7th EQAsia External Quality Assessment Trial: *Salmonella spp., Enterococcus spp., Campylobacter spp.* and *Neisseria gonorrhoeae* – 2023

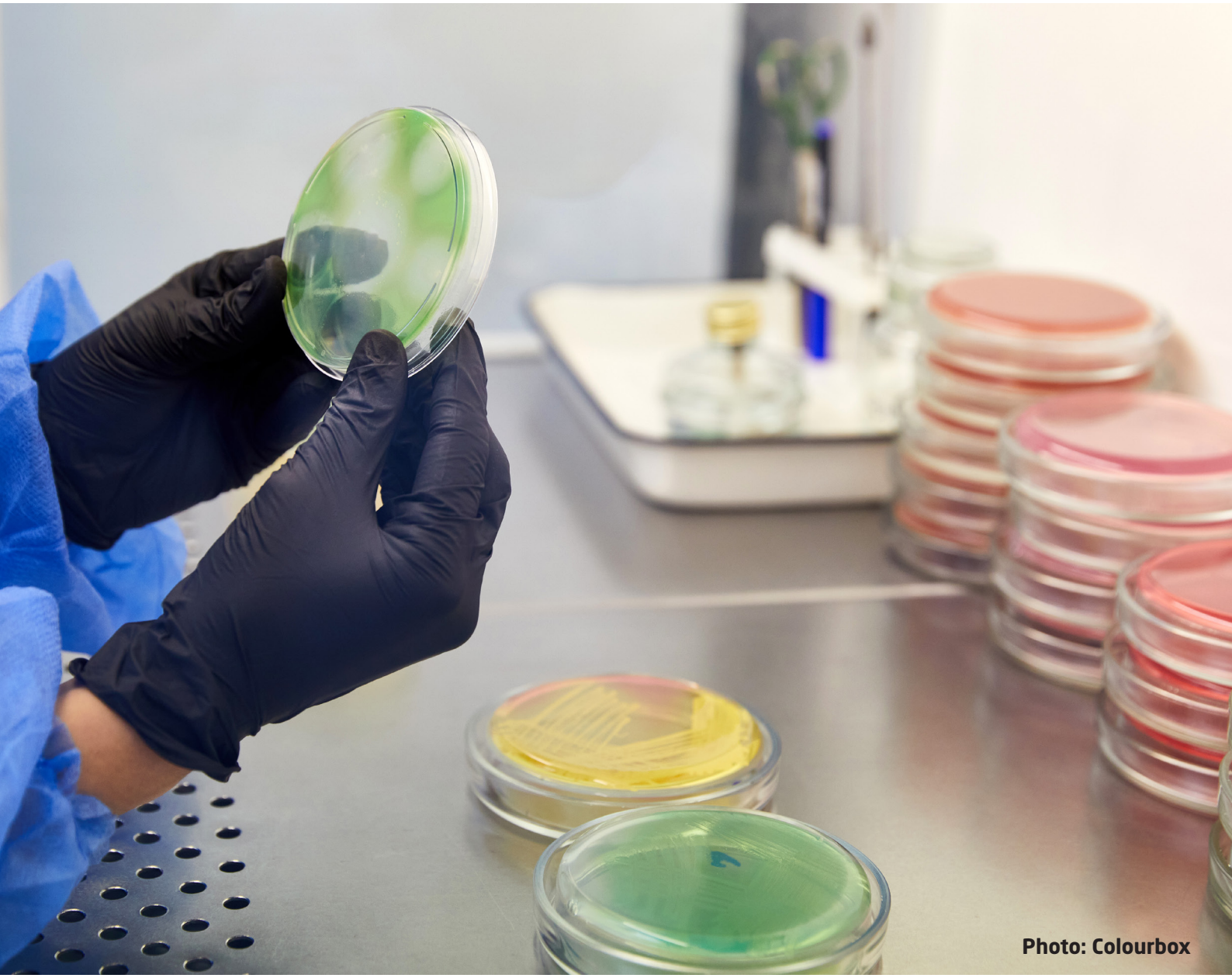


Photo: Colourbox

7th EQAsia External Quality Assessment trial:

Salmonella spp., Enterococcus spp., Campylobacter spp. and *Neisseria gonorrhoeae – 2023*

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

7th EQAsia External Quality Assessment trial:
Salmonella spp., Enterococcus spp., Campylobacter spp. and *Neisseria gonorrhoeae – 2023*

Tomislav Kostyanev[1], Hiba Al Mir[1], Rangsiya Prathan[2], Sarah Marvig Johansson[1], Pattrarat Chanchaithong[2], Elif Seyda Tosun[1], Taradon Luangtongkum[2], Freshwork A. Abegaz[3], Tobin Guarnacci[3], Nimesh Poudyal[3], Lone Brink Rasmussen[1], Soo-Young Kwon[3], Rungtip Chuanchuen[2], Rene S. Hendriksen[1]

[1]National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark
[2]Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
[3]International Vaccine Institute, Seoul, South Korea

1st edition, March 2024

Steering Committee Institutions:

World Health Organization
Food and Agriculture Organization of the United Nations
World Organisation for Animal Health
The Peter Doherty Institute for Infection and Immunity, Australia
Pacific Pathology Training Centre, New Zealand
NSW Health Pathology Microbiology, New South Wales, Australia

Index

Executive Summary

This report summarizes the results of the 7th External Quality Assessment (EQA) trial of EQAsia, the Fleming Fund Regional Grant aiming to strengthen the provision of EQA services across the One Health sector among National Reference Laboratories / Centres of Excellence in South and Southeast Asia. The EQAsia project has entered a second phase (2023 to 2025) in which it will continue to deliver the established EQA programme for both the Human Health (HH sector) and Food and Animal Health (AH sector) laboratories in the region.

The EQA trial was carried out in October - November 2023 and included bacterial identification and antimicrobial susceptibility testing (AST) of several prominent WHO and FAO priority pathogens: *Salmonella spp, Enterococcus faecalis, Enterococcus faecium, Campylobacter coli, Campylobacter jejuni,* and *Neisseria gonorrhoeae*. The latter isolate was introduced for the first time in this EQA programme since the start of the EQAsia project.

A total of 20 HH and 16 AH laboratories participated in this EQA trial. The participating laboratories were from 14 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam). Similarly to previous EQAsia EQAs, participating laboratories could choose one or more panels among the ones offered in the current EQA round. In total, data were submitted by 33 laboratories for the *Salmonella spp.* panel, 24 laboratories for the *E. faecalis/E. faecium* panel, 11 – for *Campylobacter spp.*, and 8 – for *N. gonorrhoeae*.

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

The bacterial identification component consisted in identifying the five strains of the organism in question (target organism) among a total of seven strains. The identification results from almost all laboratories that submitted data from the *Salmonella spp.* panel were conform the baseline results. Identification appeared to be more challenging in the other three panels.

On average, the AST performance of participating laboratories was the best in the *Salmonella spp.* panel (94.6%), followed by enterococci (92.6%), *Campylobacter spp.* (90.3%), and *N. gonorrhoeae* (85.2%).

Laboratories were ranked from #1 to #35 (one laboratory did not submit any data) based on their average score across the panels in which they participated. The average score varied between 78.4% (rank #35) and 99.2% (rank #1). The total average score among all 35 laboratories that submitted results was 92.8%, while the median was 93.6%.

As with previous EQAsia EQAs, many of the laboratories were struggling the most with quality control strain testing. Several laboratories (1 in the *Salmonella spp.* and *N. gonorrhoeae* panels each, 2 in the *enterococci* panel and 4 in the *Campylobacter spp.* panel) did not submit results from reference strain testing at all. The rate of laboratories whose results was conform the expected range of QC values varied across the four panels, as follows – *Salmonella spp.* (57.6%), enterococci (45.8%), *Campylobacter spp.* (27.3%), and *N. gonorrhoeae* (100%).

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

Overall, the results from this EQAsia EQA flag

once more the need to focus on both basic and more advance methodologies for culture, identification, and antimicrobial susceptibility testing within a training curriculum for the participating laboratories. Quality control testing and the use of the appropriate reference strains, as well as the translation of the QC results into corrective action by laboratories is of utmost importance to ensure a decent level of quality in a microbiology laboratory. Providing and maintaining a standardized level of credible diagnostic services would allow laboratories to generate reliable results that would ultimately feed in a pool of reliable data for surveillance purposes5

1. Introduction

The EQAsia project was launched in 2020 aiming to strengthen the provision of External Quality Assessment (EQA) services across the One Health sector among National Reference Laboratories / Centres of Excellence in South and Southeast Asia. EQAsia is supported by the Fleming Fund and strives to increase the quality of laboratory-based surveillance of WHO GLASS pathogens [1] and FAO priority pathogens [2]. EQAsia has transitioned to a second phase and will continue to deliver the established EQA programme for both the Human Health (HH) sector and Food and Animal Health (AH) sector in the region until the end of 2025.

The EQAsia Consortium includes the Technical University of Denmark, National Food Institute (DTU Food) as the Lead Grantee, the International Vaccine Institute (IVI) in South Korea, and the Faculty of Veterinary Science, Chulalongkorn University (CUVET) in Thailand.

EQAsia provides a state-of-the-art EQA program free of charge for the South and Southeast Asian region through CUVET Thailand, an existing regional provider. The EQAsia program is designed to enable the laboratories to select and participate in relevant proficiency tests of both pathogen identification and antimicrobial susceptibility testing (AST), in line with the requirements of the WHO GLASS [1]. The EQA program is supported by an informatics module where laboratories can report their results and methods used.

A total of seven 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 three 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 seventh EQA trial of the EQAsia project (EQA7) carried out in October – November 2023. 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 seventh 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 disk diffusion and MIC determination are listed in the EQA7 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 EQA7 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 EQA7

A total of 36 laboratories participated in the seventh EQA trial of the EQAsia project: 20 laboratories belonging to the HH Sector and 16 belonging to the AH Sector, located in 14 countries: Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam (**Figure 1**).

2.2 Strains

Participating laboratories could register for any of the four EQA panels. For each registration, laboratories received seven bacterial strains of which only five strains were the target species. Hence, the initial task was the identification of the bacterial species of interest using the laboratory's own routine method for bacterial identification.

The five target species of each organism were selected to represent a heterogeneous phenotypic profile. With the purpose to monitor and assess improvements and trends over time for each organism included in EQA7, 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 were tested and selected by University of New South Wales, Melbourne, Australia (UNSW). The expected MIC values are available in the appendix of this report (**Appendix 2d**).

Reference strains for the *Salmonella*, enterococci, and *Campylobacter* panels [*Escherichia coli* ATCC 25922/CCM 3954 (for disk diffusion of Salmonella strains), *E. coli* NCTC 13846/CCM 8874 (for testing colistin), *Campylobacter jejuni* ATCC 33560/ CCM 6214, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disk diffusion of the enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC)] were supplied during previous EQA rounds. The QC strains provided within EQA7 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 M100-32nd 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 EQA7 protocol (**Appendix 1**) and in **Table 1**. These antimicrobials correspond to several antimicrobial class representatives important for surveillance.

The reference values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 32nd Ed. and VET06, 1st Ed.) [3]. When not available, EUCAST clinical breakpoints (Tables v. 13.0, 2023) [5] or epidemiological cut off values [6] were used instead.

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

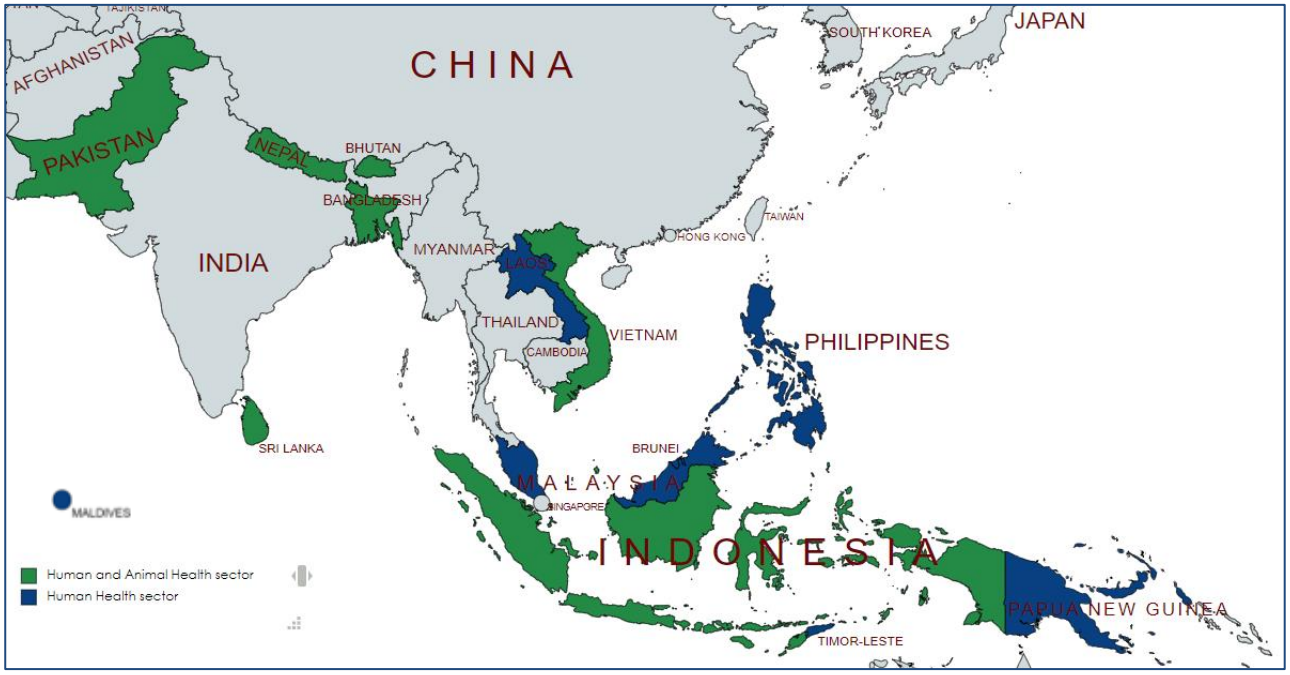


Figure 1. Countries participating in the 7th EQA of the EQAsia project. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA7 2023.

***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/	
Ciprofloxacin		dalfopristin	
Colistin		Teicoplanin	
Ertapenem		Tetracycline	
Gentamicin		Tigecycline	
Imipenem		Vancomycin	
Meropenem			
Nalidixic acid			
Sulfamethoxazole			
Tetracycline			
Trimethoprim			

2.4 Distribution

The bacterial strains were dispatched either as lyophilized strains or on swabs in transport medium in October 2023 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 EQA7 protocol (**Appendix 1**).

2.5 Procedure

Protocols and all relevant information were sent to sites and were also available at the EQAsia website [7], to allow access to all the necessary information at any time. The participants were recommended to store the lyophilized strains in a dark, dry and cool place until performance of AST.

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

Laboratories used procedures such as disk diffusion, gradient test, agar dilution and broth dilution. For the interpretation of results, only the categorisation as resistant / intermediate / susceptible (R/I/S) was evaluated, whereas MIC and inhibition zone diameter values were used as supplementary information.

All participants were invited to enter the obtained results into an informatics module designed within the EQAsia programme and adapted for this trial. The informatics module could be accessed through a secured individual login and password. After release of the results, the participants were invited to login to retrieve an individual database-generated evaluation report.

2.6 Data management

In past EQA trials, antimicrobial susceptibility testing of some of the reference strains revealed several incorrect results outside the acceptance interval for MIC determination. This is due to the use of automated instruments, which often test for an antimicrobial concentration range above the acceptance interval. For example, the quality control range for cefepime for *E. coli* ATCC 25922 is 0.016-0.12, and the laboratories using 'MIC – broth microdilution (automated)' have previously reported an MIC ≤ 1. As this is a method limitation and the laboratories cannot test for lower antimicrobial concentrations, the informatics module was adapted to score these specific occurrences as '1' (correct).

3. Results – Human Health Laboratories

3.1 Overall participation

All 20 Human Health laboratories participating in the 7th EQA of the EQAsia project, submitted results. Among these, 18, 17, 5 and 8 laboratories submitted results for *Salmonella spp.*, enterococci, *Campylobacter spp.*, and *N. gonorrhoeae* panels, respectively. The methodologies applied primarily by the laboratories varied and are summarized in **Figure 2**. The participants were invited to report inhibition zone diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each drug-bug combination. Only the categorisation was evaluated, whereas the inhibition zone diameters/MIC values were used as supplementary information. The majority of participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results (**Figure 3**).

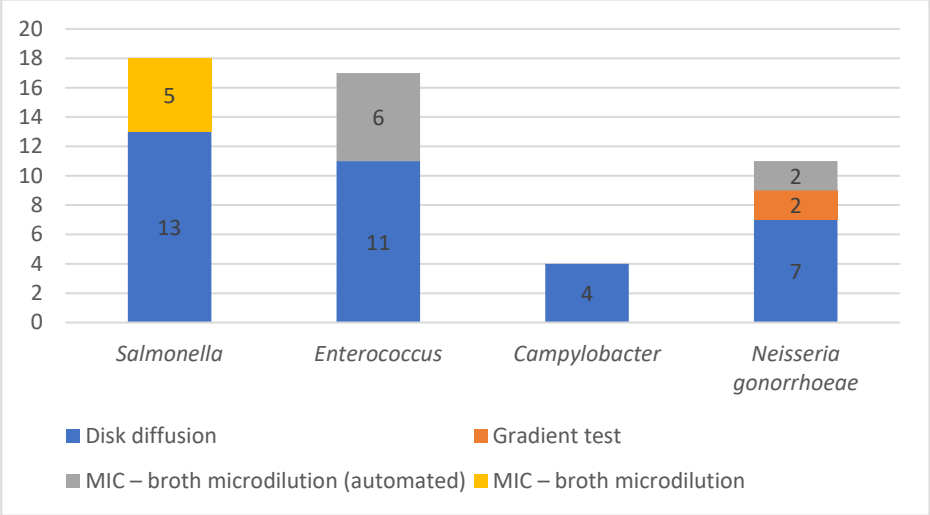


Figure 2. Methodologies primarily used by the laboratories for antimicrobial susceptibility testing in each of the panels.

