Enterococcus faecalis/faecium*	6/6
Ef EQAsia 25.2	*Enterococcus faecalis*	7/9
Ef EQAsia 25.3	*Enterococcus faecium*	8/9
Ef EQAsia 25.4	*Enterococcus faecalis*	9/9
Ef EQAsia 25.5	*Enterococcus faecalis*	9/9
Ef EQAsia 25.6	Non-*Enterococcus faecalis/faecium*	6/9
Ef EQAsia 25.7	*Enterococcus faecium*	6/7

Ef, *Enterococcus faecalis/Enterococcus Faecalis*

4.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 88.2% (strain Ef EQASIA 25.2) to 98.0% (strain Ef EQASIA 25.4) for each strain **(Table 23)**. 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.

Table 23. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 9 AH laboratories for the *E. faecium/ E. faecalis* trial.

Strain	AST in total	% Correct
Ef EQAsia 25.2	272	88.2
Ef EQAsia 25.3	296	93.9
Ef EQAsia 25.4	300	98.0
Ef EQAsia 25.5	300	93.3
Ef EQAsia 25.7	228	92.1

Ef, *Enterococcus*

Antimicrobial-based analysis

Antimicrobials with the highest deviations from the expected results were daptomycin (33.3%), followed by ciprofloxacin (26.2%), tigecycline (25.9%), ampicillin (25.3%), chloramphenicol (23.3%), and teicoplanin (20.4%). Moderate deviations were observed for linezolid (19.0%), gentamicin (18.4%), vancomycin (16.3%), and tetracycline (13.8%). The lowest deviations were recorded for erythromycin (2.5%), while quinupristin–dalfopristin showed no deviation (0%) **(Figure 17)**.

Laboratory-based analysis

Eight 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 10.7% (ranging from 3.3% to 17.9%). As the acceptance level was set to 5% deviation, Laboratories #18, # 27, 37, 38, 44, 54, and 69% did not perform within the acceptable range for the enterococci panel.

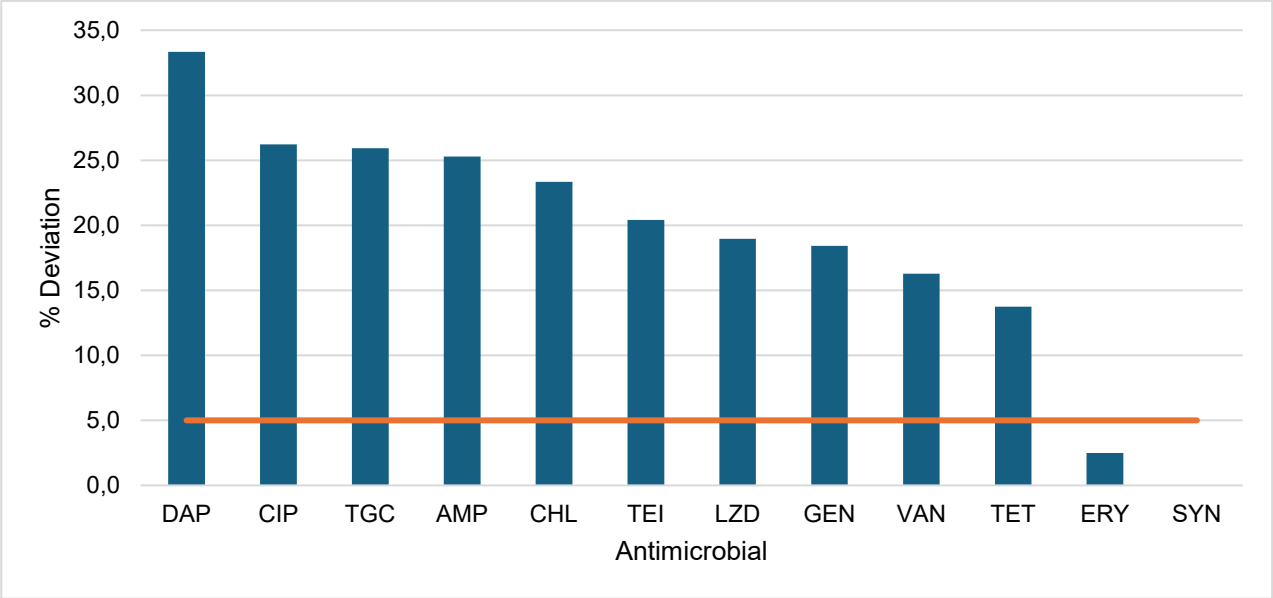

Figure 17. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=9) participating in the 11th EQA of the EQAsia project. Results are categorized according to antimicrobial agent

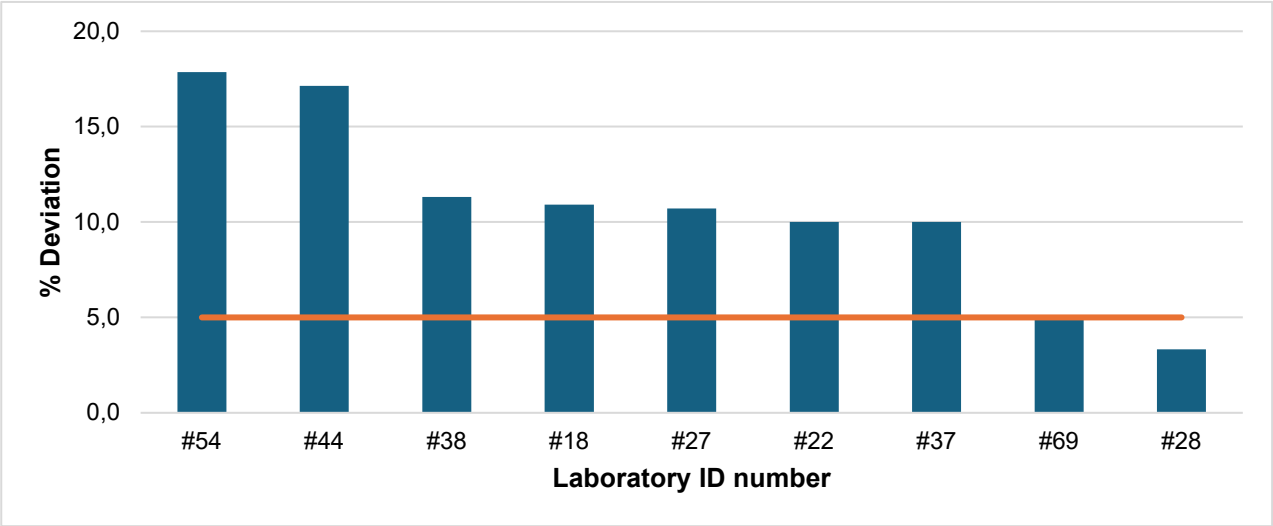

Figure 18. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=9) participating in the 11th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 disc 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 nine participating laboratories, four submitted results for the reference strain *S. aureus* ATCC 25923, while none submitted results for *E. faecalis* ATCC 29212. The highest proportion of test results outside the expected range was observed for vancomycin (1 out of 2 results) **(Table 24).** In terms of laboratory performance **(Figure 19)**, Laboratories #18, #22, and #28 showed no deviation. Laboratory #54 showed a single deviation (14.3%).

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		
	Disc Diffusion*	MIC**	Total
Ampicillin	0/3	--	**0/3**
Chloramphenicol	0/4	--	**0/4**
Ciprofloxacin	0/4	--	**0/4**
Erythromycin	0/4	--	**0/4**
Gentamicin	0/4	--	**0/4**
Linezolid	0/2	--	**0/2**
Quinupristin and Dalfopristin	0/1	--	**0/1**
Tetracycline	0/4	--	**0/1**
Tigecycline	0/1	--	**0/1**
Vancomycin	1/2	--	**1/2**

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

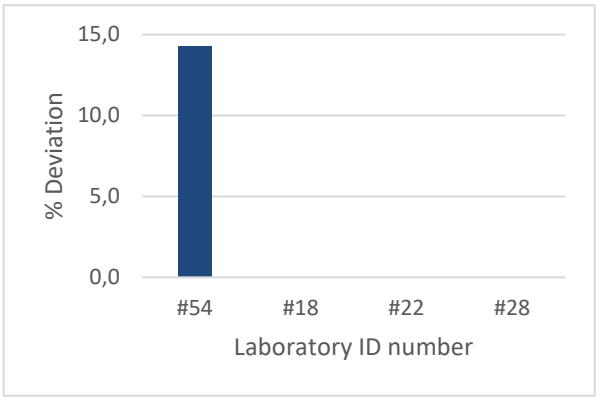

Figure 19. Percentage of deviation in the AST *S. aureus* ATCC 25923 in the *E. faecium/ E. faecalis* trial by the AH laboratories

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4.4 Campylobacter jejuni/coli trial

Six laboratories from four 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 25.2.

Table 25. Bacterial identification of each of the seven test strains provided related to the *C. jejuni/ C. coli* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Camp EQAsia 25.1	*Campylobacter coli*	5/6
Camp EQAsia 25.2	*Campylobacter jejuni*	0/5
Camp EQAsia 25.3	Non-*Campylobacter coli/jejuni*	1/3
Camp EQAsia 25.4	*Campylobacter coli*	3/5
Camp EQAsia 25.5	Non-*Campylobacter coli/jejuni*	2/2
Camp EQAsia 25.6	*Campylobacter jejuni*	4/6
Camp EQAsia 25.7	*Campylobacter coli*	3/6

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

The AST results were only submitted for four test strains and no lab submitted AST results for Camp EQAsia 25.2. The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 89% (strain Camp EQAsia 25.7) to 100% (strain Camp EQAsia 25.4) 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 6 AH laboratories for the *C. jejuni/ C. coli* trial.

Strain	AST in total	% Correct
Camp EQAsia 25.1	100	89.0
Camp EQAsia 25.4	52	100.0
Camp EQAsia 25.6	84	96.4
Camp EQAsia 25.7	64	89.0

Camp, *C. jejuni/ C. coli*

Antimicrobial-based analysis

Antimicrobials with the deviation higher than 5% threshold were ertapenem (100%), tetracycline (29.4%) ciprofloxacin (27.8%) and erythromycin (17.6%) and gentamicin (11.1%) **(Figure 20)**. Only chloramphenicol revealed no deviation from the expected results.

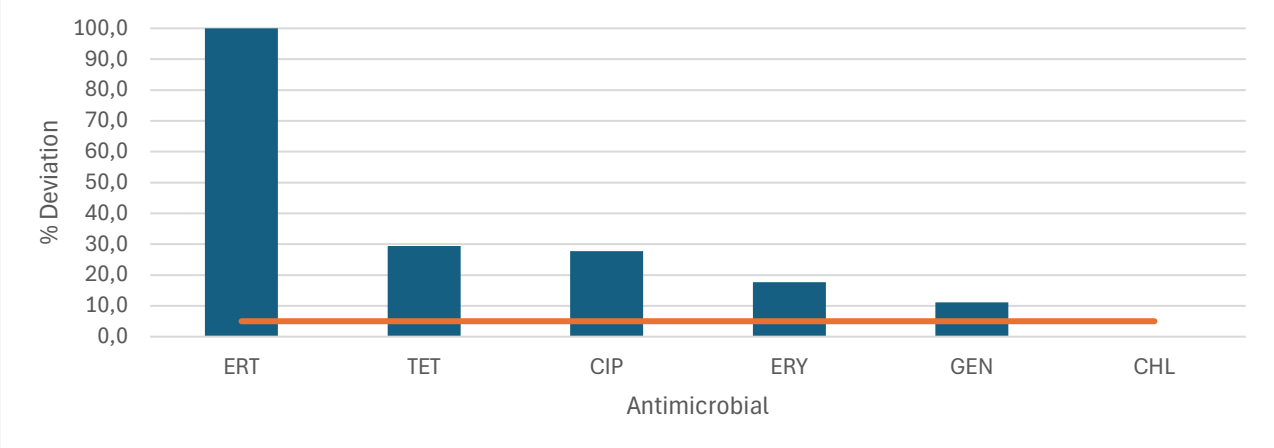


Figure 20. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=6) participating in the 11th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the percentage deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for one out of the six participants **(Figure 21)**. Laboratory #33 presented the highest deviation.

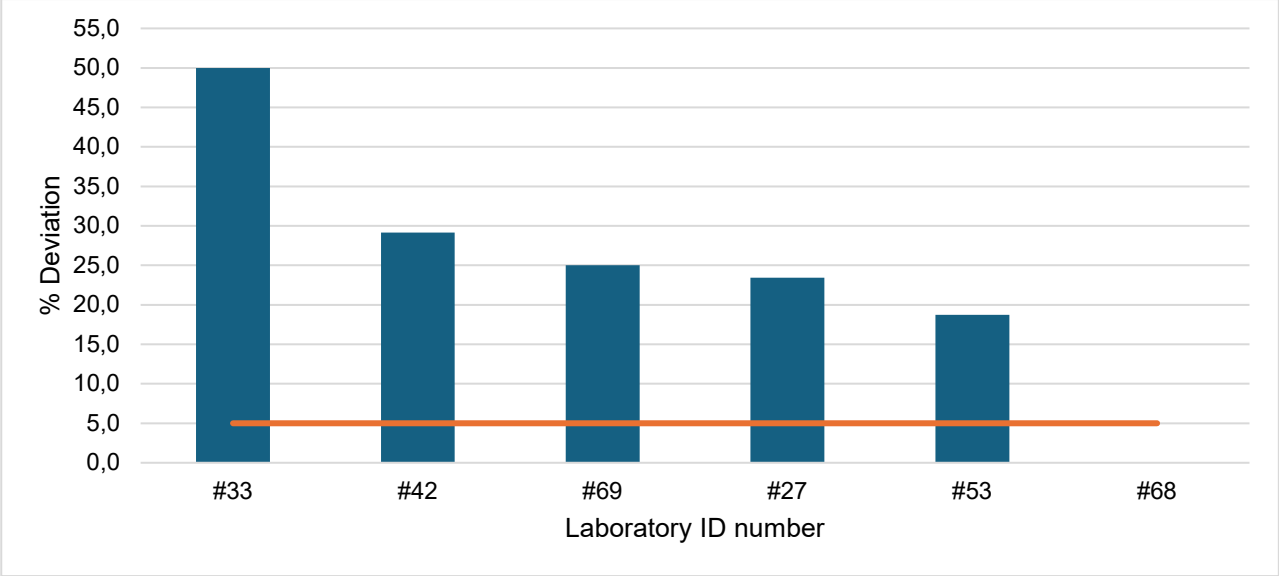


Figure 21. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=6) participating in the 11th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strain *C. jejuni* ATCC 33560

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

Among the six participating laboratories, three participating laboratories (#42, #53 and #69) that submitted AST results used disc diffusion results for *C. jejuni* ATCC 33560, acceptance intervals for disc diffusion are only available for ciprofloxacin and erythromycin **(Appendix 3b).** One of three ciprofloxacin QC results was out of range, while no deviations were observed for erythromycin in the quality control results reported by the participating laboratories **(Table 27)**.