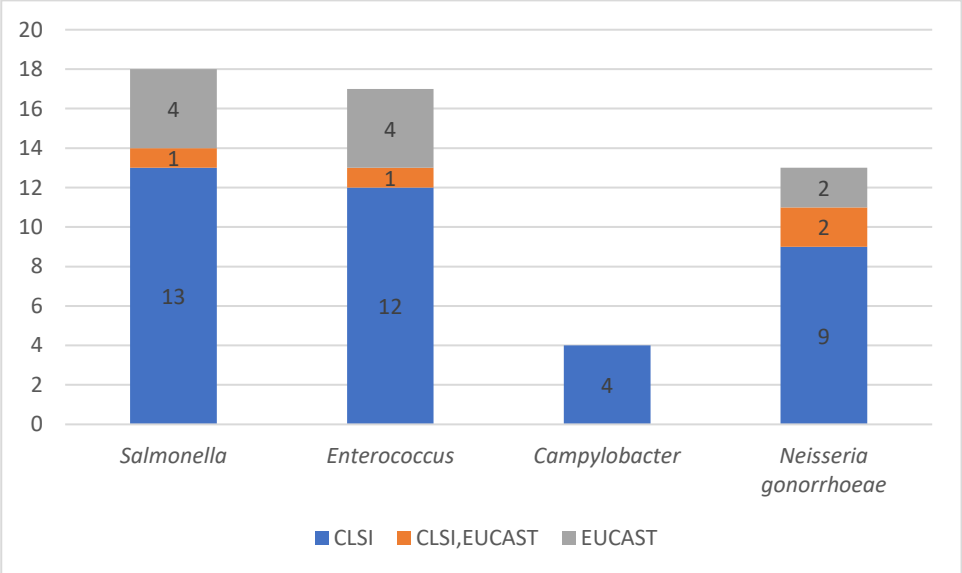


Figure 3. Use of international guidelines for interpretation of AST results by the participating laboratories.

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

The *Salmonella* panel had the highest number of total AST results (n=896) reported by 18 participating laboratories according to the recommended antimicrobials in CLSI (**Table 2**). One of the most frequently tested antibiotics were ceftazidime, ciprofloxacin, and meropenem. In the enterococci panel, participating laboratories tested and reported most frequently ampicillin, chloramphenicol, and ciprofloxacin. Only four antibiotics, ciprofloxacin, erythromycin, gentamicin, and tetracycline were tested and reported for *Campylobacter*.

In the *N. gonorrhoeae* panel, the majority of the laboratories tested all recommended antibiotics (**Table 2**).

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

	Salmonella		***Enterococcus***		***Campylobacter***		***Neisseria gonorrhoeae***	
Ampicillin	83	*9,3%*	83	*13,8%*	--	--	--	--
Azithromycin	38	*4,2%*	--	--	--	--	7	*10,4%*
Cefepime	74	*8,3%*	--	--	--	--	--	--
Cefixime	--	--	--	--	--	--	12	*17,9%*
Cefotaxime	57	*6,4%*	--	--	--	--	--	--
Cefoxitin	46	*5,1%*	--	--	--	--	--	--
Ceftazidime	84	*9,4%*	--	--	--	--	--	--
Ceftriaxone	--	--	--	--	--	--	12	*17,9%*
Chloramphenicol	72	*8,0%*	73	*12,1%*	--	--	--	--
Ciprofloxacin	84	*9,4%*	76	*12,6%*	8	*29,6%*	12	*17,9%*
Colistin	40	*4,5%*	--	--	--	--	--	--
Daptomycin	--	--	16	*2,7%*	--	--	--	--
Ertapenem	58	*6,5%*	--	--	--	--	--	--
Erythromycin	--	--	66	*11,0%*	8	*29,6%*	--	--
Gentamicin	11	*1,2%*	37	*6,2%*	3	*11,1%*	--	--
Imipenem	64	*7,1%*	--	--	--	--	--	--
Linezolid	--	--	48	*8,0%*	--	--	--	--
Meropenem	85	*9,5%*	--	--	--	--	--	--
Penicillin	--	--	--	--	--	--	12	*17,9%*
Quinupristin and dalfopristin	--	--	11	*1,8%*	--	--	--	--
Sulfamethoxazole	11	*1,2%*	--	--	--	--	--	--
Teicoplanin	--	--	36	*6,0%*	--	--	--	--
Tetracycline	66	*7,4%*	65	*10,8%*	8	*29,6%*	12	*17,9%*
Tigecycline	--	--	22	*3,7%*	--	--	--	--
Trimethoprim	23	*2,6%*	--	--	--	--	--	--
Vancomycin	--	--	68	*11,3%*	--	--	--	--
Total	**896**		**601**		**27**		**67**	

Missing data or incomplete AST results entries were observed in three out of four EQA panels among the HH laboratories participating in EQA7. A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains.

Six out of 18 laboratories had partially incomplete results submitted for the *Salmonella* panel (**Table 3**). The highest number of incomplete results in the *Salmonella* panel was seen for laboratories #05, #06, #17, and #32.

Seven out of 17 laboratories that selected the enterococci panel did not submit complete results of their own available antimicrobial agents (**Table 4**). The highest number of incomplete results in this panel were seen for laboratories #02, #32, #35, and #49.

There were no missing data in the *Campylobacter* panel data set. However, very few laboratories (n=3) reported results in this part of the EQA7 trial.

One out of 6 laboratories that submitted AST data for *N. gonorrhoeae* had incomplete results of their own available antimicrobial agents (**Table 5**).

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *Salmonella* strains reported by HH laboratories (n=18) participating in the 7th EQA of the EQAsia project.

Lab ID No.	Salm EQASIA 23.1	Salm EQASIA 23.2	Salm EQASIA 23.5	Salm EQASIA 23.6	Salm EQASIA 23.7
#01	--	--	--	--	--
#04	--	--	--	--	--
#05	FOT	FOT	FOX	FOT	FOT
#06	CIP	CIP	--	CIP	CIP
#07	--	--	--	TET	--
#10	--	--	--	--	--
#11	--	--	--	--	--
#12	--	CHL	--	--	--
#17	FOX	FOX	--	FOX	FOX
#32	CHL, SMT	CHL, ETP, GEN, SMT	--	ETP, IMI	GEN
#34	--	--	--	--	--
#35	--	--	--	--	--
#40	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--

Salm, *Salmonella*

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *E. faecalis/E. faecium* strains reported by HH laboratories (n=17) participating in the 7th EQA of the EQAsia project.

Lab ID No.	Ef EQASIA 23.1	Ef EQASIA 23.3	Ef EQASIA 23.4	Ef EQASIA 23.5	Ef EQASIA 23.7
#01	--	--	--	--	--
#02	GEN	GEN	GEN	ERY	GEN
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	--	DAP	DAP	DAP	--

#07	--	--	--	--	--
#11	--	--	--	--	--
#12	--	DAP	DAP	--	--
#17	--	TGC	TGC	TGC	TGC
#32	CIP, LZD, TEI, VAN	CHL, CIP	CHL, CIP, LZD, TEI	CHL, CIP, ERY, TEI	CHL, LZD, TEI
#34	--	--	--	--	--
#35	TET	TGC	TGC	TGC	TGC
#48	--	--	--	--	--
#49	QND	CIP, DAP, QND	CIP, DAP	DAP, QND	QND
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--

Ef, *E. faecalis/E. faecium*

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *N. gonorrhoeae* strains reported by HH laboratories (n=8) participating in the 7th EQA of the EQAsia project. Only 6 laboratories submitted AST data.

Lab ID No.	Ng EQASIA 23.2	Ng EQASIA 23.3	Ng EQASIA 23.4	Ng EQASIA 23.5	Ng EQASIA 23.6
#01	CRO	FIX	FIX, CRO	--	FIX, CRO
#02	--	--	--	--	--
#11	--	--	--	--	--
#13	--	--	--	--	--
#17	--	--	--	--	--
#34	--	--	--	--	--

Ng, *N. gonorrhoeae*

3.2 *Salmonella spp.* panel

18 laboratories from 13 countries uploaded results for the *Salmonella spp.* panel.

3.2.1 Bacterial identification

18 laboratories submitted results for bacterial identification (**Table 6**). The five target *Salmonella* strains were identified correctly by 17 laboratories.

Table 6. 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 23.1	*Salmonella*	18/18
Salm EQASIA 23.2	*Salmonella*	18/18
Salm EQASIA 23.3	Non- *Salmonella*	17/18
Salm EQASIA 23.4	Non- *Salmonella*	17/18
Salm EQASIA 23.5	*Salmonella*	17/18
Salm EQASIA 23.6	*Salmonella*	18/18
Salm EQASIA 23.7	*Salmonella*	18/18

Salm, Salmonella

3.2.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 85.7% (strain Salm EQASIA 23.7) to 93.3% (strain Salm EQASIA 23.2) (**Table 7**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were gentamicin (75.0%), sulfamethoxazole (41.7%), colistin (31.4%), cefoxitin (24.4%), ciprofloxacin (22.5%), ertapenem (10.5%), azithromycin (10.3%), and ampicillin (10.1%), whereas chloramphenicol, meropenem, and trimethoprim revealed no deviation from the expected results (**Figure 4**). The high deviation for gentamicin is likely due to

the recent changes in the CLSI breakpoints for aminoglycosides in *Salmonella spp.*

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in 4 laboratories: #11, #34, #49, and #52 (**Figure 5**). In average, the deviation was 10.0% (ranging from 0.0% to 28.3%). As the acceptance level was set to 5% deviation, 14 laboratories (#01, #04, #05, #06, #07, #10, #12, #17, #32, #35, #40, #48, #50, and #51) did not perform within the expected range for the *Salmonella* panel.

Table 7. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results submitted by 18 HH laboratories for the *Salmonella* panel.

Strain	AST in total	% Correct
Salm EQASIA 23.1	181	91.7
Salm EQASIA 23.2	178	93.3
Salm EQASIA 23.5	173	87.9
Salm EQASIA 23.6	180	90.0
Salm EQASIA 23.7	182	85.7

Salm, Salmonella

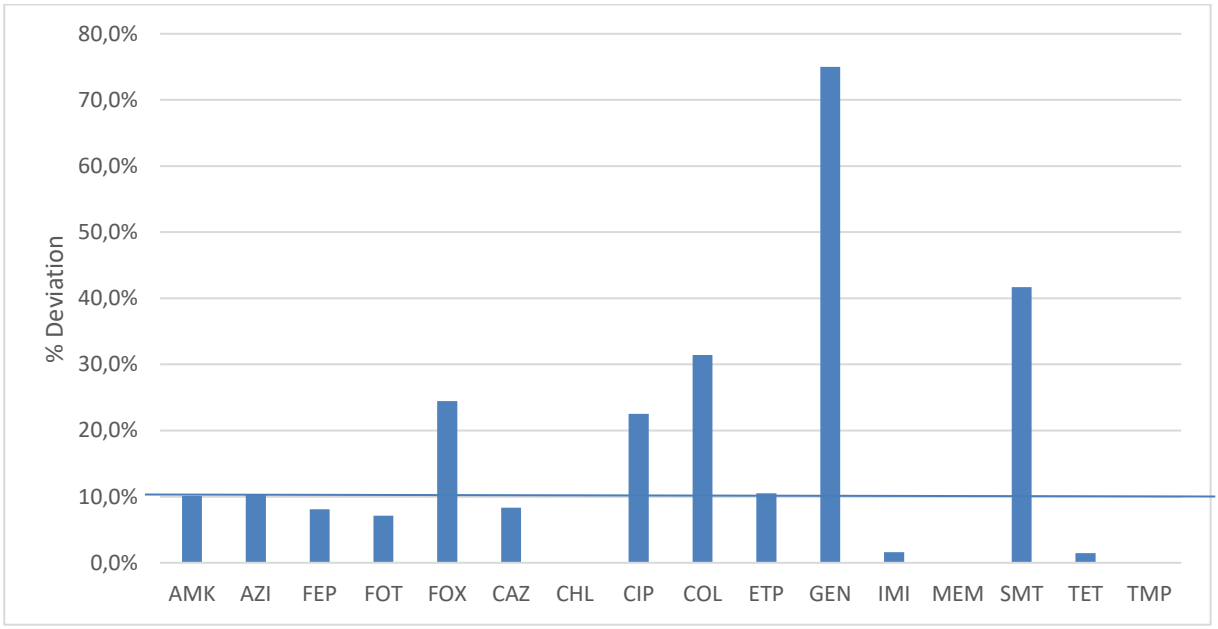


Figure 4. Percentage of deviation in the AST interpretation (R/S) among *Salmonella* strains by HH laboratories (n=18) participating in the 7th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

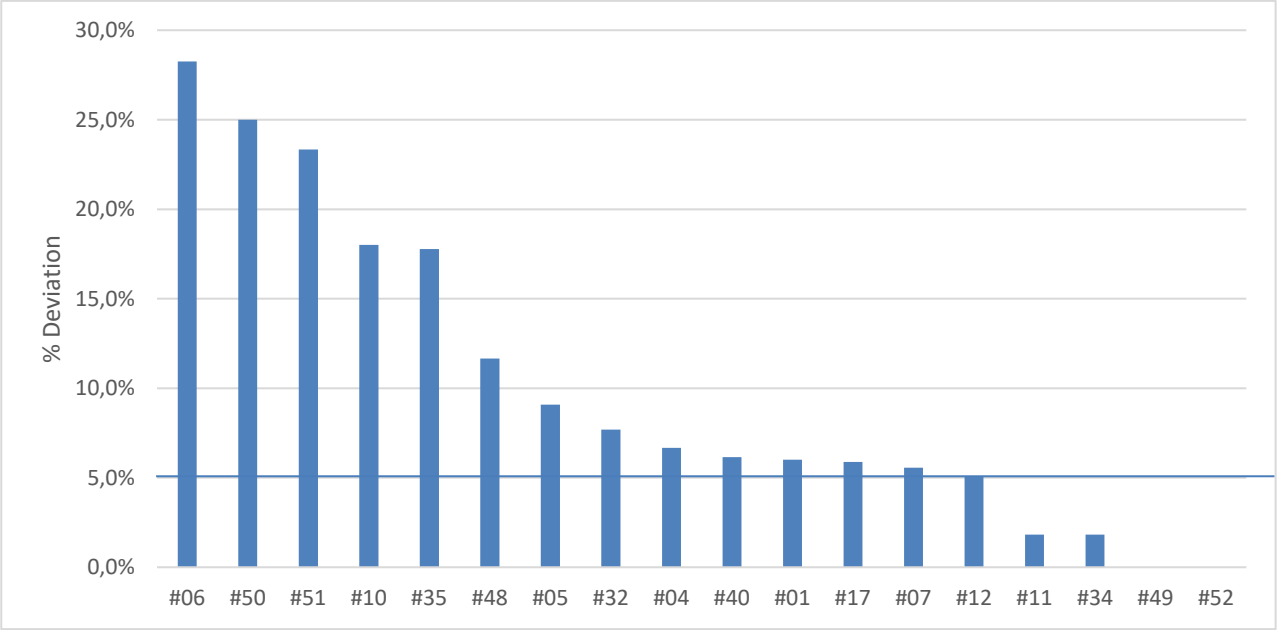


Figure 5. Percentage of deviation in the AST interpretation (R/S) among *Salmonella* strains by HH laboratories (n=18) participating in the 7th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 Serotyping

Serotyping of *Salmonella* was offered to the participants as a voluntary component. The five strains identified as *Salmonella* had to be serotyped using the method routinely used by the laboratory. If the necessary antisera for serotyping were not available, the serogroup could still be reported and further evaluated, meaning that serotype and serogroup were separately assessed in this panel. Serogroups should be reported using terms according to Kauffmann-White-Le Minor.

Of the 18 participating laboratories in this panel, six (#05, #11, #12, #34, #40, and #49) submitted results for *Salmonella* serogrouping. Two of them (#05 and #49) submitted partial results for serogrouping and did not provide serotyping results (**Table 8**). Laboratory #05 only submitted serogroup for strain Salm EQAsia 23.1; laboratory #49 reported results for the serogroup for strain Salm EQAsia 23.2. The other four laboratories were divided as follows: laboratory #11 submitted the correct serotype for all five target strains; laboratories #34 and #40 submitted the correct serotype for four out of five target strains, while laboratory #12 did not submit any correct results for this component of the panel (**Table 8**). Antigen formula data were submitted by four laboratories only.

Table 8. Serogroup, serotype and antigen of each of the 5 *Salmonella* target strains. Number of correct serogroup/serotype out of the total submitted serogroup/serotype results are presented. Results are from a total of 6 HH laboratories.

Strain	Serogroup	No. correct serogroup	Serotype	No. correct serotype
Salm EQAsia 23.1	O:4 (B)	5/5	Derby	2/4
Salm EQAsia 23.2	O:4 (B)	4/5	Typhimurium	3/4
Salm EQAsia 23.5	O:4 (B)	4/4	Schwarzengrund	2/4
Salm EQAsia 23.6	O:9 (D1)	4/4	Dublin	3/4
Salm EQAsia 23.7	O:9 (D1)	4/4	Enteritidis	3/4

Salm, *Salmonella*

3.2.4 β-lactamase-producing *Salmonella*

None of the fifteen participating laboratories uploaded results for this component of the *Salmonella* panel.

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

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

17 out of 18 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only seven performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test, agar, or broth microdilution (**Table 9**). For testing *E. coli* NCTC 13846, MIC was determined by standard method by broth microdilution.

Table 9. 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			
	Disk Diffusion	Gradient	MIC	**Total**
AMK	0/4	--	0/3	**0/7**
AMP	1/12	--	0/5	**1/17**
CAZ	2/13	--	3/3	**5/16**
CHL	1/14	--	--	**1/14**
CIP	0/11	0/1	3/3	**3/15**
COL	--	--	0/5	**0/5**
ETP	2/7	0/1	4/4	**6/12**
FEP	3/10	--	4/4	**7/14**
FOT	3/11	--	0/1	**3/12**
FOX	1/8	--	0/1	**1/9**
GEN	0/4	--	--	**0/4**
IMI	3/7	0/1	0/4	**3/12**
MEM	1/9	0/1	4/5	**5/15**
SMT	3/3	--	--	**3/3**
TET	1/12	--	0/2	**1/14**
TMP	1/3	--	0/1	**1/4**

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

Highest proportion of test results outside of the expected range was observed in sulfamethoxazole (3 out of 3) (**Table 9**).