In terms of performance, laboratories #42 and #53 showed no deviations for the two antimicrobials tested **(Figure 22)**

Table 27. AST of the reference strain *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disc Diffusion	Total
Ciprofloxacin	1/3	**1/3**
Erythromycin	0/3	**0/3**

Disc Diff. – Inhibition Zone Diameter determination by Disc Diffusion

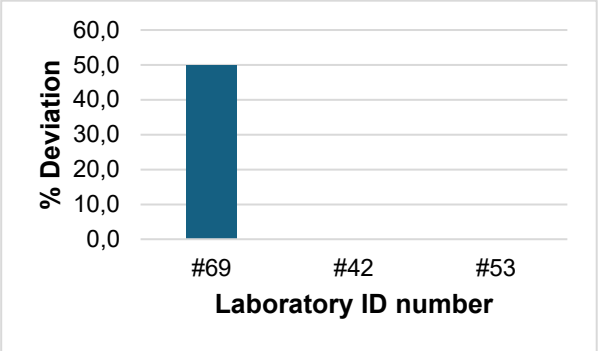


Figure 22. Percentage of deviation in the AST of *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial by AH laboratories

5. Results – Overall

5.1 Bacterial identification

A total of 33 HH and 24 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 54 laboratories for the *Salmonella* spp. panel, 29 laboratories for the *E. faecalis/E. faecium* panel, and 18 laboratories for the *Campylobacter* spp. panel.

Considering the strains tested by each laboratory in each of the trials, it was possible to calculate the percentage of incorrectly identified isolates. **Figure 23** shows the distribution of laboratories that showed deviations in identification of species for each of the panels.

For *Salmonella* spp., all 54 laboratories that submitted data reported 0% deviation, indicating complete agreement in the identification of the target strains. No laboratory showed any level of deviation for this panel, demonstrating that the *Salmonella* spp. panel was consistently and accurately identified across all submissions.

In contrast, the performance for *Enterococcus* spp. was more varied. While 20 laboratories reported 0% deviation, several laboratories showed partial or complete disagreement with the expected identifications. Specifically, 4 laboratories exhibited 20% deviation, 2 showed 40% deviation, another 2 had 60% deviation, and 1 laboratory recorded 100% deviation. These results indicate substantial variation in the ability to correctly identify *Enterococcus* spp. strains, with a notable proportion of laboratories encountering difficulties.

Similarly, the identification of *Campylobacter* spp. showed mixed outcomes. Eight laboratories reported 0% deviation, but deviations were observed among other participants: 3 laboratories showed 20% deviation, 2 laboratories 40% deviation, and 2 laboratories 100% deviation. The presence of both partial and full deviations suggests that several laboratories faced challenges in reviving and correctly identifying the *Campylobacter* strains, which may have contributed to the observed variability.

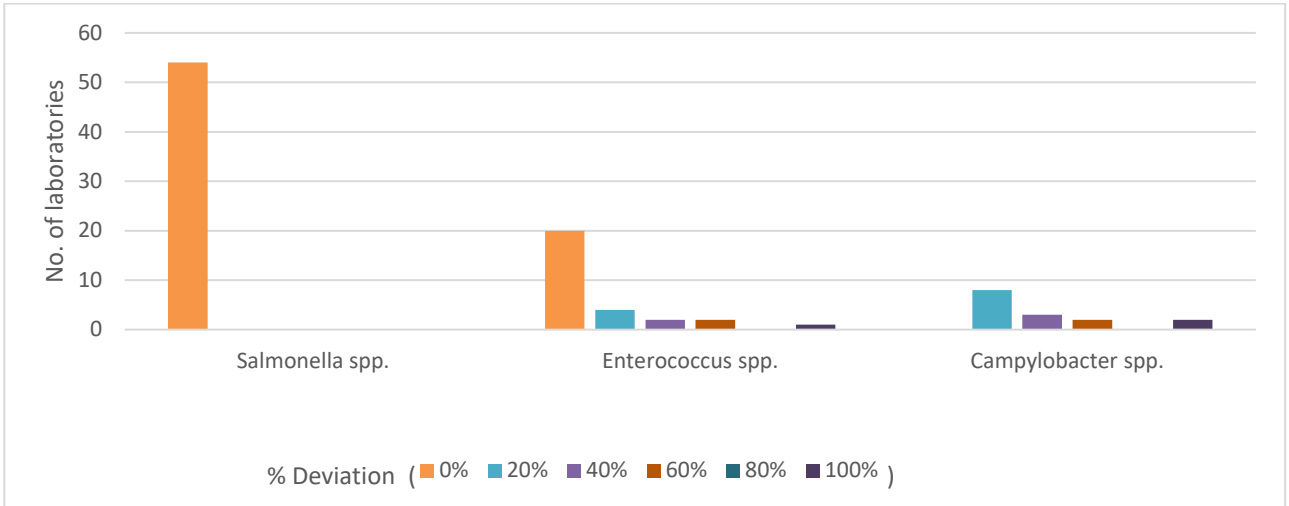


Figure 23. Percentage of deviation in the bacterial identification of target strains in the *Salmonella* spp., *E. faecalis/E. faecium* and *Campylobacter* spp., panels by the participating laboratories.

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.

For *Salmonella* spp.,the highest deviation rate was observed for levofloxacin (n=47 deviations; 71.2%), followed by colistin (n=27; 54.0%) **(Figure 24)**. Ciprofloxacin showed a lower deviation rate (n=49; 20.3%) compared with colistin but remained among the agents with notable discrepancies. Other antimicrobials with moderate deviation levels included cefoxitin (n=22; 22.2%), amikacin (n=23; 17.4%), gentamicin (n=21; 11.5%), and sulfamethoxazole (n=2; 20.0%), though the latter was based on a small sample size (n=10). All remaining antimicrobials demonstrated deviation rates below 10%, including cefepime (n=11; 8.9%), azithromycin (n=7; 8.1%), cefotaxime (n=10; 7.6%), tigecycline (n=2; 7.4%), imipenem (n=8; 6.7%), tetracycline (n=10; 6.3%), and ertapenem (n=5; 5.6%). Very low deviations were observed for trimethoprim–sulfamethoxazole (n=4; 2.0%) and chloramphenicol (n=2; 0.9%). No deviations were detected for nalidixic acid or trimethoprim (0%). Overall, deviations remained low for most antimicrobials, with fluoroquinolones, particularly levofloxacin, and colistin showing the highest discrepancy levels for *Salmonella*.

In the *Enterococcus* spp. panel, the highest deviation counts were observed for ampicillin (n=30; 24.2%), followed by ciprofloxacin (n=29; 22.4%), chloramphenicol (n=22, 22%) vancomycin (n=18; 15.8%) (**Figure 25)**. Moderate deviation levels were recorded for teicoplanin (n=13; 19.1%), linezolid (12; 13.2%), tetracycline (12; 9.8%), tigecycline (9; 16.4%) and gentamicin (8; 11.4%). Daptomycin exhibited the highest deviation rate (6; 28.6%) in from the expected AST results for *Enterococcus* panel. Minimal deviations were observed for erythromycin (4; 3.4%), and quinupristin and dalfopristin (1; 7.1%). Overall, deviation rates varied across the panel, with the highest discrepancies occurring among fluoroquinolones and daptomycin.

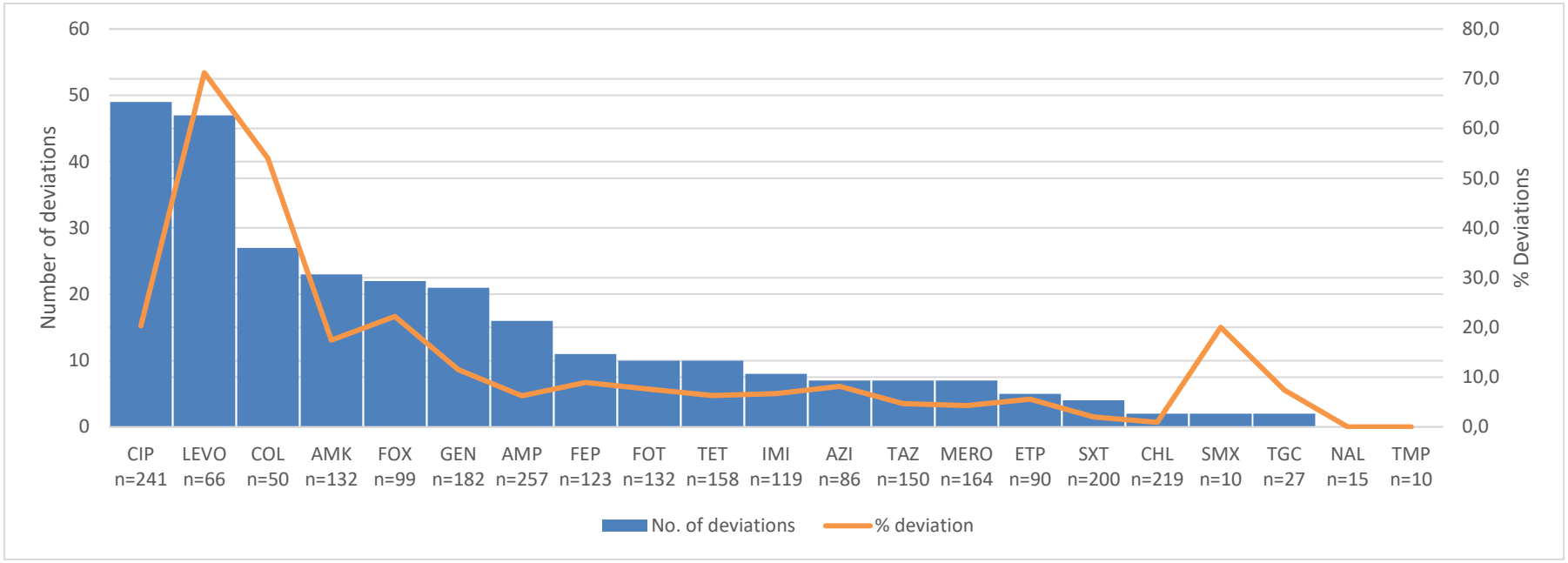


Figure 24. Distribution of the number and percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* spp. strains by the participating laboratories (n=54) in the 11th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing number of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the percentage of deviations for each antimicrobial agent.

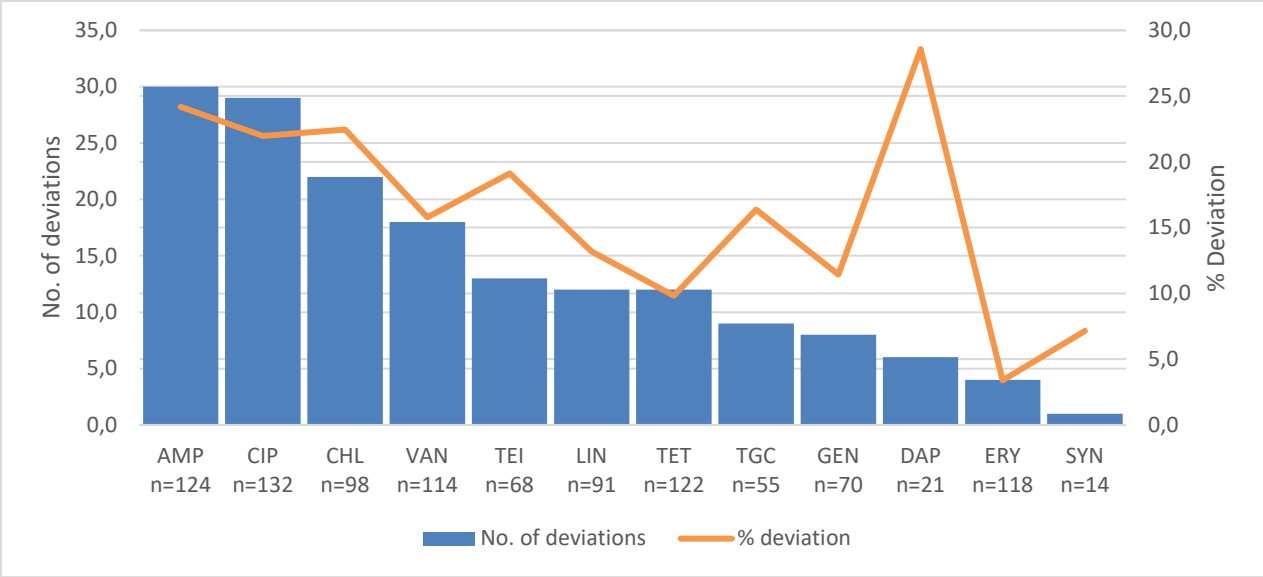


Figure 25. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. faecalis/E. faecium* strains by the participating laboratories (n=29) in the 11th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing number of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the percentage of deviations for each antimicrobial agent.

There were only 141 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 **(Figure 26)**. The highest deviation rates were observed for ertapenem (1 deviation; 100%), although this result was based on a one test (n=1) and should be interpreted cautiously. Tetracycline and ciprofloxacin both showed substantial deviation levels (13/37; 35.1% and 13/38; 34.2%, respectively), representing the largest contributors among routinely tested antimicrobials. Erythromycin exhibited a moderate deviation rate (9/37; 24.3%), while gentamicin showed relatively few discrepancies (3/24; 12.5%). No deviations were detected for chloramphenicol (0/4; 0%). Overall, the highest discrepancies occurred among tetracycline, ciprofloxacin, and erythromycin, consistent with known resistance patterns in *Campylobacter* spp.

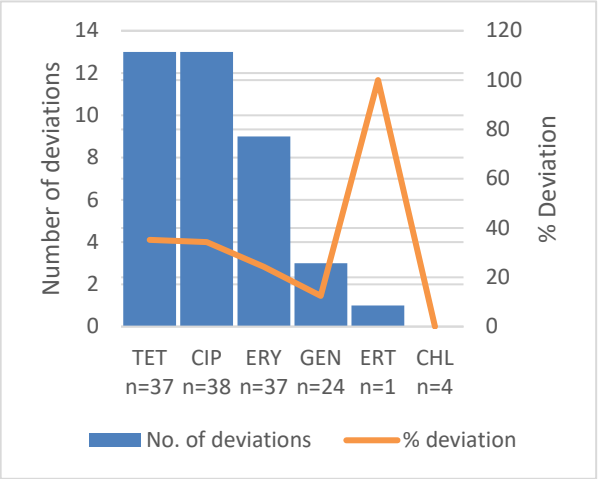


Figure 26. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among Campylobacter spp. strains by the participating laboratories (n=13) in the 11th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing number of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the percentage of deviations for each antimicrobial agent.