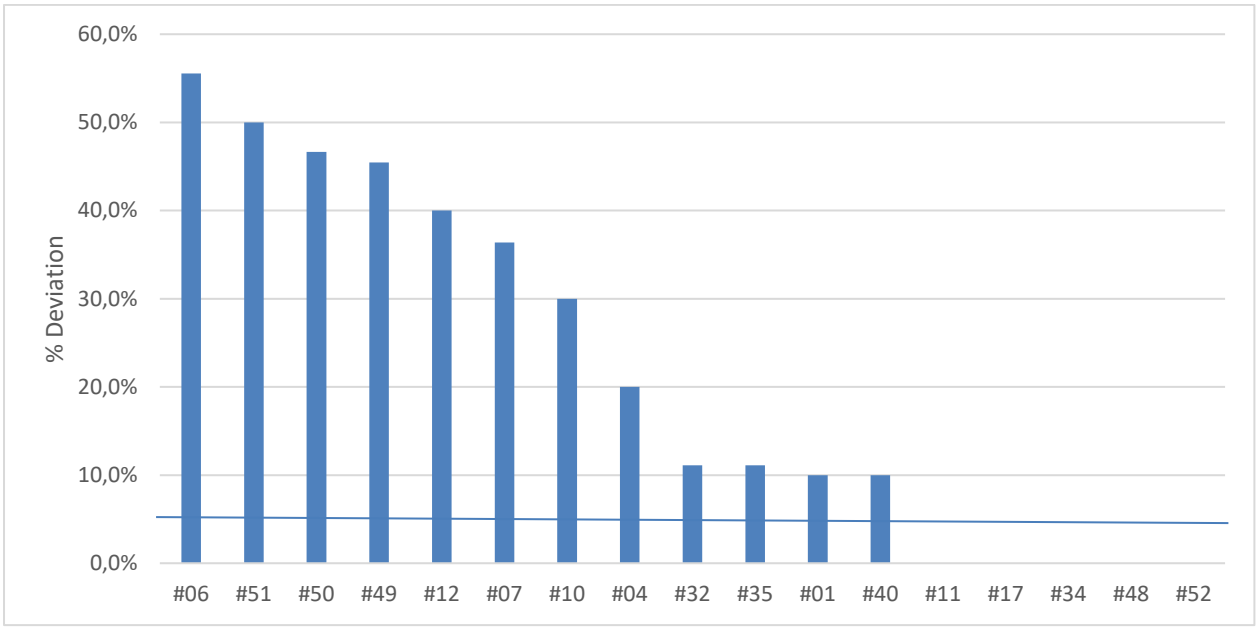


Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Salmonella* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 6).** Laboratories #11, #17, #34, #48, and #52 presented no deviation. I.e. laboratories #17, #34, and #52 used only disk diffusion, laboratory #48 applied disk diffusion and gradient test, while laboratory #11 used all three methods (MIC broth microdilution, gradient test, and disk diffusion). All other laboratories presented deviations that ranged from 10.0% to 55.6% (**Figure 6**).

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

3.3 *Enterococcus faecium/ Enterococcus faecalis* panel

17 laboratories from 12 countries uploaded results for the enterococci panel.

3.3.1 Bacterial identification

17 participating laboratories submitted results for bacterial identification (**Table 10**). The complete panel of five target *E. faecalis* and *E. faecium* strains and two non-target strains was identified correctly by 11 laboratories (64.7%).

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

Strain	Bacterial ID	No. correct
Ef EQASIA 23.1	*Enterococcus faecalis*	14/17
Ef EQASIA 23.2	Non-*Enterococcus faecalis/faecium*	14/17
Ef EQASIA 23.3	*Enterococcus faecium*	17/17
Ef EQASIA 23.4	*Enterococcus faecium*	15/17
Ef EQASIA 23.5	*Enterococcus faecium*	15/17
Ef EQASIA 23.6	Non-*Enterococcus faecalis/faecium*	16/17
Ef EQASIA 23.7	*Enterococcus faecalis*	13/17

Ef, *E. faecalis/ E. faecium*

3.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 80.0% (strain Ef EQASIA 23.1) to 94.1% (strain Ef EQASIA 23.3) (**Table 11**). The AST results submitted for the five *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species.

The highest deviation was seen for strain Ef EQAsia 23.1 (20.0%) and was caused by several instances of results' misinterpretation of the obtained results mainly for chloramphenicol, ampicillin and teicoplanin. Strains Ef EQAsia 23.4 and Ef EQAsia 23.5 also presented quite high deviations (close to 15% and 20%, respectively) that resulted from several incorrect results reported mostly by laboratories #04 and #50.

Table 11. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 17 HH laboratories for the enterococci panel.

Strain	AST in total	% Correct
Ef EQASIA 23.1	120	80.0
Ef EQASIA 23.3	118	94.1
Ef EQASIA 23.4	112	85.7
Ef EQASIA 23.5	118	80.5
Ef EQASIA 23.7	110	74.5

Ef, E. faecalis/ E. faecium

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were quinupristin and dalfopristin (45.5%), daptomycin (42.9%), chloramphenicol (32.4%), tigecycline (31.8%), vancomycin (25.4%), teicoplanin (22.2%), gentamicin (20.0%), nalidixic acid (12.0%), and linezolid (10.6%) (**Figure 7**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in laboratories #01, #02, and #34 (**Figure 8**). In average, the deviation was 15.9% (ranging from 4.0 to 47.3%). As the acceptance level was set to 5% deviation, 14 laboratories (#04, #05, #06, #07, #11, #12, #17, #32, #35, #48, #49, #50, #51, and #52) did not perform within the expected range for the enterococci panel.

Laboratory #50 presented the highest deviation observed for this panel. Half of the submitted results were not in accordance with the expected outcome, resulting in penalties (score of 0, 1 or 3 instead of 4) and in the observed deviation.

The deviations in the results submitted by laboratory #04 were exclusively in the AST of Ef EQASIA 23.4 and 23.5, leading to a performance score of 68% for this part of the trial.

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

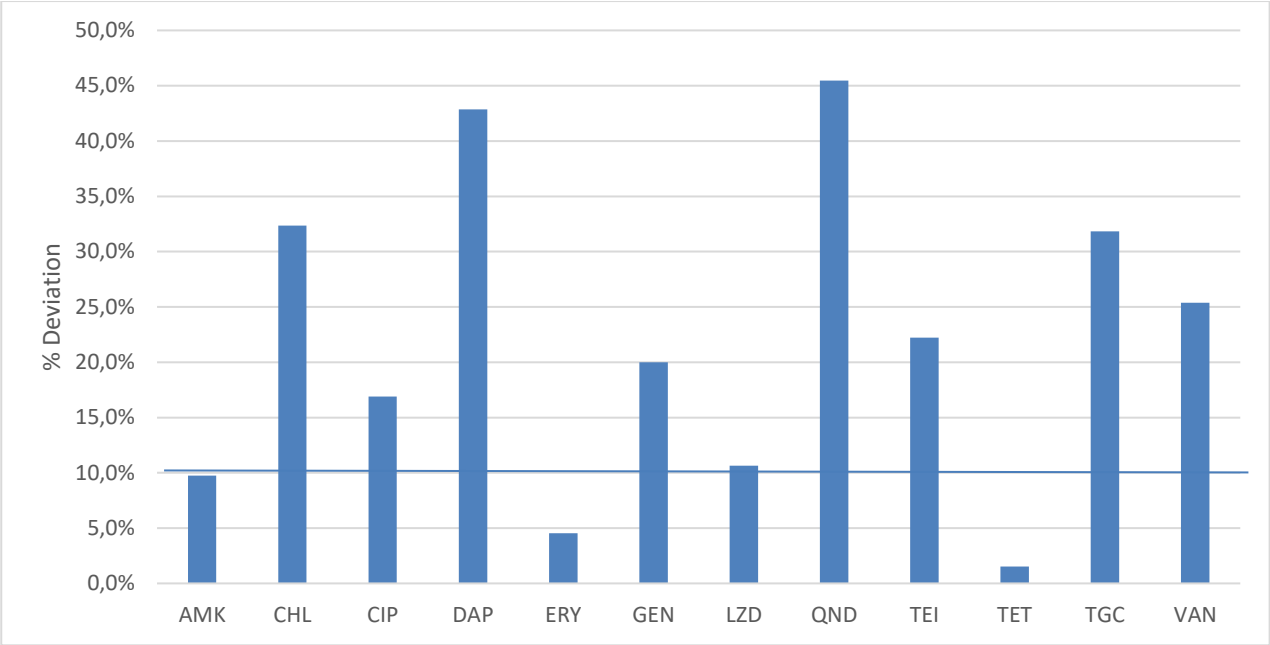


Figure 7. Percentage of deviation in the AST interpretation (R/S) among enterococci strains by HH laboratories (n=17) participating in the 7th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

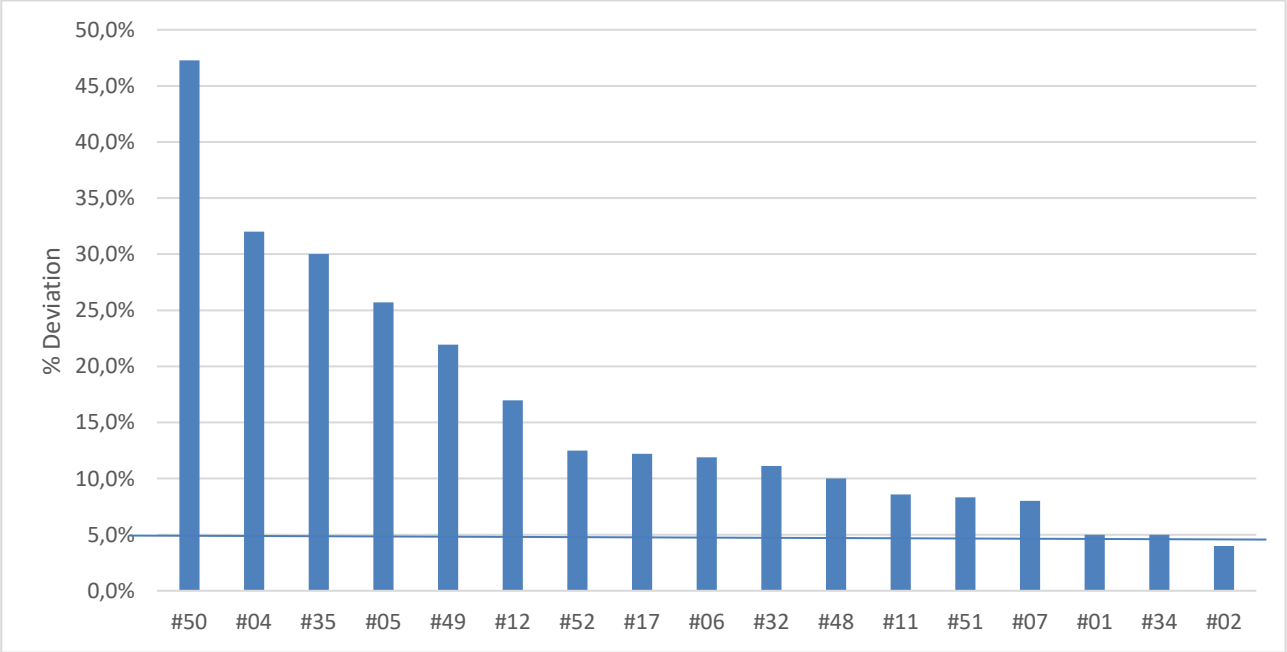


Figure 8. Percentage of deviation in the AST interpretation (R/S) among *E. faecalis/E. faecium* strains by HH laboratories (n=17) participating in the 7th EQA in the EQAsia project. Results are categorized by laboratory ID number.

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

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

16 out of 17 participating laboratories submitted results for this part of the enterococci panel. 12 laboratories reported results for the reference strain *S. aureus* ATCC 25923. Eight laboratories entered results also for *E. faecalis* ATCC 29212. Both disk diffusion and MIC test results were reported for both reference strains by some laboratories. However, it should be noted that the reference strain *S. aureus* ATCC 25923 could only be used to determine inhibition zone diameters by disk diffusion, while *E. faecalis* ATCC 29212 is recommended for MIC testing.

Highest proportion of test results outside of the expected range was observed in tigecycline (2 out of 3) (**Table 12**). Ampicillin and vancomycin have also showed high deviations (6 out of 14 and 5 out of 13, respectively).

Table 12. AST of the reference strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff. *	MIC **	Total
AMP	4/8	2/6	**6/14**
CHL	0/9	1/3	**1/12**
CIP	0/8	0/5	**0/13**
DAP	--	0/2	**0/2**
ERY	0/7	0/5	**0/12**
GEN	0/5	1/2	**1/7**
LZD	1/5	0/5	**1/10**
QND	1/1	0/2	**1/3**
TEI	1/5	1/3	**2/8**
TET	1/9	0/3	**1/12**
TGC	1/1	1/2	**2/3**
VAN	3/7	2/6	**5/13**

Disk Diff. – inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution
**S. aureus* ATCC 25923 for disk diffusion
***E. faecalis* ATCC 29212 for MIC

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains. Laboratories #01, #02, #07, #11, #17, #34, #48, and #49 presented no deviations. I.e. laboratories #01, #02, #17, #34, and #48 used only disk diffusion method, while the other 3 laboratories did primarily MIC testing. The remaining 8 laboratories presented deviations that ranged from 18.2% to 63.6% (**Figure 9**). Overall, the average deviation for this part of the panel was 18.0%.

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

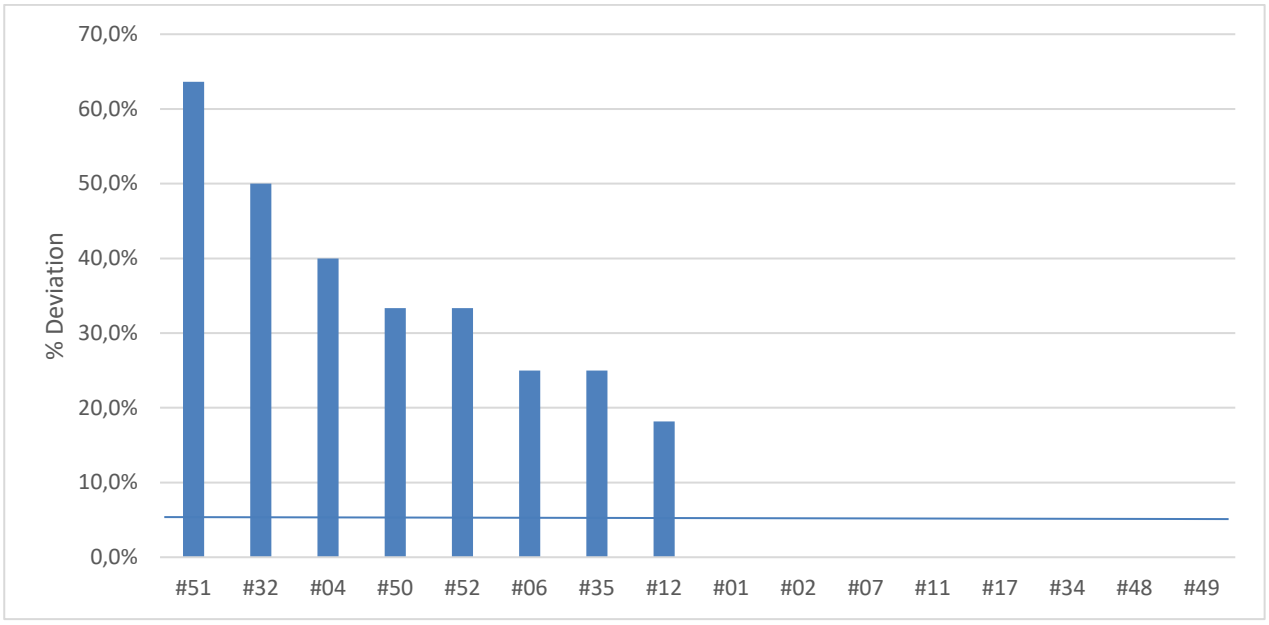


Figure 9. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 reference strains in the *E. faecium/ E. faecalis* panel by the HH laboratories.

3.4 *Campylobacter jejuni/coli* panel

Only 6 HH laboratories signed up for this part of the EQA7 panel. Overall, 5 laboratories submitted partial data. One laboratory could not revive any of the panel strains and did not submit any data at all.

3.4.1 Bacterial identification

Five participating laboratories submitted results for bacterial identification (**Table 13**). None of the laboratories could revive and identify correctly all seven strains of this panel. Of the five laboratories, #17 had identified correctly 6 out of 6 reported isolates. The other four laboratories could revive between two and five isolates only.

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 23.1	*Campylobacter jejuni*	1/3
Camp EQAsia 23.2	Non-*Campylobacter coli/jejuni*	3/3
Camp EQAsia 23.3	*Campylobacter jejuni*	2/3
Camp EQAsia 23.4	*Campylobacter jejuni*	2/3
Camp EQAsia 23.5	*Campylobacter coli*	1/2
Camp EQAsia 23.6	Non-*Campylobacter coli/jejuni*	2/3
Camp EQAsia 23.7	*Campylobacter coli*	2/4

Camp, C. jejuni/ C. coli

3.4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) for four of the target strains was completely in line (100.0%). Strain Camp EQASIA 23.5 had a much lower percentage – 57.1% (**Table 14**).

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

Strain	AST in total	% Correct
Camp EQASIA 23.1	4	100.0
Camp EQASIA 23.3	3	100.0
Camp EQASIA 23.4	6	100.0
Camp EQASIA 23.5	7	57.1
Camp EQASIA 23.7	3	100.0

Camp, C. jejuni/ C. coli

Antimicrobial-based analysis

The total number of antimicrobials tested was four (ciprofloxacin, erythromycin, gentamicin, and tetracycline). In total, there were only 23 available AST results to evaluate for the entire panel from the three labs that submitted AST data. Antimicrobials with deviations from the expected results higher than 10% were ciprofloxacin (14.3%), erythromycin (14.3%), and tetracycline (14.3%) (**Figure 10**).