5.2.2 Laboratories performance

Across the three panels included in this EQA round, participating laboratories demonstrated a wide range of performance levels. As in previous trials, the degree of heterogeneity varied considerably between panels. The *Campylobacter* spp. panel showed the greatest variability, with performance scores ranging from 43.8% to 100%, indicating substantial differences in laboratory proficiency. (**Figure 27**). The highest overall performance was recorded for the *Salmonella* spp. panel. Several laboratories achieved perfect scores, and the average performance across all laboratories reporting results for this panel was high. The lowest score in the *Salmonella* spp. panel was 82.7%, while the majority of laboratories (n=49) scored 90% or above.

For the *Enterococcus* spp. panel, results showed moderate heterogeneity. While some laboratories scored above 95%, the lowest observed score was 50%. The performance spread indicates that while many laboratories perform consistently well, others still face challenges in achieving reliable results for the *Enterococcus* spp. panel.

The *Campylobacter* spp. panel exhibited the lowest minimum score among all panels, with results ranging from a high of 100% to a low of 43.8%. This confirms that *Campylobacter* detection and identification remain particularly complex for many laboratories, contributing significantly to the overall variability in performance.

Laboratories were ranked from #1 to #57 based on their average score across all panels for which they submitted results. The highest-ranking laboratories (rank #1) achieved an average score of 100%, while the lowest-ranking laboratory (rank #57) obtained a performance score of 68.9%. The total average score across all laboratories submitting results was 93.5%, while the median was 94.5%. Among the 57 laboratories, 14.0% achieved 100%, 47.4% scored ⩾95%, and 84.2% scored ⩾90%. This distribution indicates that although many laboratories demonstrated excellent proficiency, there are marked performance gaps between the top and lower scoring laboratories.

Overall, the results of this EQA trial reveal a

substantial degree of heterogeneity, especially within the *Campylobacter* spp. panel. These findings highlight once again that proficiency levels vary greatly among participating laboratories, emphasizing the importance of continuous training, methodological harmonization, and quality assurance efforts across the region.

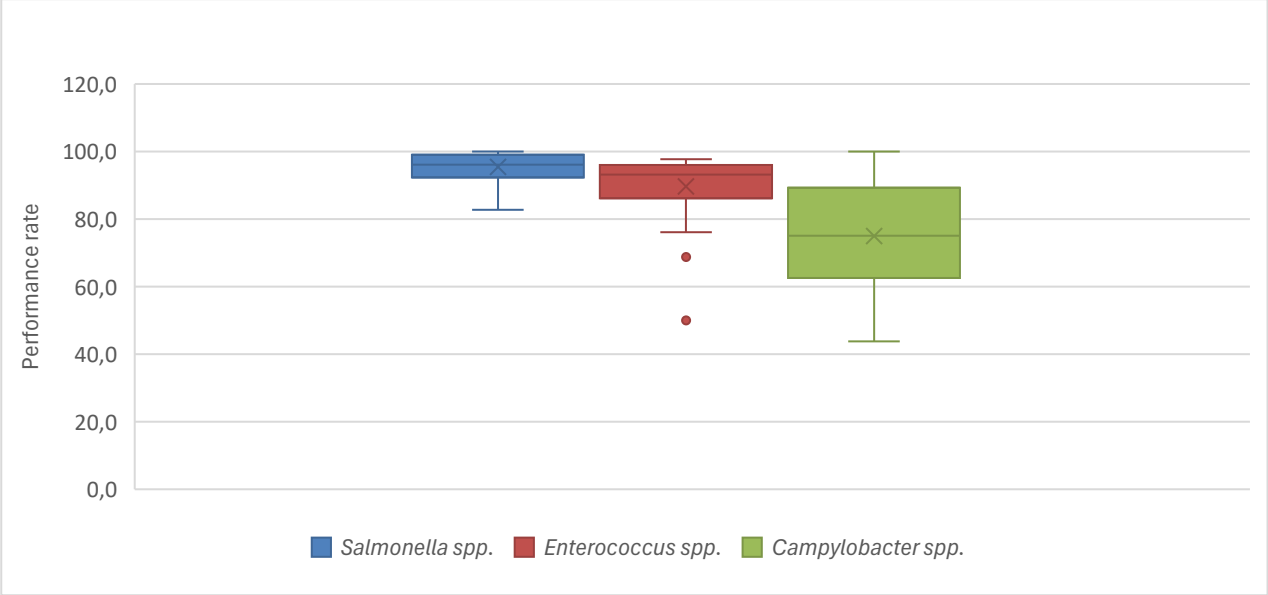


Figure 27. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 11th EQA of the EQAsia project.

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 *Salmonella* spp. panel, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disc 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 (14 in the *Enterococcus* spp. panel, 13 in the *Campylobacter* spp. panel, 1 in the *Salmonella* spp. panel) did not submit results from reference strain testing at all.

For the *Salmonella* spp. panel, 23 laboratories showed no deviation among those that submitted QC data (n=52). The remaining laboratories presented deviations ranging from 8.3% to 100%, illustrating substantial variability in performance within this panel.

In the Enterococcus spp. panel, 15 laboratories submitted QC results. Of these, 9 laboratories achieved 100% accordance with the expected ranges. The remaining 6 laboratories showed deviations, with scores ranging from 33.3% to 81.8%, indicating moderate heterogeneity and highlighting laboratories which need performance improvement.

For the *Campylobacter* spp. panel, a total of 4 laboratories submitted QC results. Three laboratories achieved 100%, while one laboratory scored 50%. While performance among these few submissions was mostly consistent, the very small number of participants limits broader interpretation of panel-wide proficiency.

Overall, when comparing reference strain testing

with target strain AST results, the QC component remained more heterogeneous, leading to lower performance for a subset of laboratories. The variability was most apparent in the *Enterococcus* results (due to the spread from 33.3% to 100%.

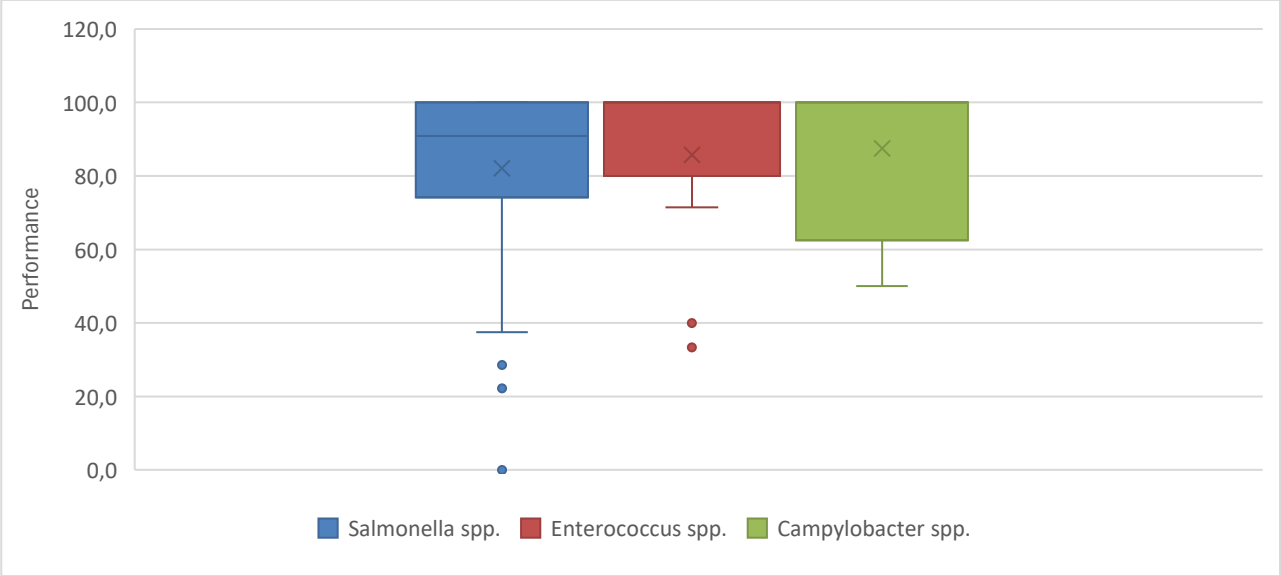


Figure 28. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 11th EQA of the EQAsia project.