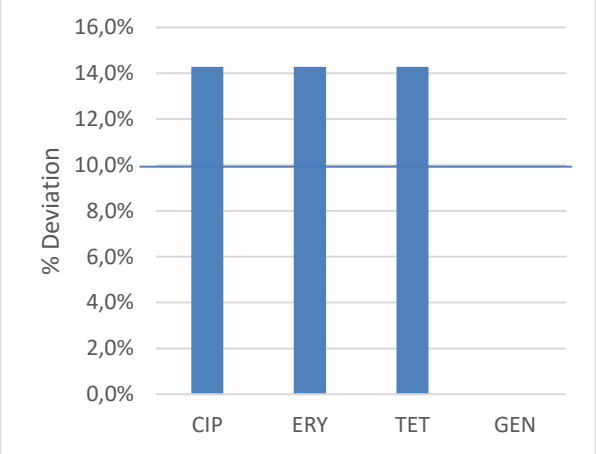


Figure 10. Percentage of deviation in the AST interpretation (R/S) among *C. jejuni/C. coli* strains by HH laboratories (n=3) participating in the 7th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in one laboratory - #40 had a deviation of the AST results of 37.5%.

The other two laboratories (#17 and #35) showed no deviations (**Figure 11**). In average, the deviation was 12.5%.

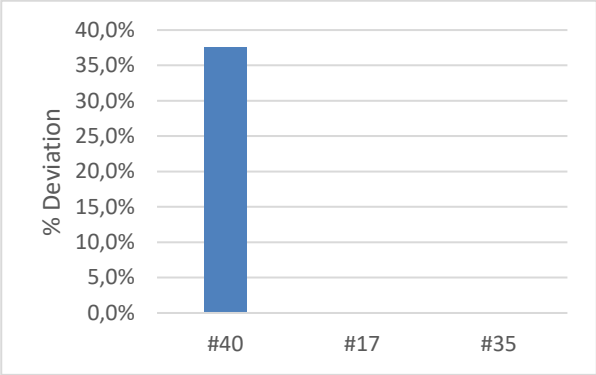


Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among C. jejuni/ C. coli strains by HH laboratories (n=3) participating in the 7th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.4.3 Quality control strain *C. jejuni* ATCC 33560

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

The three participating laboratories (#17, #35 and #40) that submitted AST results used disk diffusion results for *C. jejuni* ATCC 33560 when grown at 42°C for 24h; for these conditions, acceptance intervals for disk diffusion are only available for ciprofloxacin and erythromycin (**Appendix 3c**). Therefore, even though the laboratories submitted results for other antimicrobials, those results could not be assessed (**Table 15**).

Laboratories #17 and #40 had no deviations in their expected results for the reference strain for these two antibiotics. To the contrary, the values for ciprofloxacin and erythromycin, reported by laboratory #35 were not within the expected range of values (deviation was 100%) (**Figure 12**).

Table 15. AST of the reference strains *C. jejuni* ATCC 33560 in the *C. jejuni/ C. coli* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diffusion	Total
CIP	1/3	1/3
ERY	1/3	1/3

Disk Diffusion – inhibition zone diameter determination by disk diffusion.

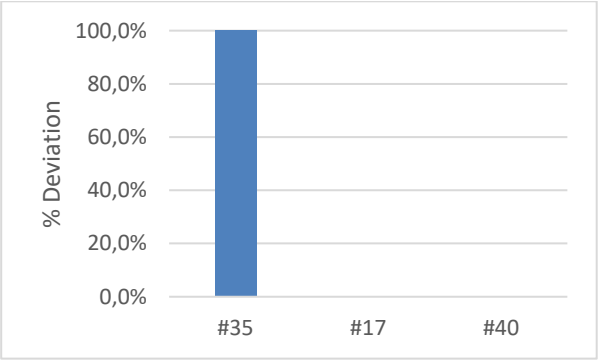


Figure 12. Percentage of deviation in the AST of *C. jejuni* ATCC 33560 in the *Campylobacter spp.* panel by the HH laboratories (n=3).

3.5 *Neisseria gonorrhoeae* panel

14 laboratories from 12 countries participated in this part of the EQA7 trial.

3.5.1 Bacterial identification

Eight laboratories that selected the *N. gonorrhoeae* panel submitted results for bacterial identification. The remaining laboratories reported difficulties reviving the isolates or did not submit any data. The majority of the laboratories reporting data, submitted partial results for only some of the isolates from the panel (**Table 16**). Strains Ng EQASIA 23.4 and Ng EQASIA 23.6 were reported correctly by all the labs that could revive the isolates. No growth for both strains was reported by nine laboratories. The only laboratory that could revive isolate Ng EQASIA 23.7, reported it as a non-target strain. The success rate for the identification of the other test strains varied between laboratories.

Table 16. Bacterial identification of each of the 7 test strains provided within the *N. gonorrhoeae* 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
Ng EQASIA 23.1	*Neisseria mucosa*	4/5
Ng EQASIA 23.2	*Neisseria gonorrhoeae*	1/1
Ng EQASIA 23.3	*Neisseria gonorrhoeae*	1/6
Ng EQASIA 23.4	*Neisseria gonorrhoeae*	4/4
Ng EQASIA 23.5	*Haemophilus influenzae*	2/3
Ng EQASIA 23.6	*Neisseria gonorrhoeae*	4/4
Ng EQASIA 23.7	*Neisseria gonorrhoeae*	0/1

Ng, *N. gonorrhoeae*

3.5.2 AST performance

The AST performance for the *N. gonorrhoeae* panel is analysed from a strain-, antimicrobial-, and laboratory-based perspective to allow for a broader interpretation of the results.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 0.0% (strain Ng EQASIA 23.2) to 80.0% (strain Ng EQASIA 23.6) in this panel (**Table 17**). The only laboratory (#01) that reported results for strain Ng EQASIA 23.2 had wrong or slightly incorrect results for all reported antibiotics. There was no AST data to be evaluated for strain Ng EQASIA 23.7.

Table 17. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/S). Results are from 6 HH laboratories that submitted AST data for the *N. gonorrhoeae* panel.

Strain	AST in total	% Correct
Ng EQASIA 23.2	4	0.0
Ng EQASIA 23.3	4	50.0
Ng EQASIA 23.4	20	80.0
Ng EQASIA 23.6	20	60.0
Ng EQASIA 23.7	0	--

Ng, *N. gonorrhoeae*

Antimicrobial-based analysis

All of the reported antimicrobials had deviations higher than 10%, as follows penicillin (60.0%), tetracycline (60.0%), ciprofloxacin (30.0%), azithromycin (25.0%), cefixime (14.3%), and ceftriaxone (14.3%) (**Figure 13**).

Laboratory-based analysis

For the *N. gonorrhoeae* panel, all six laboratories that submitted AST data had deviations higher than 5% compared to the baseline results (**Figure 14**). The average deviation was 34.6% (ranging from 10.0% to 64.3%).

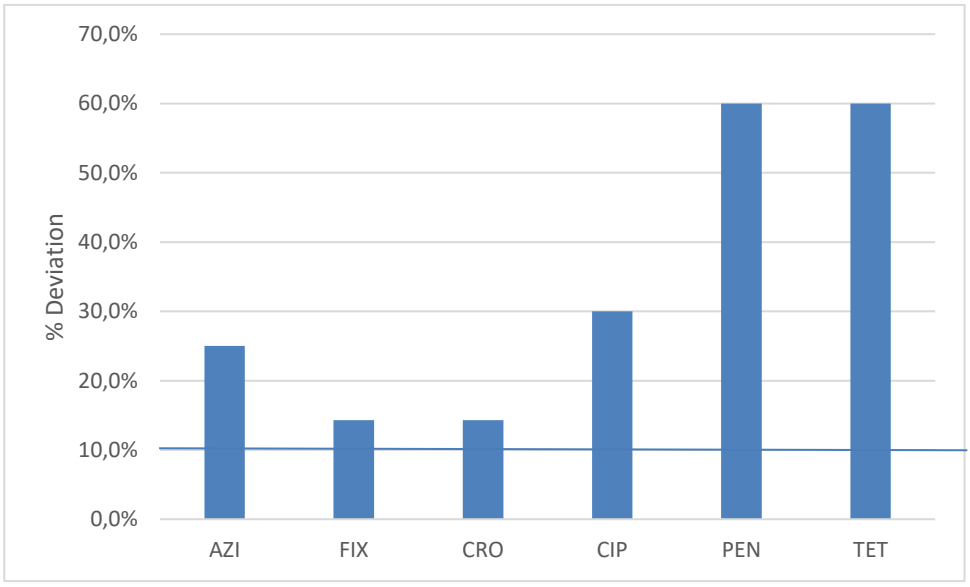


Figure 13. Percentage of deviation in the AST interpretation (R/S) among *N. gonorrhoeae* strains by HH laboratories (n=6) participating in the 7th EQA of the EQAsia project. Results are categorized by antimicrobial agent.

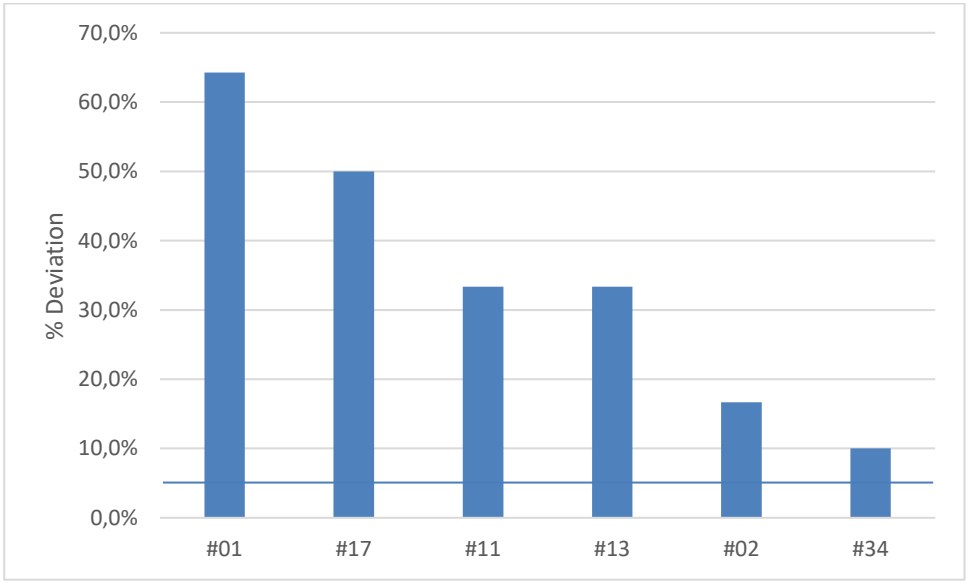


Figure 14. Percentage of deviation in the AST interpretation (R/S) among *N. gonorrhoeae* strains by HH laboratories (n=6) participating in the 7th EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.5.3 Quality control strains *N. gonorrhoeae*

The QC strains provided to participating laboratories within EQA7 included *Neisseria gonorrhoeae* ATCC49226, WHO G, WHO L, WHO O and WHO P.

Among the 14 participating laboratories, 7 laboratories submitted results for this part of the EQA panel. Four laboratories submitted data for the reference strain *Neisseria gonorrhoeae* ATCC49226, four laboratories – for strain WHO G, and two laboratories – for WHO L. Some of the laboratories tested more than one QC strains. There were no deviations in the QC results reported by the participating laboratories (**Table 18**).

Table 18. AST of the reference strains *Neisseria gonorrhoeae* ATCC49226, WHO G and WHO L in the *N. gonorrhoeae* panel. The test results outside of expected range are presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk diff.	Gradient	MIC	**Total**
AZI	0/4	0/2	0/2	**0/8**
FIX	0/5	0/2	0/2	**0/9**
CRO	0/6	0/2	0/2	**0/10**
CIP	0/6	0/2	0/2	**0/10**
PEN	0/5	0/3	0/2	**0/10**
TET	0/6	0/2	0/2	**0/10**

Disk diff. – inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth macro- and microdilution

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 17 Animal Health laboratories participating in the 7th EQA of the EQAsia programme, 15 laboratories submitted results for the *Salmonella* panel, 7 for the *Enterococcus faecium/ E. faecalis* panel and 6 laboratories submitted results for the *Campylobacter jejuni/ C. coli* panel (**Figure 15**).

Applied AST methodologies for the three panels are presented in **Figure 15**. Disk diffusion as the sole method was the preferred choice for all the panels. Laboratory #18 was the only participant that used broth microdilution (automated). Laboratories #26, #28, #36 and #37 used a combination of disk diffusion and broth microdilution. The remaining laboratories (#53) applied disk diffusion in combination with broth macrodilution method. Laboratory #37 and #38 did not report AST results for *C. jejuni/ C. coli.*

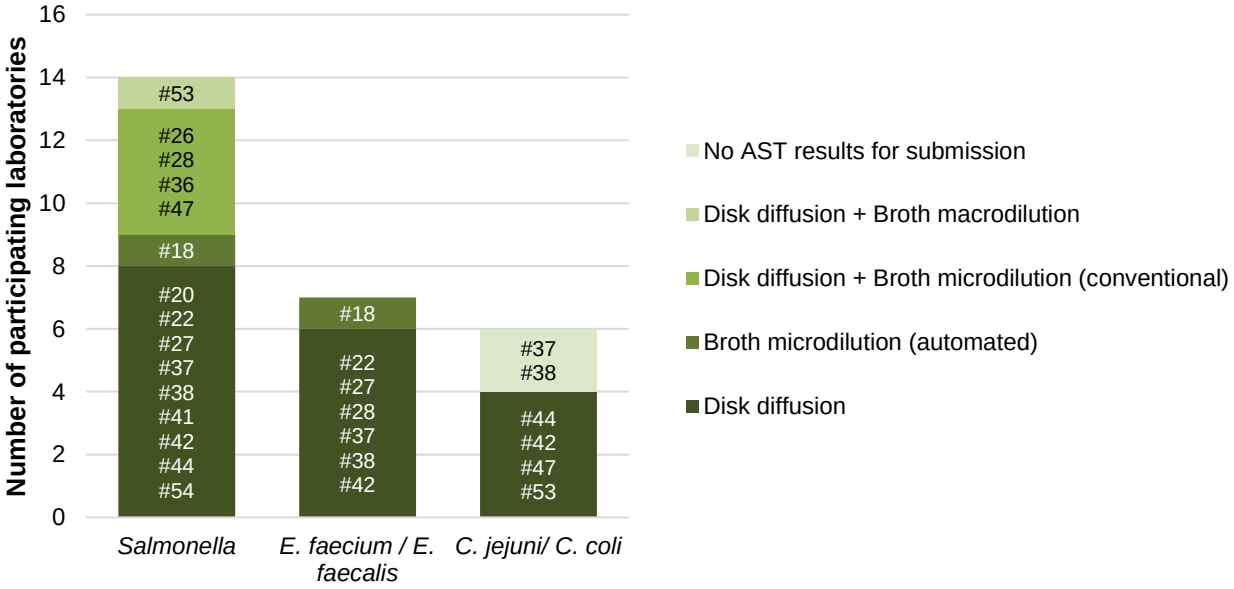


Figure 15. Methodologies applied by the AH laboratories participating for each of the panels.

The participants were invited to report inhibition zone diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each strain/antimicrobial combination. Only the categorisation was evaluated, whereas the inhibition zone diameters/MIC values were used as supplementary information. The EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested drugs (**Table 1**).

For Gram-negative bacteria *Salmonella* panel (**Table 19**), ampicillin, ciprofloxacin and tetracycline were tested by most of the laboratories. In contrast, cefoxitin, colistin, cefepime and azithromycin were tested by less than half of the participating laboratories. For Gram-positive bacteria, ciprofloxacin, ampicillin, erythromycin and gentamicin were tested by most laboratories in the *E. faecium/ E. faecalis* panel, whereas daptomycin and teicoplanin were tested by only one AH laboratory. Lastly, in the *C. jejuni/ C. coli* panel, ciprofloxacin, erythromycin and tetracycline were tested by all laboratories that submitted data, whereas ertapenem was tested by only one AH laboratory.

Table 19. Total of ASTs performed for each antimicrobial and in total for each of the panels by AH laboratories

	Salmonella		***E. faecium/ E. faecalis***		***C. jejuni/ C. coli***	
Ampicillin	75	10.7%	29	11.6%	-	-
Azithromycin	30	4.3%	-	-	-	-
Cefepime	30	4.3%	-	-	-	-
Cefotaxime	55	7.9%	-	-	-	-
Cefoxitin	25	3.6%	-	-	-	-
Ceftazidime	35	5.0%	-	-	-	-
Chloramphenicol	60	8.6%	21	8.4%	3	11.5%
Ciprofloxacin	73	10.4%	34	13.6%	6	23.1%
Colistin	30	4.3%	-	-	-	-
Daptomycin	-	-	2	0.8%	-	-
Ertapenem	20	2.9%	-	-	1	3.8%
Erythromycin	-	-	29	11.6%	6	23.1%
Gentamicin	35	5.0%	29	11.6%	4	15.4%
Imipenem	40	5.7%	-	-	-	-
Linezolid	-	-	20	8.0%	-	-
Meropenem	45	6.4%	-	-	-	-
Sulfamethoxazole	42	6.0%	-	-	-	-
Quinupristin/dalfopristin	-	-	10	4.0%	-	-
Teicoplanin	-	-	5	2.0%	-	-
Tetracycline	64	9.2%	26	10.4%	6	23.1%
Tigecycline	-	-	20	8.0%	-	-
Trimethoprim	40	5.7%	-	-	-	-
Vancomycin	-		24	9.6%	-	-
Total	**699**		**249**		**26**	

Scattering of missing data or incomplete AST results entries were observed in the *Salmonella* and enterococci panels (**Tables 20** and **21**). One of the 15 laboratories selecting *Salmonella* did not submit complete results.

Regarding the *E. faecium/ E. faecalis* panel, two out of the seven participating laboratories revealed incomplete results of their own available antimicrobial agents (**Table 21**). Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance.

Table 20. Distribution of incomplete or missing data of antimicrobial agents among *Salmonella* strains reported by AH laboratories (n=15) participating in the 7th EQA of the EQAsia project.

Lab ID No.	Salm EQAsia 23.1	Salm EQAsia 23.2	Salm EQAsia 23.5	Salm EQAsia 23.6	Salm EQAsia 23.7
#27	CIP, SMX, TET	CIP	SMX	SMX	-

Salm, *Salmonella*

Table 21. Distribution of incomplete or missing data of antimicrobial agents among *E. faecium/ E. faecalis* strains reported by AH laboratories (n=7) participating in the 7th EQA of the EQAsia project.

Lab ID No.	Ef EQAsia 23.1	Ef EQAsia 23.3	Ef EQAsia 23.4	Ef EQAsia 23.5	Ef EQAsia 23.7
#18	SYN	DAP, GEN, SYN	DAP, SYN	DAP, SYN	-
#27	CHL, GEN, LZD, TET, TGC	CHL, LZD, TGC	GEN	CHL, GEN, LZD, TET, TGC	GEN, LZD, TET, TGC

Ef, *E. faecium/ E. faecalis*

4.2 *Salmonella spp.* panel

Fifteen laboratories from nine countries uploaded results for the *Salmonella* panel.

4.2.1 Bacterial identification

All 15 participating laboratories correctly identified the five target *Salmonella* strains. Two non-*Salmonella* strains (strain Salm EQAsia 23.3 and Salm EQAsia 23.4) were misidentified as *Salmonella* by laboratory #41 (**Table 22**).

Table 22. Bacterial identification of each of the seven test strains provided related to the *Salmonella* panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Salm EQAsia 23.1	*Salmonella*	15/15
Salm EQAsia 23.2	*Salmonella*	15/15
Salm EQAsia 23.3	Non-*Salmonella*	13/14
Salm EQAsia 23.4	Non-*Salmonella*	13/14
Salm EQAsia 23.5	*Salmonella*	15/15
Salm EQAsia 23.6	*Salmonella*	15/15
Salm EQAsia 23.7	*Salmonella*	15/15

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.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 89.5% (strain Salm EQAsia 23.5) to 96.9% (strain Salm EQAsia 23.1) for each strain (**Table 23**).

Table 23. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from fifteen AH laboratories for the *Salmonella* panel.

Strain	AST in total	% Correct
Salm EQAsia 23.1	552	96.9
Salm EQAsia 23.2	560	94.8
Salm EQAsia 23.5	560	89.5
Salm EQAsia 23.6	560	93.9
Salm EQAsia 23.7	564	92.2

Salm, *Salmonella*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were sulfamethoxazole (25.6%), followed by gentamicin (12.9%) and colistin (12.5%), while ertapenem, imipenem and meropenem revealed no deviation from the expected results (**Figure 16**).

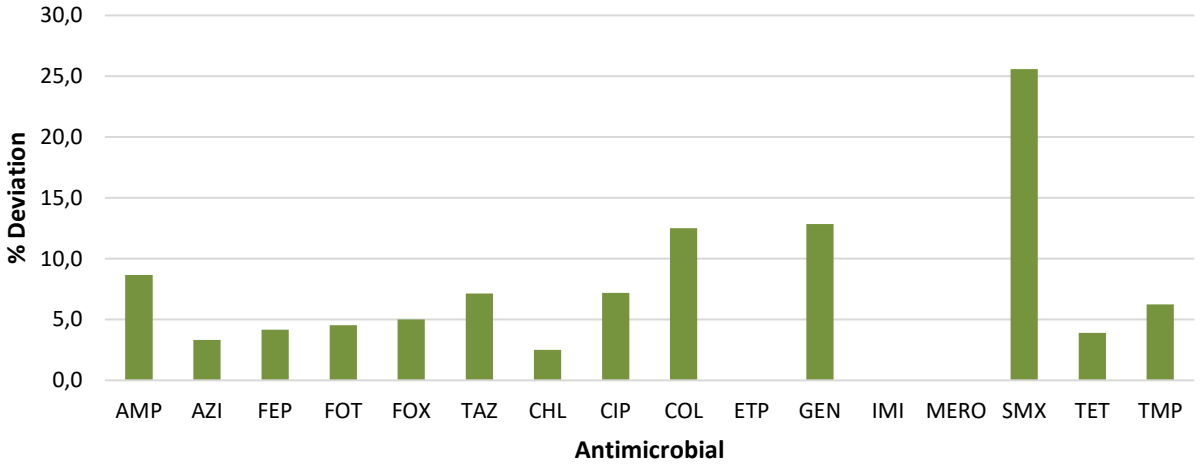


Figure 16. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=15) participating in the 7th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for 8 out of the 15 participants (**Figure 17**). In average, the deviation was 6.7% (ranging from 1.2% to 17.9%). As the acceptance level was set to 5% deviation, 7 laboratories did not perform within the expected range for the EQA trial.

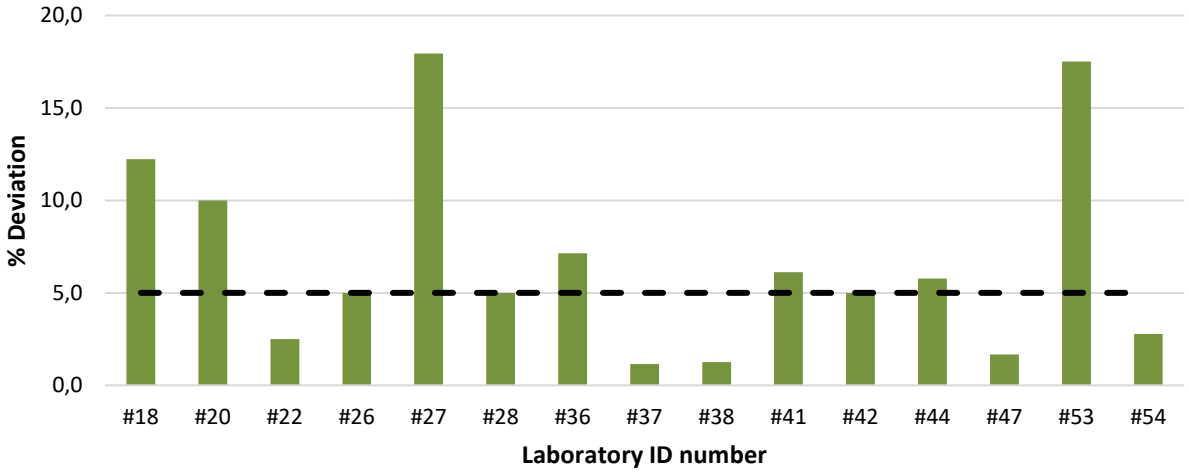


Figure 17. Percentage of deviation in the AST interpretation (R/I/S) among *Salmonella* strains by AH laboratories (n=15) participating in the 7th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 Serotyping

Serotyping of *Salmonella* was offered to the participants as a voluntary component. In this component, the five strains identified as *Salmonella* should be serotyped using the method routinely used by the laboratory. If the necessary antisera for serotyping were not available, serogroup could still be reported and further evaluated, meaning that serotype and serogroup were separately assessed in this trial. Serogroups should be reported using terms according to Kauffmann-White-Le Minor.

Of the 15 participating laboratories in the trial, four (#26, #27, #36 and #47) submitted results for *Salmonella* serogrouping, but only three laboratories (#26, #36 and #47) provided serotyping results (**Table 24**). Laboratory #36 only submitted serogroup and serotype results for strain Salm EQAsia 23.6; laboratory #27 reported results for five strains and correctly identified the serogroup for all five strains; Laboratory #47 only submitted serogroup and serotype results for strain Salm EQAsia 23.2 and Salm EQAsia 23.7 and correctly identified; lastly, laboratory #26 not only was the sole participant correctly identifying the serogroup of all five *Salmonella* strains, as it was also the only one submitting serotyping data, and completely accurate as well (**Table 24**).

Table 24. Serogroup, serotype and antigen of each of the 5 *Salmonella* strains. Number of correct serogroup/serotype out of the total submitted serogroup/serotype results are presented. Results are from a total of 4 AH laboratories.

Strain	Serogroup	No. correct Serogroup	Serotype	No. correct Serotype
Salm EQAsia 23.1	O:4 (B)	2/2	Derby	1/1
Salm EQAsia 23.2	O:4 (B)	3/3	Typhimurium	2/2
Salm EQAsia 23.5	O:4 (B)	2/2	Schwarzengrund	1/1
Salm EQAsia 23.6	O:9 (D1)	3/3	Dublin	1/2
Salm EQAsia 23.7	O:9 (D1)	3/3	Enteritidis	2/2

Salm, *Salmonella*

4.2.4 β-lactamase-producing *Salmonella*

None of the fifteen participating laboratories uploaded results for this component of the *Salmonella* panel.

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

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

Among the 15 participating laboratories, 14 submitted results for the reference strain *E. coli* ATCC 25922 and only two (#18 and #53) performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: inhibition zone diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated and conventional). For testing *E. coli* NCTC 13846, MIC was determined by microdilution and macrodilution methods. The highest proportion of test results outside of the expected range was observed for trimethoprim (3 out of 8) and ciprofloxacin (5 out of 14) (**Table 25**). Regarding the laboratories' performance (**Figure 18**), laboratories #22, #37, #38 and #47 presented no deviation. While laboratories #22, #37 and #38 applied disk diffusion, laboratory #47 used broth microdilution. The remaining ten laboratories presented deviations that ranged from 7.7% to 66.7% (**Figure 18**).

Table 25. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *Salmonella* panel. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diffusion	MIC	Total
AMK	0/4	0/1	**0/5**
AMP	1/10	0/4	**1/14**
FEP	1/5	1/1	**2/6**
FOT	2/8	1/1	**3/9**
FOX	0/3	-	**0/3**
TAZ	0/5	0/1	**0/6**
CHL	2/9	0/2	**2/11**
CIP	2/10	3/4	**5/14**
COL		1/2	**1/2**
ETP	0/2	1/1	**1/3**
GEN	1/7	1/4	**2/11**
IMI	1/6	0/2	**1/8**
MERO	0/6	1/2	**1/8**
SMX	2/5	0/2	**2/7**
TET	1/9	0/3	**1/12**
TMP	1/5	2/3	**3/8**

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

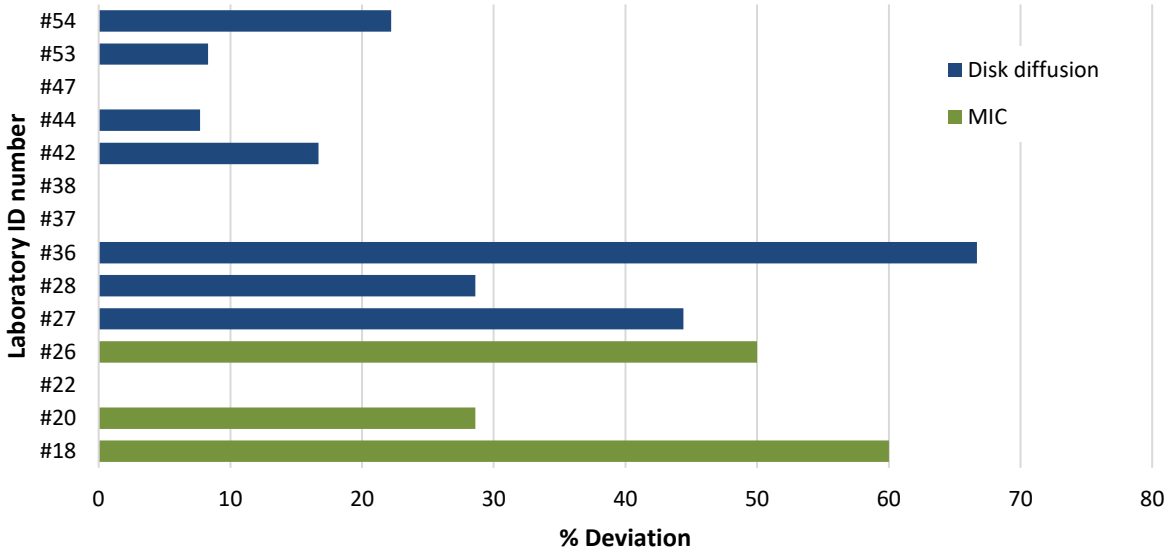


Figure 18. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Salmonella* panel by the AH laboratories.

4.3 *Enterococcus faecium/ Enterococcus faecalis* panel

A total of seven laboratories from seven countries uploaded results for the enterococci panel.

4.3.1 Bacterial identification

All seven participating laboratories submitted results for bacterial identification (**Table 26**). Four out of seven laboratories correctly identified all seven test strains provided. Strain Ef EQAsia 23.6 was misidentified as non-*Enterococcus faecalis/faecium* by laboratories #37 and #42, whereas the *E. faecium* strain Ef EQAsia 23.3 was reported as non-*Enterococcus faecalis/faecium* by laboratory #37. Laboratory #27 was unable to perform bacterial identification and reported all 7 strains as *E. faecalis*.

Table 26. Bacterial identification of each of the seven test strains provided related to the *E. faecium/ E. faecalis* panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ef EQAsia 23.1	*Enterococcus faecalis*	7/7
Ef EQAsia 23.2	Non-*Enterococcus faecalis/faecium*	5/6
Ef EQAsia 23.3	*Enterococcus faecium*	5/7
Ef EQAsia 23.4	*Enterococcus faecium*	6/7
Ef EQAsia 23.5	*Enterococcus faecium*	6/7
Ef EQAsia 23.6	Non-*Enterococcus faecalis/faecium*	4/7
Ef EQAsia 23.7	*Enterococcus faecalis*	7/7

Ef, *Enterococcus*

4.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 93.6% (strain Ef EQASIA 23.1) to 98.2% (strain Ef EQASIA 23.3) for each strain (**Table 27**).

Table 27. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 7 AH laboratories for the *E. faecium/ E. faecalis* panel.

Strain	AST in total	% Correct
Ef EQAsia 23.1	204	93.6
Ef EQAsia 23.3	164	98.2
Ef EQAsia 23.4	216	97.2
Ef EQAsia 23.5	200	97.5
Ef EQAsia 23.7	212	93.9

Ef, *Enterococcus*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were quinupristin and dalfopristin (15.0%) and daptomycin (12.5%), whereas erythromycin, linezolid and tetracycline revealed no deviation from the expected results (**Figure 19**).

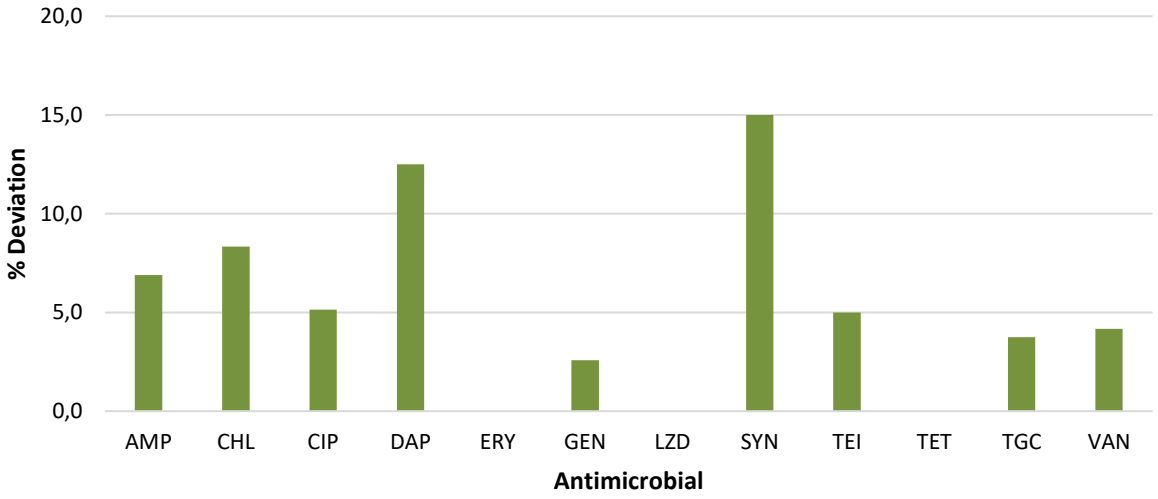


Figure 19. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=7) participating in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for six participants (**Figure 20**). In average, the deviation was 4.0% (ranging from 1.7 to 8.1%). As the acceptance level was set to 5% deviation, only one laboratory (#37) did not perform within the expected range for the trial.

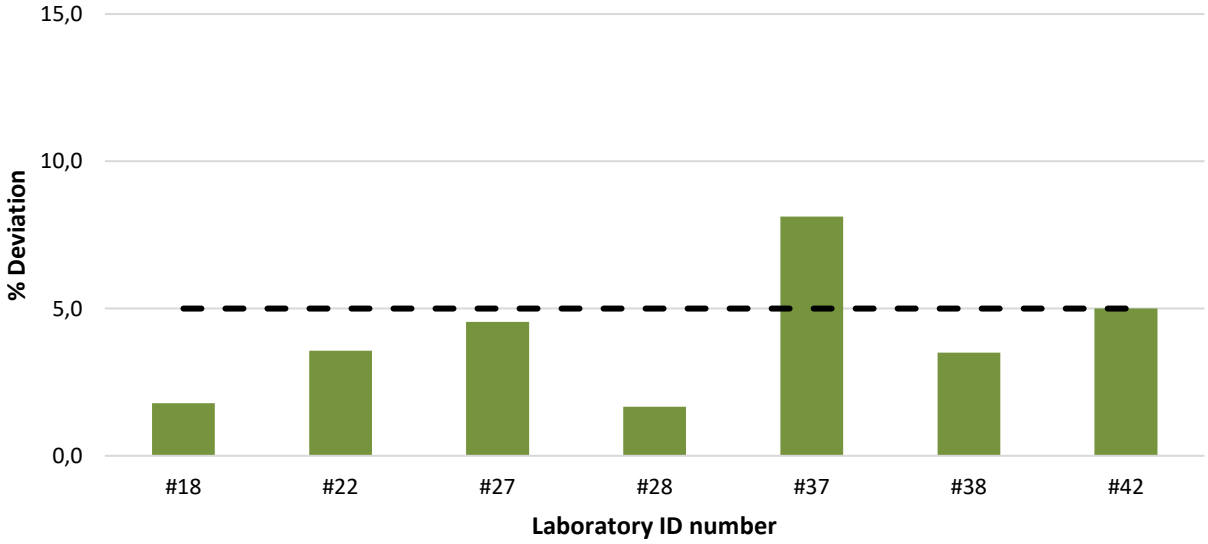


Figure 20. Percentage of deviation in the AST interpretation (R/S) among *E. faecium/ E. faecalis* strains by AH laboratories (n=7) participating in the 7th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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

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

Among the seven participating laboratories, four submitted results for the reference strain. Different methodologies for testing the reference strain *E. faecalis ATCC 29212* were applied: MIC was determined by broth microdilution (**Table 28, ****). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine inhibition zone diameters by disk diffusion (**Table 28, ***).