6. Discussion

6.1 Human Health Laboratories

In the 11th EQAsia EQA round, 33 Human Health laboratories participated and submitted data for at least one panel, with disc diffusion remaining the most frequently used AST methodology. While several laboratories applied broth microdilution or gradient diffusion, methodological consistency varied across the region. Revival of strains was generally successful except in the *Neisseria gonorrhoeae* panel, where preservation issues resulted in 15 out of 17 laboratories being unable to culture the isolates. As confirmed in the post-trial review, this was related to transport viability rather than laboratory capability, highlighting the need to strengthen shipment and pre-distribution viability checks for fastidious organisms.

All laboratories that performed bacterial identification in the *Salmonella* spp. and *Enterococcus* spp. 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 expected 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 or inconsistent reporting of AST data persisted in both the *Salmonella* and *Enterococcus* panels, where multiple laboratories did not test all available antimicrobials across all five target strains. Ensuring complete datasets is essential for accurate assessment of laboratory performance and for distinguishing methodological errors from data entry gaps.

Bacterial identification performance differed substantially between panels. *Salmonella* identification was excellent, with all 31 laboratories correctly identifying all five target strains and both non-target organisms. In contrast, *Enterococcus* identification was more challenging. Correct identification of target strains ranged from 15/20 to 18/20, while non-target strains were correctly identified by only 16–17 laboratories, indicating persistent difficulties in differentiating *E. faecalis, E. faecium*, and related species. *Campylobacter* identification represented the greatest challenge; no laboratory correctly identified all seven strains, and for some strains, such as Camp EQAsia 25.2, none of the nine laboratories providing results achieved correct identification. These findings reflect the well-known sensitivity of *Campylobacter* spp. to suboptimal culture conditions and the technical complexity of their phenotypic differentiation.

Overall AST performance of HH laboratories varied substantially between panels. In the *Salmonella* panel, most laboratories performed well, although notable deviations were associated with fluoroquinolones and colistin, drug classes known to be method-sensitive.

The high deviation observed for colistin can be explained by the fact that AST results for this antimicrobial should be interpreted only as susceptible or resistant, in accordance with the EUCAST breakpoints tables and as stated in the EQA11 protocol. However, several laboratories reported intermediate results instead of susceptible, resulting in a score penalty (a score of 3 instead of the maximum score of 4). In the context of EQA, the laboratories must adhere to the guidelines provided in the protocol for the interpretation of AST results.

In the *Enterococcus* panel, high deviation rates for AST results were observed for several clinically important antimicrobials, including teicoplanin and ampicillin. This suggests that, beyond identification challenges, laboratories continue to face difficulty in reliably interpreting resistance profiles for Gram-positive organisms. The small number of laboratories submitting

Campylobacter AST data limited the strength of conclusions for this panel, although the results indicated significant strain-specific variation and methodological gaps.

Performance on quality control strains further emphasized the variability across laboratories. Some laboratories demonstrated strong QC concordance, while others showed repeated deviations, particularly when using MIC-based methods for *Salmonella* QC strains or when testing glycopeptides for *Enterococcus* QC strains. Routine and systematic QC testing remains essential for ensuring reliability of AST results, and the inconsistent submission of QC data indicates that these practices require improvement.

Despite the challenges observed, participation in this round reflects strong regional engagement and a willingness among national laboratories to strengthen their AST capacities. The difficulties encountered with *N. gonorrhoeae* strains provided valuable lessons for improving pre-distribution viability testing and shipping procedures for future EQAs. Continued technical support, targeted training, particularly in *Enterococcus* and *Campylobacter* identification and AST, and strengthened QC implementation will be essential for further improving laboratory performance across the region.

6.2 Animal Health Laboratories

For the Animal Health sector, 24 laboratories participated in the 11th EQA of the EQAsia project. The participating laboratories mostly applied disc diffusion alone for determining Inhibition Zone Diameters. Several laboratories relied solely on MIC determination methods or a combination of disc diffusion and MIC testing by broth microdilution.

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.

As mentioned above, bacterial identification was the first component of each panel. No major issues were observed in the identification of the five target strains among the seven isolates provided in the *Salmonella* panel. However, the identification and differentiation between *E. faecium*, *E. faecalis*, and other *Enterococcus* species revealed limitations in the bacterial identification capacity of some participating laboratories. Several isolates in the *Campylobacter* panels could not be revived by certain laboratories, or the reported identification of revived isolates did not always match the baseline results. Attention should therefore be paid to the use of appropriate culture media and strict adherence to the protocol for reconstituting lyophilized bacteria, as these factors may have contributed to the inability of some laboratories to successfully cultivate *Campylobacter* spp. isolates.

Regarding AST performance, several antimicrobials showed high deviations from the expected results in the *Salmonella* panels, including levofloxacin (77.5%), colistin (48.0%), and ciprofloxacin (27.8%). Similar to the HH laboratories, the high deviation observed for colistin can be explained by the fact that AST results for this antimicrobial should be interpreted only as susceptible or resistant, in accordance with the EUCAST breakpoints tables and as stated in the EQA11 protocol. In addition, colistin testing requires the use of broth microdilution, whereas some laboratories applied disc diffusion and may lack sufficient knowledge or experience with the appropriate methodology. The high deviation observed for sulfamethoxazole was mainly driven by the small number of tests performed for this antimicrobial.

In the *E. faecium/E. faecalis* trial, AST results submitted for the five *E. faecium/E. faecalis* strains were still considered for evaluation even when the species were incorrectly identified by the laboratories (limited to cases where *E. faecalis* was identified as *E. faecium*, or vice versa), as the interpretation criteria do not differ substantially between these two species. In this trial, the highest deviations were observed for

ampicillin (16.2%) and teicoplanin (15.8%). In the *C. jejuni/C.* coli trial, the highest AST deviation was observed for ertapenem (100%), which reflects the very low number of tests performed (single reported test).

Regarding laboratory performance, laboratories were ranked according to the percentage of deviating results in antimicrobial susceptibility testing. A deviation of 5% or less in result interpretation (R/I/S) was observed for 4 of the 20 participating laboratories in the *Salmonella* panel, 3 of the 9 laboratories in the *E. faecium/E. faecalis* trial, and only one laboratory in *the C. jejuni/C. coli* trial.

Lastly, laboratories performed antimicrobial susceptibility testing (AST) of the quality control strains relevant to each panel. In the *Salmonella* panels, 21 of the 23 participating laboratories submitted results for the reference strains. In the *E. faecium/E. faecalis* trial, five laboratories did not submit results for either the *S. aureus* ATCC 25923 or *E. faecalis* ATCC 29212 reference strains. In the *C. jejuni/C. coli* trial, three of the six participating laboratories submitted results for *C. jejuni* ATCC 33560.