The highest proportion of test results outside of the expected range was observed for chloramphenicol (1 out of 3) and linezolid (1 out of 4) (**Table 28**).

Regarding the laboratories' performance (**Figure 21**), laboratories #18, #22 and #37 presented no deviation. While laboratories #22 and #37 applied disk diffusion. laboratory #27 presented two deviations (tested eight antimicrobials); this laboratory reported that disk diffusion was the methodology applied for testing the test strains and the reference strain.

Table 28. AST of the reference strains *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* panel. Proportion of test results outside of expected range is presented by methodology used.

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

Disk Diffusion – inhibition zone diameter determination by disk diffusion;
MIC – MIC determination by broth macro- or microdilution, or by agar dilution.
**S. aureus* ATCC 25923 for disk diffusion
***E. faecalis* ATCC 29212 for MIC

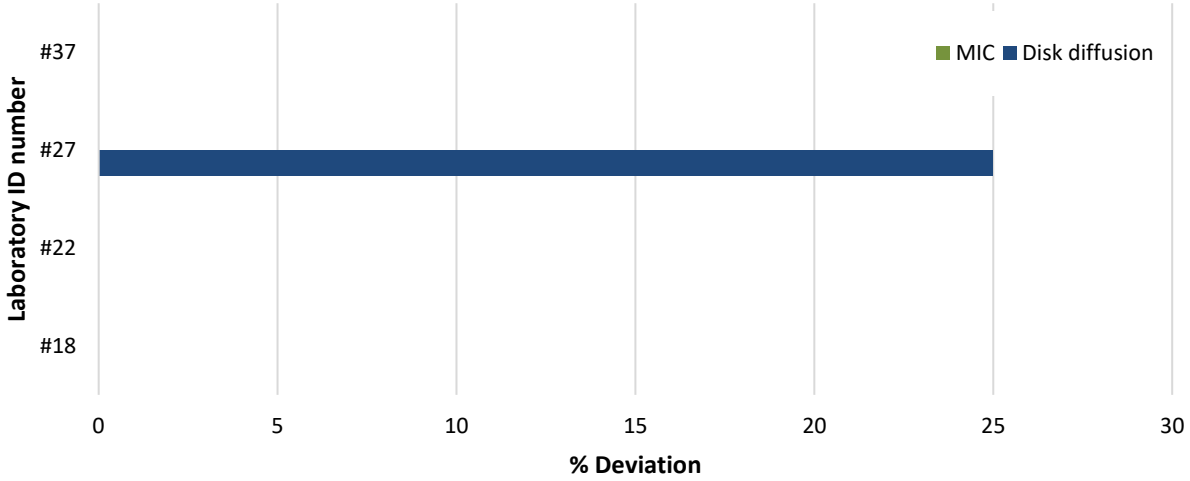


Figure 21. Percentage of deviation in the AST *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 in the *E. faecium/ E. faecalis* panel by the AH laboratories.

4.4 *Campylobacter jejuni/coli* panel

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

4.4.1 Bacterial identification

Of the six participating laboratories, only laboratories #37 and #47 submitted results for bacterial identification and correctly identified for all seven strains. Laboratory #38 submitted data for only two strains (Camp EQAsia 23.3 and Camp EQAsia 23.5) and misidentified as non-*Campylobacter coli/jejuni*. Laboratory #44 submitted data for only two (Camp EQAsia 23.2 and Camp EQAsia 23.4) and misidentified strain Camp EQAsia 23.2 as *C. coli.* Laboratory #53 did not submit results for strains Camp EQAsia 23.3, Camp EQAsia 23.4 and Camp EQAsia 23.6 and misidentified strain Camp EQAsia 23.1 as non-*Campylobacter coli/jejuni.* Lastly, laboratory #42 submitted data for only three strains (Camp EQAsia 23.2, Camp EQAsia 23.6 and Camp EQAsia 23.7) and misidentified strains Camp EQAsia 23.2 and Camp EQAsia 23.7 as *C. jejuni* **(Table 29)**.

Table 29. Bacterial identification of each of the seven test strains provided related to the *C. jejuni/ C. coli* panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Camp EQAsia 23.1	*Campylobacter jejuni*	2/3
Camp EQAsia 23.2	Non-*Campylobacter coli/jejuni*	3/5
Camp EQAsia 23.3	*Campylobacter jejuni*	2/3
Camp EQAsia 23.4	*Campylobacter jejuni*	3/3
Camp EQAsia 23.5	*Campylobacter coli*	3/4
Camp EQAsia 23.6	Non-*Campylobacter coli/jejuni*	3/3
Camp EQAsia 23.7	*Campylobacter coli*	3/4

Camp, *C. jejuni/ C. coli*

4.4.2 AST performance

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

Strain-based analysis

Laboratories #37 and #38 did not submit the results for AST. The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 85.4% (strain Camp EQAsia 23.7) to 100.0% (strain Camp EQAsia 23.4 and Camp EQAsia 23.5) for each strain (**Table 30**). The results from one strain revealed more than 10% deviation (Camp EQAsia 23.7) (**Table 30**).

Table 30. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 4 AH laboratories for the *C. jejuni/ C. coli* panel.

Strain	AST in total	% Correct
Camp EQAsia 23.1	-	-
Camp EQAsia 23.3	-	-
Camp EQAsia 23.4	36	100.0
Camp EQAsia 23.5	20	100.0
Camp EQAsia 23.7	48	85.4

Camp, *C. jejuni/ C. coli*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were gentamicin (18.8%) and ciprofloxacin (16.7%) (**Figure 22**). Chloramphenicol, ertapenem, erythromycin and tetracycline revealed no deviation from the expected results.

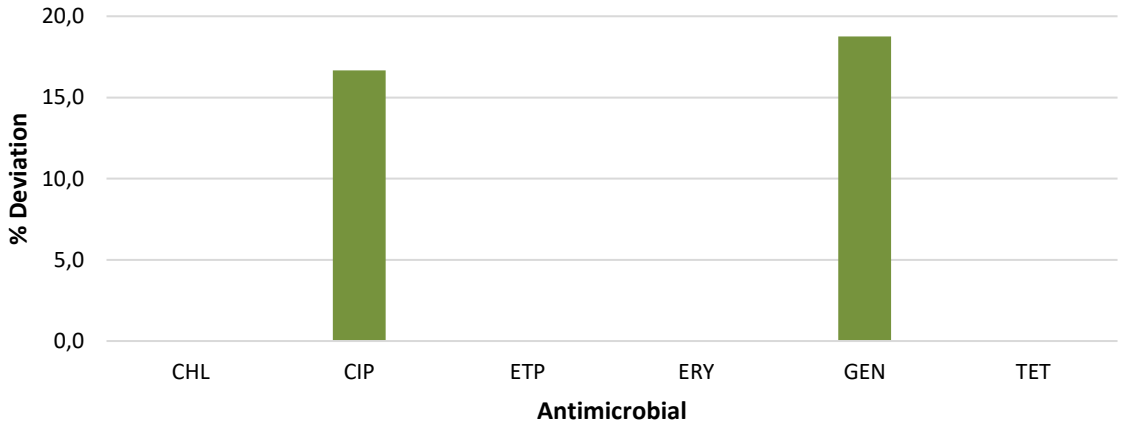


Figure 22. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=4) participating in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for three out of the four participants (**Figure 23**). Laboratory #42 presented the highest deviation.

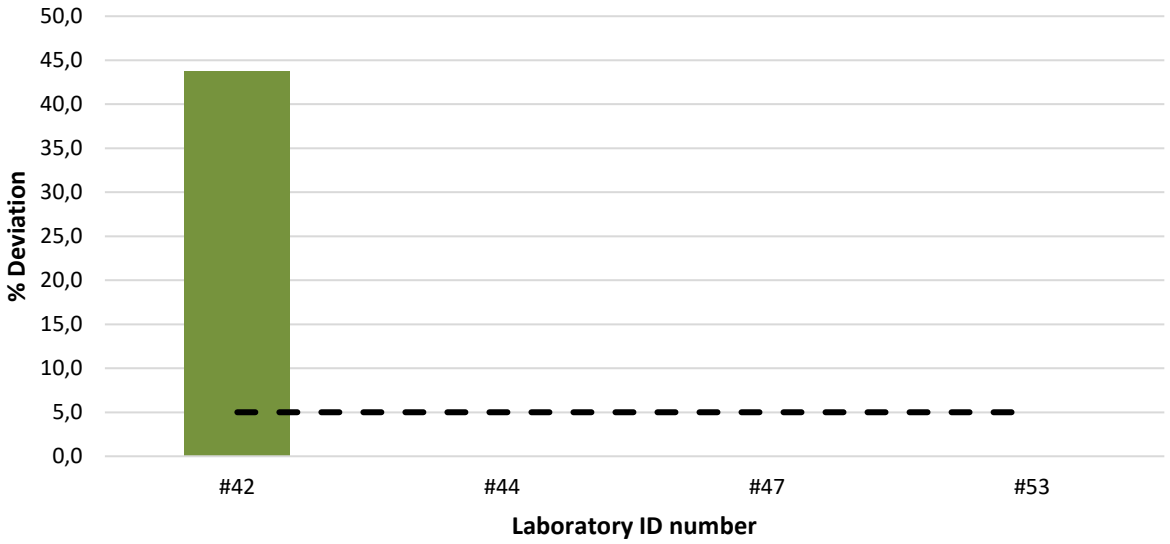


Figure 23. Percentage of deviation in the AST interpretation (R/I/S) among *C. jejuni/ C. coli* strains by AH laboratories (n=4) participating in the 7th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strain *C. jejuni* ATCC 33560

The quality control strain *C. jejun*i ATCC 33560 was sent to all participating laboratories free of charge (in a previous trial) to be used as a reference strain for the *C. jejuni/ C. coli* panel. Among the four participating laboratories, three submitted results for the reference strain *C. jejun*i ATCC 33560.

The highest proportion of test results outside of the expected range were observed for ciprofloxacin (2 out of 3) and erythromycin (1 out of 3) (**Table 31**).

In terms of performance, laboratory #53 presented no deviation for the two antimicrobials tested. Inversely, laboratory #42 and #44 had one and two deviations, respectively (**Figure 24**). Laboratory #42 and #44 presented deviations above the acceptance interval.

Table 31. AST of the reference strain *C. jejun*i ATCC 33560 in the *C. jejuni/ C. coli* panel. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diffusion	Total
CIP	2/3	2/3
ERY	1/3	1/3

Disk Diffusion – inhibition zone diameter determination by disk diffusion

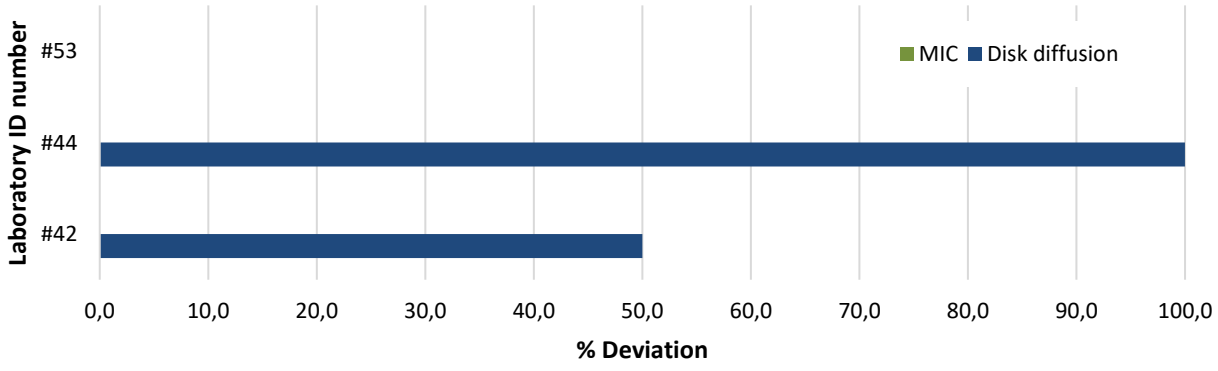


Figure 24. Percentage of deviation in the AST of *C. jejun*i ATCC 33560 in the *C. jejuni/ C. coli* panel by the AH laboratories.

5. Results – Overall

5.1 Bacterial identification

A total of 20 HH and 16 AH laboratories participated in this EQA trial. The participating laboratories were from 14 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Laos People Democratic Republic, Malaysia, the Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Timor-Leste, and Vietnam). In total, data were submitted by 33 laboratories for the *Salmonella spp.* panel, 24 laboratories for the *E. faecalis/E. faecium* panel, 11 – for *Campylobacter spp.*, and 8 – for *N. gonorrhoeae*.

Considering the test strains tested by each laboratory in each of the trials, it was possible to calculate the percentage of incorrectly identified isolates. **Figure 25** shows the distribution of laboratories that had a deviation for each of the panels.

Minor deviations were observed in the submitted data by very few laboratories for the bacterial identification component of the target strains in the *Salmonella spp.* panel. To the contrary, laboratories were divided in the data they reported for the *E. faecalis/E. faecium*, *Campylobacter spp.,* and *N. gonorrhoeae* panels. The difficulty to revive several *Campylobacter* and *N. gonorrhoeae* have led to skewed results in addition to the challenge faced by several laboratories to identify the target strains correctly.

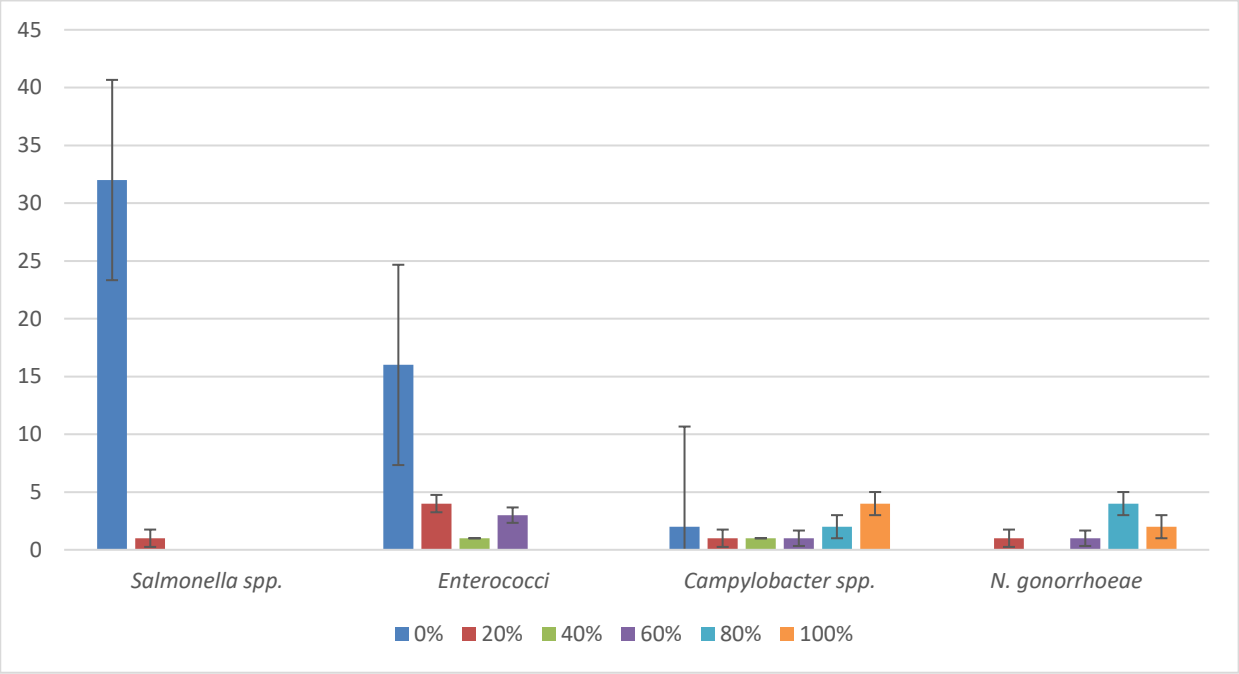


Figure 25. Percentage of deviation in the bacterial identification of target strains in the *Salmonella spp.*, *E. faecalis/E. faecium*, *Campylobacter spp.,* and *N. gonorrhoeae* panels by the participating laboratories.

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.

Figures 26-29 show the distribution of deviations presented by the laboratories submitting results for the respective antimicrobial (number of laboratories is indicated under each antimicrobial abbreviated name).

There were several deviations from the expected results in the *Salmonella spp.* panel mainly attributed to gentamicin and colistin (57.4% and 40.0%, respectively). The recent update in the CLSI guidelines reflecting new breakpoints for aminoglycosides for Salmonella might partially explain this deviation (**Figure 26**). All other antimicrobials showed deviations below 40%.

The results submitted for the enterococci panel showed most deviations for daptomycin and quinupristin and dalfopristin (44.4% and 42.9%, respectively) mainly because of the low number of tests performed (**Figure 27)**. Other antimicrobials with high percentage of deviations were chloramphenicol (32.6%) and vancomycin (23.1%).