For laboratories that reported quality control data, deviations in this component were defined as AST results for the reference strains that fell outside the established quality control acceptance intervals. These findings suggest that handling and use of reference strains need to be strengthened to ensure consistent laboratory performance.

7. Conclusions

This report presents the results of the EQAsia 11th EQA trial, which was carried out in September – November 2025 and included bacterial identification and AST of several prominent WHO and FAO priority pathogens: *Salmonella* spp., *E. faecalis/ E. faecium*, *C coli/ C. jejuni,* and *N. gonorrhoeae.*

The results of the 11th EQAsia EQA trial demonstrate clear progress in laboratory capacity across South and Southeast Asia while also highlighting several persistent challenges that require continued attention. Overall, participation was strong among both HH and AH laboratories, reflecting sustained regional commitment to improving bacterial identification, AST, and quality assurance systems. The high performance observed in several panels, particularly *Salmonella*, shows that many laboratories maintain robust diagnostic capability. However, substantial heterogeneity across organisms, antimicrobials, and laboratory sectors underlines the need for further harmonization and targeted capacity building.

Bacterial identification performance varied considerably between panels. Accuracy for *Salmonella* was consistently high across both HH and AH, with all laboratories correctly identifying the target strains. In contrast, identification of *Enterococcus* spp. and especially *Campylobacter* spp. remained more challenging, with multiple laboratories reporting incorrect or incomplete results. The inability of laboratories to consistently revive and identify *Campylobacter* strains persisted, and no laboratory correctly identified all seven *Campylobacter* strains in HH; one *Campylobacter* strain (Camp EQAsia 25.2) had 0/9 correct IDs in HH and 0/5 in AH submissions. Similarly, the *N. gonorrhoeae* panel could not be completed due to strain viability issues — 15 of 17 HH laboratories were unable to culture the isolates, underscoring the need for strengthened pre-distribution viability testing and improved shipping procedures for fastidious organisms.

AST performance also revealed notable variability. While overall average scores were high, 93.5% across laboratories, significant deviations were observed for specific antimicrobials. Fluoroquinolones, including levofloxacin and ciprofloxacin, and colistin accounted for the highest error rates in both HH and AH laboratories. For Gram-positive organisms, several laboratories struggled with accurate interpretation for ampicillin, teicoplanin, vancomycin, and other key agents included in the *Enterococcus* panel. The *Campylobacter* panel showed the greatest AST variability overall, driven partly by the small number of laboratories submitting results but also by limitations in methodological capacity for microaerophilic organisms.

Quality control (QC) strain testing remained one of the weakest components of laboratory performance. Many laboratories either failed to submit QC results or submitted results falling outside the expected ranges. Since QC accuracy is essential for validating AST outcomes, these findings point to gaps in internal quality systems and a need for greater routine use and maintenance of reference strains. Importantly, laboratories that showed higher deviation rates for QC strains often also demonstrated poorer performance in test strain AST, emphasizing the direct relationship between QC implementation and diagnostic reliability.

Incomplete or inconsistent data submission was another recurring issue, particularly within the *Salmonella* and *Enterococcus* panels. Missing or incomplete AST entries limited the ability to fully assess performance and underscored the need for meticulous data entry within the informatics module. Ensuring complete datasets is essential for accurate evaluation and for enabling laboratories to identify true methodological errors rather than administrative inconsistencies.

Taken together, the findings of this EQA trial reaffirm that strong foundational capacity exists across the region, especially for commonly tested pathogens such as *Salmonella*. However, they also demonstrate the necessity of continued investment in training, methodological

harmonization, and quality assurance. Attention must be directed toward improving the culture, identification, and AST of fastidious organisms, most notably *Campylobacter* spp. and *Neisseria gonorrhoeae,* as well as enhancing technical competencies related to AST interpretation for both Gram-negative and Gram-positive bacteria. As the EQAsia programme approaches its conclusion under the Fleming Fund, this final EQA round underscores the long-term importance of sustaining regional quality assessment systems. Strengthened QC practices, consistent adherence to international standards (CLSI/EUCAST), and systematic corrective actions will be crucial for ensuring reliable, comparable, and high-quality AMR surveillance data across One Health sectors. Continued national and regional support will be essential to preserve and further advance the substantial progress achieved during successive EQAsia EQA trials.

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.

[3] Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 35th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2025.

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

[5] Unemo M, Golparian D, Sánchez-Busó L, Grad Y, Jacobsson S, Ohnishi M, Lahra MM, Limnios A, Sikora AE, Wi T, Harris SR. 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. Epub 2016 Jul 17. PMID: 27432602; PMCID: PMC5079299.

[6] The European Committee on Antimicrobial Susceptibility Testing. *Routine and extended internal quality control for MIC determination and disc diffusion as recommended by EUCAST*. Version 15.0, 2025. http://www.eucast.org.

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

[8] EQAsia Website: https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eqasia/eqa-trials

9. Appendices

Appendix 1: EQA11 Protocol

EQAsia EQA11 trial

Protocol
Identification and antimicrobial susceptibility testing (AST) of *Salmonella* 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 EQA11 trial** includes four EQA panels each composed of seven test strains – *Salmonella* 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 disc 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 disc diffusion of the Enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC).

The QC strains provided within EQA11 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 *Salmonella* 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 EQA11 OUTLINE

3.1 Shipping and receipt of strains

Your laboratory is one of the 63 human health and animal health laboratories from South and Southeast Asia participating in EQA11. In September 2025, you are expected to receive a parcel containing one or more of the following panels:

- ***Salmonella* panel** - seven test strains of which five are *Salmonella* 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 disc 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 Salmonella and Enterococcus strains are shipped on media in transport tubes (swabs).

3.2 Reviving and storing the strains

On receipt of the **lyophilized Neisseria** samples, prompt processing is recommended. If not performing testing immediately, store the un-reconstituted vials at 2-8ºC and protect them from direct light. The **lyophilized campylobacter 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 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.

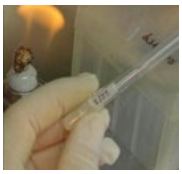
   Note: To maintain the vacuum condition, **do not break the tip of the ampoule**. Otherwise, the air will enter the ampoule and the cotton wool plug will be pushed down and in contact with dried bacterial culture. If it happens, please simply remove the cotton plug with forceps.

   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 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 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.

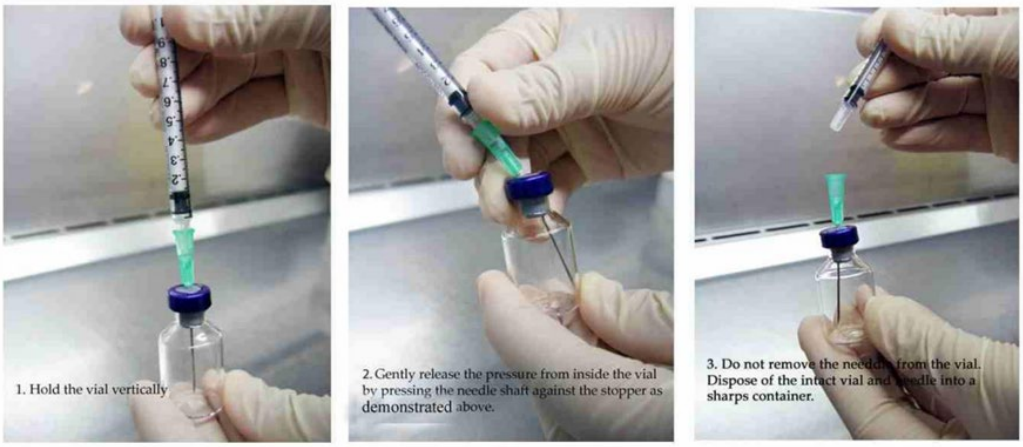
Do not mouth pipette and do not reconstitute the specimens until you are ready to plate them out.

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.

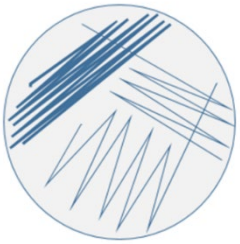


- **Reviving *Salmonella* and *Enterococcus* isolates**

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 *Salmonella* spp., *Enterococcus* spp., *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 *Salmonella* spp., *Enterococcus* spp., *Campylobacter* spp. and *Neisseria gonorrhoeae* test strains, and of the reference strains

The strains identified as *Salmonella* 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 disc diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 35th Ed.). When not available, EUCAST clinical breakpoints (Tables v. 15.1, 2025) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Salmonella* 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 *Salmonella*

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

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disc diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK*	≤ 18	-	≥ 18	≥ 8	-	< 8
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP*	≤ 1	-	> 4	≥ 27	-	< 24
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ*	≤ 1	-	> 4	≥ 22	-	< 19
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.06	0.12-0.5	≥ 1	≥ 31	21-30	≤ 20
Levofloxacin	≤ 0.12	0-25-1	≥ 2	≥ 21	17-20	≤ 16
Colistin, COL*	≤ 2	-	> 2	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
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, 35th 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 15.1, 2025. http://www.eucast.org."

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 Disc 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, 35th 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 15.1, 2025. 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 Disc 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 Disc diffusion (mm)		
	S	I	R	S	I	R
Azithromycin, AZI	≤ 1	-	-	≥ 30	-	-
Cefixime, CFM	≤ 0.25	-	-	≥ 31	-	-
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, 35th 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 (Disc diffusion and MIC QC ranges for ATC49226) in CLSI M100, 35th 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 following link:

https://sciencedata.dk/shared/25dfa151c3b5f841b3030fd42441570a

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 October 27th, 2025.

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) – *Salmonella spp.*

	Amikacin (AMK)		Ampicillin (AMP)		Azithromycin (AZI)		Cefepime (FEP)		Cefotaxime (FOT)		Cefoxitin (FOX)	
Salm EQAsia 25.1	<=4	S	>32	R	4	S	0.12	S	<=0.25	S	4	S
Salm EQAsia 25.2	<=4	S	2	S	8	S	0.25	S	0.25	S	16	I
Salm EQAsia 25.3	<=4	S	>32	R	4	S	0.25	S	≤0.25	S	4	S
Salm EQAsia 25.4	<=4	S	<=1	S	4	S	0.12	S	≤0.25	S	4	S
Salm EQAsia 25.5	<=4	S	>32	R	64	R	16	R	>64	R	4	S

R, Resistant; I, Intermediate; S, Susceptible

	Ceftazidime (TAZ)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)		Ertapenem (ETP)		Gentamicin (GEN)	
Salm EQAsia 25.1	0.5	S	≤8	S	≤0.015	S	≤0.25	I	≤0.015	S	≤0.5	S
Salm EQAsia 25.2	<=1	S	≤8	S	0.03	S	4	R	0.03	S	>16	R
Salm EQAsia 25.3	1	S	≤8	S	8	R	≤0.25	I	≤0.015	S	>16	R
Salm EQAsia 25.4	0.5	S	≤8	S	0.03	S	>4	R	≤0.015	S	≤0.5	S
Salm EQAsia 25.5	16	R	≤8	S	1	R	≤0.25	I	0.03	S	>16	R

R, Resistant; I, Intermediate; S, Susceptible

	Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Nalidixic acid (NAL)		Sulfamethoxazole (SMX)		Tetracycline (TET)	
Salm EQAsia 25.1	0.25	S	≤1	I	≤0.03	S	≤4	S	>512	R	>32	R
Salm EQAsia 25.2	0.25	S	≤1	I	≤0.03	S	≤4	S	>512	R	≤2	S
Salm EQAsia 25.3	0.25	S	8	R	≤0.03	S	>64	R	>512	R	>32	R
Salm EQAsia 25.4	0.5	S	≤1	I	≤0.03	S	≤4	S	16	S	≤2	S
Salm EQAsia 25.5	0.25	S	2	R	≤0.03	S	>64	R	>512	R	≤2	S

R, Resistant; I, Intermediate; S, Susceptible

	Tigecycline (TGC)		Trimethoprim (TMP)		Trime/Sulfa (SXT)	
Salm EQAsia 25.1	≤0.25	S	>16	R	>4	R
Salm EQAsia 25.2	≤0.25	S	≤0.25	S	<=0.12	S
Salm EQAsia 25.3	0.5	S	≤0.25	S	<=0.12	S
Salm EQAsia 25.4	≤0.25	S	≤0.25	S	<=0.12	S
Salm EQAsia 25.5	≤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 25.2	4	S	8	S	>16	R	0.5	S	>128	R	>1024	R
Ef EQAsia 25.3	8	S	8	S	1	S	1	S	>128	R	<=8	S
Ef EQAsia 25.4	2	S	64	R	>16	R	2	S	>128	R	>1024	R
Ef EQAsia 25.5	1	S	8	S	1	S	2	S	>128	R	<=8	S
Ef EQAsia 25.7	>64	R	8	S	>16	R	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 25.2	1	S	8	R	16	R	32	R	0.12	S	64	R
Ef EQAsia 25.3	1	S	32	R	<=0.5	S	128	R	0.25	S	<=1	S
Ef EQAsia 25.4	1	S	16	R	<=0.5	S	64	R	0.25	S	64	R
Ef EQAsia 25.5	1	S	16	R	<=0.5	S	64	R	0.12	S	16	R
Ef EQAsia 25.7	1	S	1	S	<=0.5	S	<=1	S	0.06	S	<=1	S

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 25.1	8	S	32	R	4	R	4	S	0.5	S	1	S
Camp EQAsia 25.2	4	S	32	R	0.5	S	>512	R	>16	R	64	R
Camp EQAsia 25.4	<=2	S	8	R	1	R	<=1	S	0.5	S	<=0.5	S
Camp EQAsia 25.6	<=2	S	<=012	S	0.25	S	<=1	S	0.5	S	<=0.5	S
Camp EQAsia 25.7	4	S	32	R	1	R	512	R	<=0.25	S	64	R

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 25.2	0.25	S	<0.002	S	<0.016	S	0.004	S	0.032	S	0.25	S
NG EQAsia 25.3	1	S	0.5	S	2	S	>32	R	2	R	4	R
NG EQAsia 25.5	>256	R	0.5	S	2	S	>32	R	1	I	128	R
NG EQAsia 25.6	4	R	0.004	S	<0.016	S	0.004	S	0.25	I	1	I
NG EQAsia 25.7	0.5	S	0.008	S	0.008/ 0.016	S	0.002/ 0.004	I	0.5	I	0.5	I

R, Resistant; I, Intermediate; S, Susceptible; *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 disc diffusion ranges are according to CLSI M100 35th 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 disc diffusion as recommended by EUCAST. Version 13.0, 2025. 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 disc diffusion ranges are according to CLSI M100 35th 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

Disc 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 disc diffusion ranges are according to CLSI M100 35th 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 disc diffusion ranges are according to CLSI M100 35th edition, Tables 4B and 5C

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