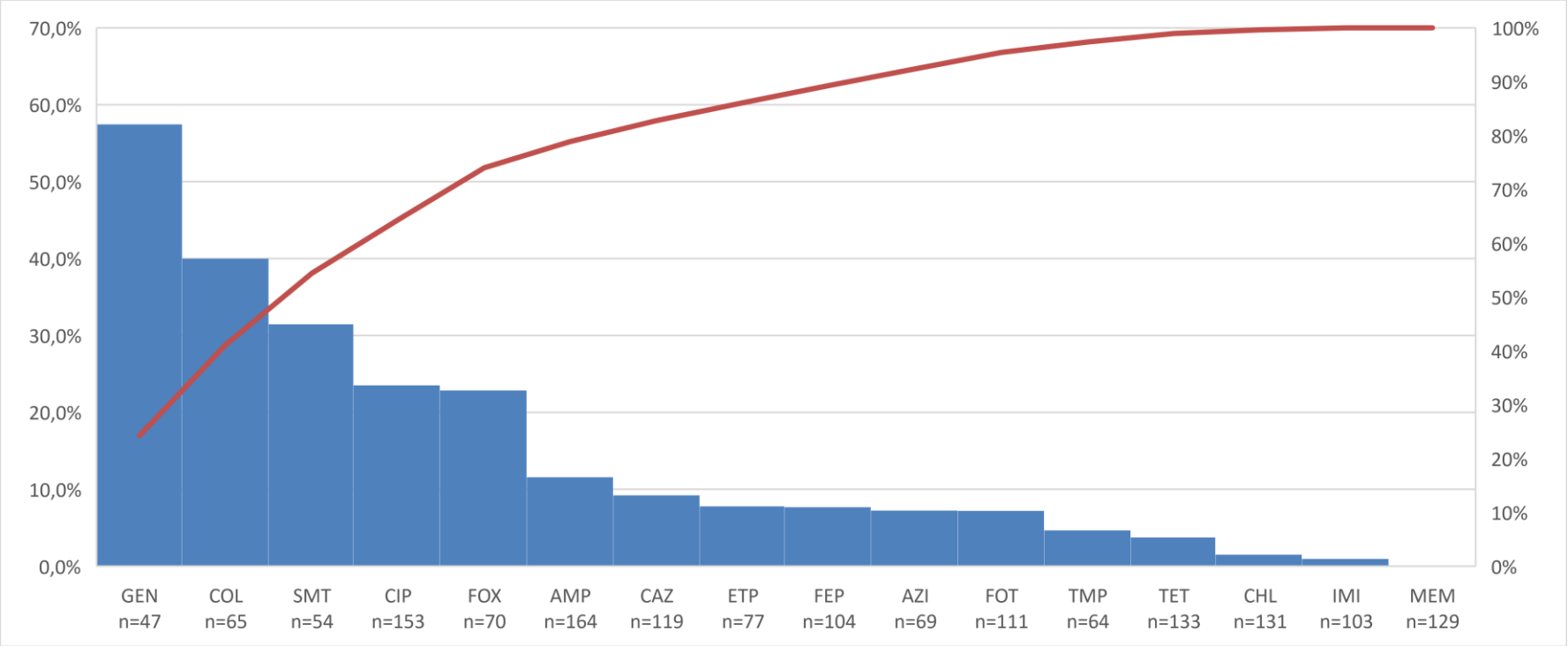


Figure 26. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Salmonella spp.* strains by the participating laboratories (n=33) in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

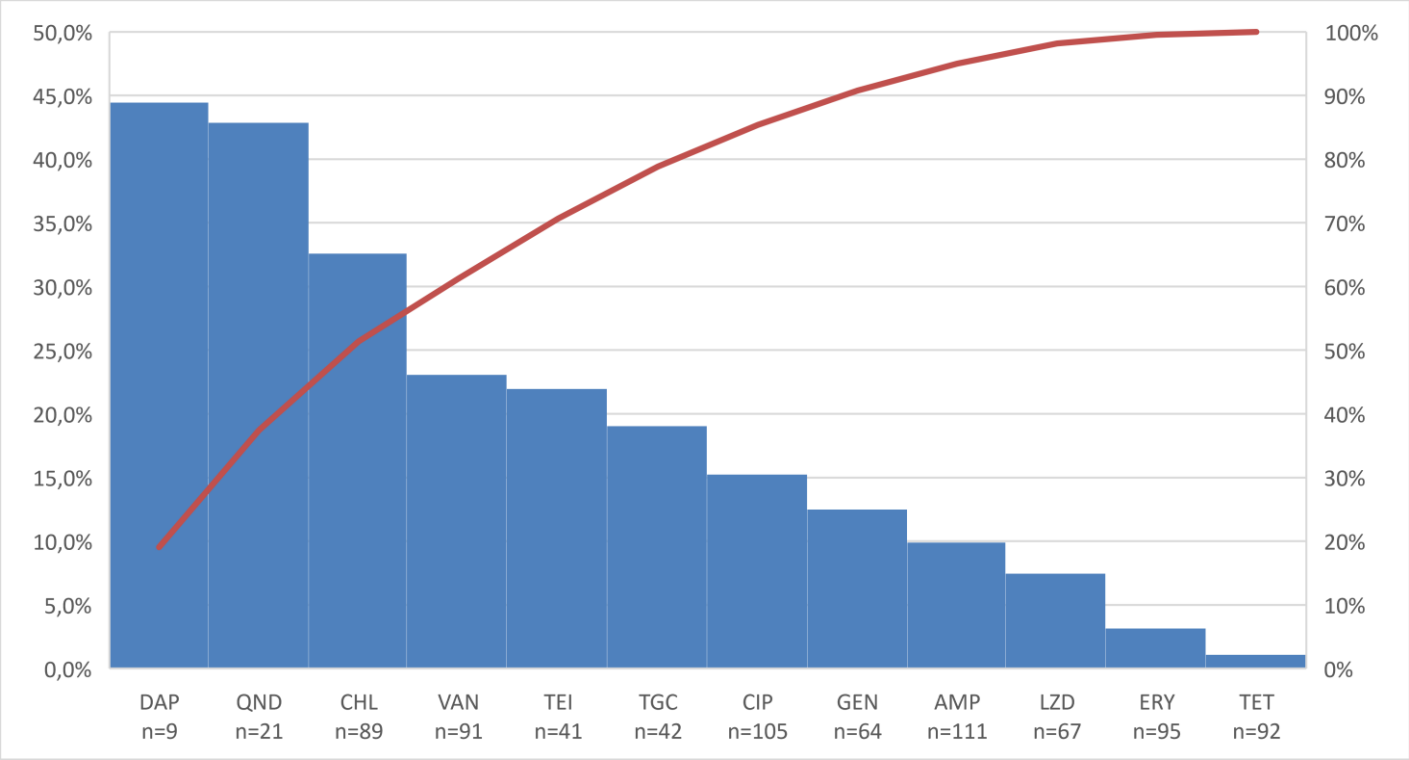


Figure 27. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. faecalis/E. faecium* strains by the participating laboratories (n=24) in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

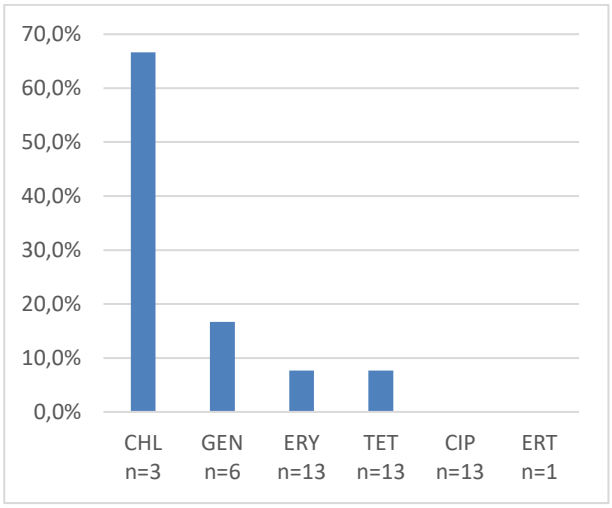


Figure 28. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Campylobacter spp.* strains by the participating laboratories (n=11) in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation.

There were only 49 AST results that were submitted and scored in the *Campylobacter spp.* panel. The low overall number of results is partially the reason for high percentage of deviations, mostly for chloramphenicol (66.7%) and gentamicin (16.7%) (**Figure 28)**. All other results showed deviations of less than 10%.

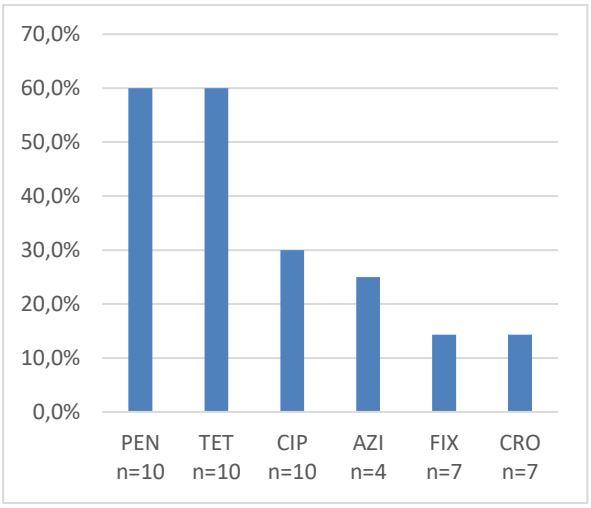


Figure 29. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *N. gonorrhoeae* strains by the participating laboratories (n=8) in the 7th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation.

The results submitted in the *N. gonorrhoeae* panel (n=48) showed deviations for all reported antimicrobials, mostly for penicillin and tetracycline (60.0% for each), as well as ciprofloxacin (30.0%) and azithromycin (25.0%) (**Figure 29)**.

5.2.2 Laboratories performance

In each of the panels, the overall performance of laboratories varied according to their performance score. There was more heterogeneity between the laboratories in the *Campylobacter spp.* and *N. gonorrhoeae* panels (**Figure 30**).

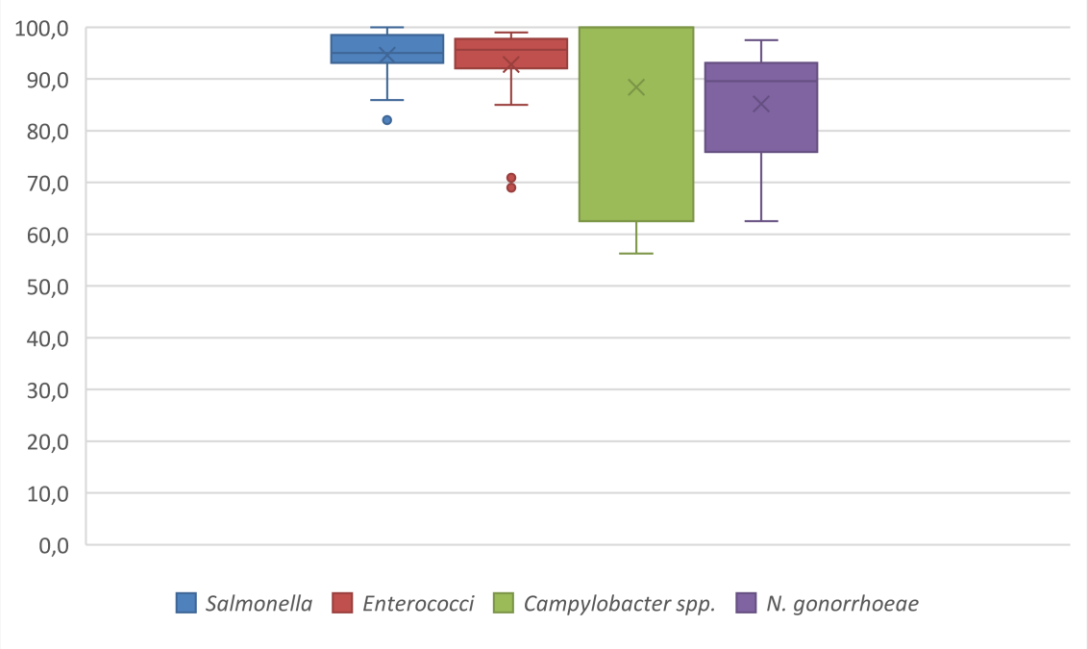


Figure 30. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 7th EQA of the EQAsia project. Most laboratories' performance rate was clustered between 82.1% and 97.5%, being more homogenous for the *Salmonella spp.* panel.

Out of the four panels included in this trial, the obtained results were the best for the *Salmonella spp.* and enterococci panels (average score 94.6% and 92.6%, respectively). The labs with minimum score in these two panels had a performance rate of 82.1% and 69%, respectively. The lowest performance score in the *Campylobacter spp.* panel was 56.3%, while for the gonococci panel – 62.5%.

Laboratories were ranked (#1 to #35) based on their average score across the panels in which they participated. The average score varied between 78.4% (rank #35) and 99.2% (rank #1). The total average score among all 35 laboratories that submitted results was 92.8%, while the median was 93.6%.

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

5.3 Quality control strains

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

As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (2 in the *Salmonella spp.* panel, 2 in the enterococci panel, 5 in the *Campylobacter spp.* panel, and 1 in the *N. gonorrhoeae* panel) did not submit results from reference strain testing at all. For the *Salmonella*

spp. EQA round, there were 9 laboratories (5 HH and 4 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=22) presented deviations between 7.7% and 50.0%. 11 laboratories (8 HH and 3 AH) showed no deviations in the reference strain testing in the enterococci panel. The remaining 13 laboratories submitted results that deviated between 10% and 63.6%. There was large heterogeneity in the *Campylobacter spp.* panel where the deviations were up to 100.0%. To the contrary, all the results submitted in the reference strain testing in the *N. gonorrhoeae* panel were according to the expected ranges.

Compared to the submitted AST results of the target strains, the results from the testing of the quality control strains were more heterogeneous and led to a much lower performance score in this component of the EQA trial. The greatest heterogeneity was observed in the *Campylobacter spp.* panel and partly also in the enterococci panel (**Figure 31**). The minimum score in the *Salmonella spp.* panel was 50.0%, while in the enterococci panel it was 36.4%. The testing of the *Campylobacter jejuni ATCC 33560/ CCM 6214* caused a substantial variety in the results submitted by the participating laboratories. Two of the laboratories did not submit any results conform to the expected results' range. The laboratories participating in the *N. gonorrhoeae* panel submitted a set of results that was within the expected values regardless of what reference strain was tested for that panel.

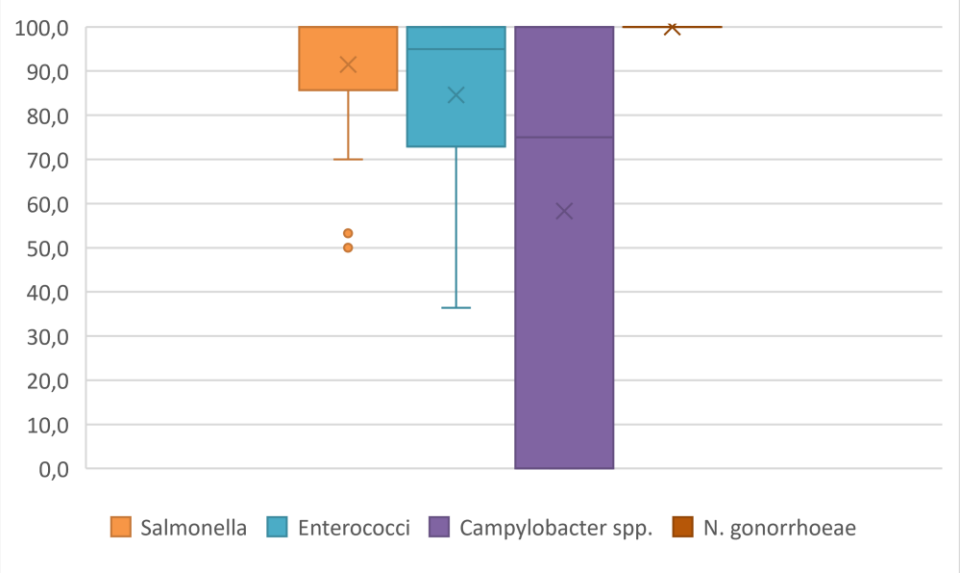


Figure 31. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 7th EQA of the EQAsia project.

6. Discussion

6.1 Human Health Laboratories

Overall, 20 Human Health laboratories participated in the 7th EQA of the EQAsia project and submitted EQA results for one or more EQA panels. Disk diffusion was chosen most frequently as a methodology for testing the recommended antimicrobials in each of the panels. Several laboratories relied solely on MIC determination methods or a combination of disk diffusion and MIC testing by either gradient test or broth microdilution.

All laboratories that performed bacterial identification in the *Salmonella spp.* and enterococci panels have also submitted AST results. However, this was not the case in the *Campylobacter spp.* and *N. gonorrhoeae* panels. Several isolates in these panels could not be revived by some of the laboratories or the reported identification of the revived isolates did not always match the baseline results. Attention should be paid to the use of appropriate media and following the protocol to reconstitute lyophilized bacteria, as these could be some of the main reasons why several laboratories were not able to cultivate isolates from the *Campylobacter spp.* and *N. gonorrhoeae* panels. Detection of gonorrhoea and particularly multidrug-resistant gonococci is of utmost importance for clinical microbiology laboratories. Hence, the need for special emphasis on this pathogen within the EQAsia project.

Incomplete AST results' entries were observed in all panels, except *Campylobacter spp.* where only 3 HH laboratories participated. Seven out of 17 HH laboratories that selected the enterococci panel did not submit complete results of their own available antimicrobial agents. It would be expected that the isolates of each trial would be tested against the same panel of antimicrobials, allowing for a solid assessment of the laboratories' performance and capacity.

The EQA participants showed high proficiency in correctly identifying the isolates in the *Salmonella spp.* panel. In the other three panels, the bacterial identification success rate varied. The identification and differentiation between *E. faecium, E. faecalis* and other *Enterococcus* species appeared to be challenging for 6 out of 17 laboratories whose results did not match the baseline for this panel. This underlines the need for targeted training on this particular species and the importance of the correct identification also related with antimicrobial susceptibility testing and possible resistance mechanisms.

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

For the *Salmonella spp.* panel, some antimicrobials presented a high deviation from the expected results, such as: gentamicin (75.0%), sulfamethoxazole (41.7%), and colistin (31.4%). The AST results in the enterococci panel also showed deviations from the baseline expected results, particularly for quinupristin and dalfopristin (45.5%), daptomycin (42.9%), chloramphenicol (32.4%), and tigecycline (31.8%). The total number of tested antimicrobials in the *Campylobacter spp.* and *N. gonorrhoeae* panels was relatively low due to the inability of some laboratories to revive and identify all isolates correctly.

On average, the AST performance of participating laboratories was the best in the *Salmonella spp.* panel (95.7%), followed by enterococci (91.5%), *Campylobacter spp.* (87.5%), and *N. gonorrhoeae* (85.2%).

Detection and confirmation of presumptive beta-lactamase producing *Salmonella spp.* was an optional component of this EQA and laboratories opted out and did not submit data for it. *Salmonella* serotyping was also voluntary. Four HH laboratories submitted results for *Salmonella* serogrouping, and only three laboratories provided serotyping results.

Among all HH laboratories, there were a few that did not submit antimicrobial susceptibility testing

results for the quality control strains across all four panels. According to the CLSI recommendations, quality of laboratory performance is determined by the quality control management, indicating accuracy and precision of data produced by an individual laboratory. Therefore, the correct AST results of test strains without quality control may not imply a reliable laboratory AST performance.

6.2 Animal Health Laboratories

For the Animal Health sector, 15 laboratories participated in the 7th EQA of the EQAsia project. The participating laboratories mostly applied disk diffusion alone for determining inhibition zone diameters, others opted for broth microdilution or a combination of the two methodologies.

The participants were asked to firstly perform bacterial identification and then proceed with AST of the target strains. Incomplete AST results' entries were observed in all panels, except the *C. jejuni/ C. coli* panel. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance. Although laboratories #37 and #38 performed bacterial identification, they did not submit AST results for the *C. jejuni/ C. coli* panel.
As mentioned above, bacterial identification was the first component in each of the panels. There were no major issues with bacterial identification of the five target strains among the seven isolates provided for the *Salmonella spp.* panel. The identification and differentiation between *E. faecium*, *E. faecalis* and other *Enterococcus* species revealed some limited capacity of the participating laboratories to perform bacterial identification, where the *E. faecalis* isolates were correctly identified, but not the remaining strains, suggesting that advice and training on the subject may be required among the AH laboratories. Similarly, three out of six laboratories that participated and submitted results to the *C. jejuni/ C. coli* trial demonstrated limitations on differentiation between *C. jejuni*, *C. coli* and other *Campylobacter* species.

For the antimicrobial susceptibility testing performance, sulfamethoxazole presented quite high deviations in the S*almonella spp.* panel (25.6%). In the *E. faecium/ E. faecalis* panel, the AST results submitted for the five *E. faecium/ E. faecalis* strains were still considered for evaluation, even if incorrectly identified by the laboratories (only for *E. faecium* strains identified as *E. faecalis*, and vice-versa), since the interpretation criteria is not substantially different for these two species; here, the highest deviations (quinupristin/dalfopristin and daptomycin) can be explained by the fact that these antimicrobials were tested by few laboratories. The AST deviations observed in the *C. jejuni/C. coli* trial were quite high for two of the six tested drugs (ciprofloxacin and gentamicin).
Regarding laboratories' performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. A deviation below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for seven out of the fifteen participants in the *Salmonella spp.* panel and for only one participant in the *E. faecium/ E. faecalis* panel and *C. jejuni/C. coli* panel.
None out of the fifteen participating laboratories in the *Salmonella spp.* panel submitted results for the detection and confirmation of presumptive beta-lactamase producing bacteria. Serotyping of *Salmonella* was also a component with voluntary participation, for which only four of the fifteen participating laboratories reported results. Of those, however, only three submitted data for both serogroup and serovars. Based on the results, it is notable that some laboratories could only identify certain serogroups. This could be due to limited technical capacity, but also lack of antisera supply.
Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the panels. All participating laboratories submitted results for the reference strains in the *Salmonella spp.* panel. Three laboratories did not submit results for the *S.*

aureus ATCC 25923 or *E. faecalis* ATCC 29212 reference strain in the *E. faecium/ E. faecalis* panel. Laboratory #47 did not submit results for *C. jejuni* ATCC 33560. For the laboratories reporting data, the deviations in this component were defined as AST results of the reference strain that were outside the quality control acceptance intervals, which suggests that handling of reference strains needs to be strengthened to ensure the laboratories' good performance.

7. Conclusions

This report presents the results of the EQAsia 7th EQA trial, which was carried out in October – November 2023 and included bacterial identification and antimicrobial susceptibility testing (AST) of several prominent WHO and FAO priority pathogens: *Salmonella spp., Enterococcus faecalis/ Enterococcus faecium, Campylobacter coli/ Campylobacter jejuni,* and *Neisseria gonorrhoeae.*

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

Performance issues in terms of bacterial identification and antimicrobial susceptibility testing were detected for both sectors, demonstrating the ongoing need for support, with training and building further capacity in the reference laboratories in the South and Southeast Asian region.

In terms of bacterial identification, the pathogens included in this trial had a higher degree of difficulty compared to previous panels. Identification proved to be challenging in the enterococci and *Campylobacter spp.* panels. *N. gonorrhoeae* panel was introduced for the first time since the start of the EQAsia project.

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

In this EQA trial, there were several misinterpretations of the MIC/ inhibition zone diameter values in the reported results, especially in the enterococci and *Campylobacter spp.* panels, demonstrating lower level of proficiency of some of the participating laboratories. This EQA exercise also revealed the need to place a special emphasis on detecting and identifying fastidious microorganisms. Capacity building is further needed in this direction since several laboratories were unable to reconstitute and isolate a large number of strains from the *Campylobacter spp.* and *N. gonorrhoeae* panels. It is also a requirement that all participating laboratories follow the same protocol and interpretation criteria to allow for comparison of results.

Antimicrobial susceptibility testing of the reference strains is also highly important and, therefore, largely recommended. Relevant reference strains have been sent to the participating laboratories during previous EQA rounds free of charge to be used not only in the EQAsia EQAs, but also in the routine work. Several reference strains for the microbiology diagnostics of gonococci were sent to participating laboratories for the first time within

this EQA round. Laboratories need to make sure they have all necessary quality control strains that should be tested on a regular basis. Proper storage and maintenance of these reference strains is recommended. Routine testing is required for quality control purposes, as deviating results for the quality control strains imply invalidation of the AST results for the test strains. Furthermore, action needs to be taken every time the results from the quality control testing deviate from the ranges set in the methodological standards used. EQAsia has also prioritized quality control of AST as a training topic and is offering continuous support on this matter.

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

8. References

[1] Annex 8: Pathogen-antimicrobial combinations under GLASS-AMR surveillance. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

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

[3] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

[4] 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.

[5] The European Committee on Antimicrobial Susceptibility Testing. *Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST*. Version 13.0, 2023. http://www.eucast.org.

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

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

9. Appendices

Appendix 1: EQA7 Protocol

EQAsia EQA7 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 EQA7 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 disk diffusion of Salmonella strains), *E. coli* NCTC 13846/CCM 8874 (for testing colistin), *Campylobacter jejuni* ATCC 33560/ CCM 6214, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disk diffusion of the Enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC).

The QC strains provided within EQA7 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. EQA7 OUTLINE

3.1.Shipping and receipt of strains

Your laboratory is one of the 37 human health and animal health laboratories from South and Southeast Asia participating in EQA7. In October 2023, 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 disk diffusion) and *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) reference strains have been provided in previous EQA rounds.

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

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

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

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

3.2.Reviving and storing the strains

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

- **Reviving Enterococcus and Campylobacter lyophilised cultures**

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

Needed material:

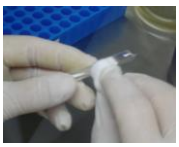
- An ampoule cutter or a file
- Sterile Luria Bertani (LB) broth
- LB agar plates (5 to 6 plates per one strain)
- Columbia broth for Campylobacter
- mCCDA agar plates (5 to 6 plates per one strain) for Campylobacter
- Autopipette with tips or Pasture pipettes
- Inoculating loop

1. Carefully take the ampoule out of the wrap.

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

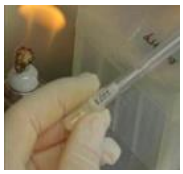
   Note: The ampoule can be cut in the middle or below the cotton wool plug.

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



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

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



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

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

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

Needed material:

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

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

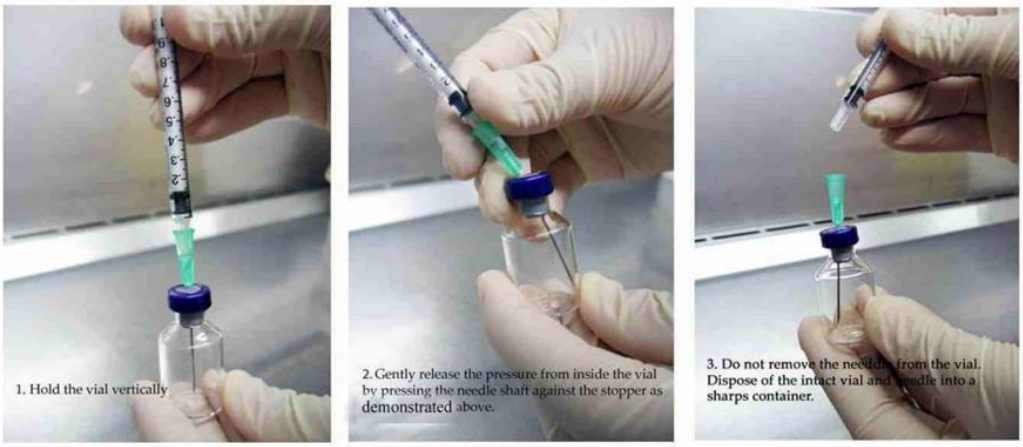
Do not mouth pipette and do not reconstitute the specimens until you are ready to plate them out.

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

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

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

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

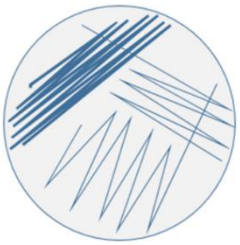


- **Reviving Salmonella isolates**

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., Enterococci, Campylobacter spp.* and *Neisseria gonorrhoeae* test strains

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

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

3.4. Serotyping of *Salmonella spp*.

The five identified *Salmonella* strains should be serotyped by using the method routinely used in your own laboratory. In addition, serogroup results will be evaluated. Therefore, if you do not have all the necessary antisera for serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

3.5. Antimicrobial susceptibility testing of *Salmonella spp., Enterococci, 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 disk diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 33rd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 13.1, 2023) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *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 Disk diffusion (mm)		
	S	I	R	S	I	R
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.06	0.12-0.5	≥ 1	≥ 31	21-30	≤ 20
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10

Reference values are based on Enterobacterales breakpoints from CLSI M100, 33rd Ed.

*****Aminoglycosides may appear active in vitro for *Salmonella spp.* but are not clinically effective and should not be reported as susceptible. They are not required to be reported for this EQA panel.

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

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

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

Reference values are based on *Enterococcus spp.* breakpoints from CLSI M100, 33rd Ed.
*Reference values are based on *Enterococcus* spp. clinical breakpoints from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org."

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

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

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

Reference values are based on *Campylobacter jejuni/coli* breakpoints from CLSI M45, 3rd Ed.
*Reference values are based on *C. jejuni* and *C. coli* epidemiological cut off values from https://mic.eucast.org/ in August 2023.
**Reference values are based on EFSA (European Food Safety Authority) recommendation.

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

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

Reference values are based on *N. gonorrhoeae* breakpoints from CLSI M100, 33rd Ed.

N.B. For the interpretation of the AST results for *N. gonorrhoeae* quality control strains provided with this EQA panel (ATCC49226, WHO G, WHO L, WHO O and WHO P) please refer to Table 4B and 5C (Disk diffusion and MIC QC ranges for ATC49226) in CLSI M100, 33rd 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 hiami@food.dtu.dk

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

Results must be submitted no later than November 24th, 2023.

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 1: EQA7 protocol

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

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

	Ampicillin (AMP)		Azithromycin (AZI)		Cefepime (FEP)		Cefotaxime (FOT)		Cefoxitin (FOX)		Ceftazidime (TAZ)	
Salm EQAsia 23.1	<=1	S	8	S	≤0.06	S	≤0.25	S	4	S	0.5	S
Salm EQAsia 23.2	>32	R	4	S	≤0.06	S	≤0.25	S	2	S	≤0.25	S
Salm EQAsia 23.5	> 32	R	64	R	> 32	R	> 64	R	4	S	16	R
Salm EQAsia 23.6	2	S	4	S	0.12	S	≤0.25	S	8	S	0.5	S
Salm EQAsia 23.7	4	S	8	S	0.25	S	≤0.25	S	16	I	1	S

R, Resistant; I, Intermediate; S, Susceptible

	Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)		Ertapenem (ETP)		Imipenem (IMI)		Meropenem (MERO)	
Salm EQAsia 23.1	4	S	≤ 0.015	S	≤ 0.25	I	≤0.015	S	0.25	S	≤0.03	S
Salm EQAsia 23.2	4	S	0.03	S	≤0.25	I	≤0.015	S	0.25	S	≤0.03	S
Salm EQAsia 23.5	4	S	2	R	0.5	I	≤0.015	S	0.5	S	≤0.03	S
Salm EQAsia 23.6	4	S	0.03	S	8	R	≤0.015	S	0.5	S	0.06	S
Salm EQAsia 23.7	8	S	0.03	S	2	I	0.03	S	0.25	S	≤0.03	S

R, Resistant; I, Intermediate; S, Susceptible

	Nalidixic acid (NAL)		Sulfamethoxazole (SMX)		Tetracycline (TET)		Tigecycline (TGC)		Trimethoprim (TMP)	
Salm EQAsia 23.1	≤ 4	S	16	S	≤ 2	S	≤0.25	S	≤0.25	S
Salm EQAsia 23.2	≤ 4	S	> 512	R	>32	R	0.5	S	≤0.25	S
Salm EQAsia 23.5	> 64	R	> 512	R	≤ 2	S	≤ 0.25	S	>32	R
Salm EQAsia 23.6	≤ 4	S	≤ 8	S	≤ 2	S	≤ 0.25	S	≤0.25	S
Salm EQAsia 23.7	≤ 4	S	> 512	R	≤ 2	S	≤ 0.25	S	≤ 0.25	S

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 23.1	2	S	16	I	>16	R	1	S	>128	R	>1024	R
Ef EQAsia 23.3	> 64	R	8	S	>16	R	2	S	>128	R	256	R
Ef EQAsia 23.4	> 64	R	8	S	>16	R	8	R	>128	R	≤ 8	S
Ef EQAsia 23.5	64	R	16	I	8	R	2	S	>128	R	>1024	R
Ef EQAsia 23.7	1	S	8	S	1	S	2	S	>128	R	16	S

R, Resistant; I, Intermediate; S, Susceptible

	Linezolid (LZD)		Quinu/Dalfo (SYN)		Teicoplanin (TEI)		Tetracycline (TET)		Tigecycline (TGC)		Vancomycin (VAN)	
Ef EQAsia 23.1	1	S	8	R	16	I	32	R	0.25	S	64	R
Ef EQAsia 23.3	2	S	1	S	> 64	R	64	R	0.12	S	> 128	R
Ef EQAsia 23.4	2	S	2	I	2	S	64	R	0.12	S	> 128	R
Ef EQAsia 23.5	2	S	16	R	≤ 0.5	S	32	R	0.12	S	2	S
Ef EQAsia 23.7	2	S	16	R	≤ 0.5	S	64	R	0.12	S	16	I

R, Resistant; I, Intermediate; S, Susceptible

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

	Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Ertapenem (ETP)		Erythromycin (ERY)		Gentamicin (GEN)		Tetracycline (TET)	
Camp EQAsia 23.1	4	S	0.06	S	0.06	S	≤ 1	S	≤ 0.25	S	≤ 0.5	S
Camp EQAsia 23.3	4	S	>32	R	0.5	S	> 512	R	> 16	R	64	R
Camp EQAsia 23.4	4	S	0.25	S	≤ 0.12	S	1	S	≤ 0.25	S	≤ 0.5	S
Camp EQAsia 23.5	4	S	32	R	1	R	> 512	R	0.5	S	> 64	R
Camp EQAsia 23.7	8	S	32	R	1	R	2	S	0.5	S	1	S

R, Resistant; I, Intermediate; S, Suscept

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

	Azithromycin (AZI)		Ceftriaxone (CRO)		Cefixime (CFM)		Ciprofloxacin (CIP)		Penicillin (PEN)		Tetracycline (TET)	
NG EQAsia 23.2	0.25	S	0.032	S	0.016	S	0.008	S	PPNG	R	2	R
NG EQAsia 23.3	0.25	S	≤0.016	S	≤0.016	S	0.25	I	PPNG	R	16	R
NG EQAsia 23.4	1	S	0.5	R	2	R	≥32	R	2	R	4	R
NG EQAsia 23.6	16	R	0.032	S	0.125	S	≥32	R	2	R	1	I
NG EQAsia 23.7	>256	R	1	R	1	R	≥32	R	2	R	≥32	R

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

Appendix 3: Quality control ranges for the reference strains

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

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

MIC ranges and disk diffusion ranges are according to CLSI M100 33rd edition, Tables 4A-1 and 5A-1

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

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

Appendix 3b: Quality control ranges for *Campylobacter jejuni* ATCC 33560

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

MIC ranges and disk diffusion ranges are according to CLSI M100 33rd edition, Tables 4A-1 and 5A-1

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

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

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

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

MIC and disk diffusion ranges are according to CLSI M100 33rd edition, Tables 4A-2 and 5A-1

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

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

MIC ranges and disk diffusion ranges are according to CLSI M100 33rd edition, Tables 4B and 5C

DTU National Food Institute
Henrik Dams Allé
Building 202
2800 Kgs. Lyngby
Denmark

Tel: +45 35 88 70 00

www.food.dtu.dk

ISBN: 978-87-7586-028-9