DTU Fødevareinstituttet
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10th EQAsia External Quality Assessment trial:

Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* – 2025

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Executive Summary

This report presents the findings from the 10th External Quality Assessment (EQA) trial conducted by the EQAsia project in March - May 2025. The trial evaluated the performance of laboratories in the South and Southeast Asia region in the bacterial identification and antimicrobial susceptibility testing (AST) of four priority pathogens: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus.*

The EQAsia project, supported by the Fleming Fund, aims to strengthen laboratory capacity for antimicrobial resistance (AMR) surveillance within a One Health framework. A total of 61 laboratories (34 Human Health, HH; 27 Animal Health, AH) from 14 countries participated, submitting results for at least one of the four bacterial panels.

The bacterial identification component consisted of identification of the five strains of the organism in question (target organism) among a total of seven strains. Bacterial identification was generally accurate across all panels, with most laboratories correctly identifying the target species. However, challenges remained in distinguishing non-target strains in *the E. coli* and *S. aureus* panels, indicating a need for reinforced identification protocols.

The AST performance revealed significant variability. The *S. aureus* panel demonstrated the highest overall proficiency (average score 96%), followed by *E. coli* and *K. pneumoniae* (94% each). In contrast, the *P. aeruginosa* panel showed the lowest performance (average score 90%), with particularly high error rates for critical antibiotics like ceftazidime (47.8% deviation) and piperacillin-tazobactam (33.0% deviation). Detection of complex resistance mechanisms (e.g., ESBL, AmpC, carbapenemase) proved challenging, especially for strains with combined resistance profiles.

A major concern identified was the performance in testing quality control (QC) strains. While some laboratories achieved perfect scores, others reported significant deviations, with error rates reaching 100% for certain QC strains in the *E. coli* and *K. pneumoniae* panels. This highlights fundamental procedural deficiencies in basic AST methodologies within a subset of laboratories. The laboratories need to ensure they have all necessary quality control strains that should be tested on a regular basis. 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.

In conclusion, while many laboratories demonstrated satisfactory performance, the results underscore persistent and critical gaps in AST accuracy, particularly for Gram-negative pathogens and last-resort antimicrobials. The findings emphasize the indispensable role of ongoing EQA programs like EQAsia in identifying areas for improvement. Targeted interventions, continuous training, and strict adherence to quality control practices are essential to enhance the reliability of laboratory data, which is crucial for effective clinical decision-making and AMR surveillance in the region.

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 World Health Organization (WHO) Global Antimicrobial Resistance and Use Surveillance System (GLASS) priority pathogens [1] and Food and Agricultural Organization (FAO) priority pathogens [2]. EQAsia has transitioned to a second phase and continues 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 at no cost for the South and Southeast Asian region distributed through CUVET Thailand, a leading 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 accordance 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 nine 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 project and, as recommended by FAO, to evaluate the capacity of veterinary laboratories 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 10th EQA trial of the EQAsia project (EQA10) carried out in March – May 2025. The trial included four EQA panels, each containing seven test strains. Of these, five were the organism in question (target organism, i.e., *K. pneumoniae*), whereas the other two test strains were different from the targeted species (reported as non-target, i.e., non-*K. pneumoniae*). For each of the seven test strains, participants were requested to report which five strains belong to the expected target organism. For these five test strains of the target organism, AST results were requested from the participating laboratories. There was no additional testing was needed for the two strains of non-target species.

This 10th EQA trial includes identification and AST of *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*. The aim of this EQA trial was to

monitor the quality of AST results produced by the participating laboratories and identify underperforming laboratories requiring additional support and assistance to improve their performance in bacterial identification and AST.

The evaluation of the participants' results is based on international guidelines, specifically 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 EQA10 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 described in the EQA10 protocol (**Appendix 1**). This standardized interpretation of results is necessary to allow comparison of performance between laboratories. Laboratory performance is considered acceptable if there is < 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. Since this report assesses the interpretation of MIC/zone diameter rather than the actual 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 in the high quality of their AST performance.

In this report, results from laboratories affiliated with the human health (HH) or animal health (AH) sectors are presented separately. The laboratories are identified by codes and each code is known only by the corresponding laboratory and the organizers. The full list of laboratory codes is confidential and known only by the EQAsia consortium.

This report, in its final version, is approved by a Technical Advisory Group consisting of members from 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 EQA10

A total of 63 laboratories signed up for 10th EQA trial of the EQAsia project and 61 laboratories submitted the results for evaluation. Out of 61 laboratories, 34 laboratories belonging to the HH Sector and 27 belonging to the AH Sector, located in 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 were given the opportunity to register for any of the four EQA panels. For each registration, the laboratory 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, one of the test strains is used as an internal control strain that will also be included in future EQAs with varying strain code.

Candidate strains for this EQA were tested at DTU National Food Institute and additionally verified by the external partner (The Peter Doherty Institute for Infection and Immunity, Australia). Expected MIC values (**Appendix 2a-d**) of the selected strains for this EQA were further confirmed by CUVET.

Reference strains [*Escherichia coli* ATCC 25922/CCM 3954, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC)] were provided at no cost during previous EQA rounds with instructions for storage and maintenance for quality assurance purposes and to be used in future EQA trials. The expected quality control ranges for the reference strains (**Appendix 3a-c**) were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-35th Ed., tables 4A-1 and 5A-1 [3].

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all four panels are outlined in the EQA10 protocol (**Appendix 1**) and in **Table 1**. These antimicrobial agents represent several antimicrobial classes crucial for surveillance, as well as antimicrobials required for detection and confirmation of ESBL-, AmpC-, and carbapenemase-producing phenotypes.

The reference values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 35th Ed. and VET06, 1st Ed.) [3, 4]. When not available, CLSI M100 33rd Ed., EUCAST clinical breakpoints (Tables v. 15.0, 2025) [4] or epidemiological cut off values (February 2025) [5] were used instead. Cefotaxime / clavulanic acid and ceftazidime / clavulanic acid results (*E. coli* and *K. pneumoniae* panel) were not scored, as these drug combinations are mostly important for confirmation of ESBL-, AmpC-, and carbapenemase-producing phenotypes. Results for presumptive beta-lactam resistance mechanisms were interpreted according to the most recent EFSA (European Food Safety Authority) [6] and EUCAST recommendations for surveillance, also included in the EQA10 protocol.

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

Figure 1: Countries participating in the 10th 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 EQA10 2025. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Amikacin	Amikacin	Amikacin	Cefoxitin
Ampicillin	Ampicillin	Aztreonam	Chloramphenicol
Azithromycin	Azithromycin	Cefepime	Ciprofloxacin
Cefepime	Cefepime	Ceftazidime	Clindamycin
Cefotaxime	Cefotaxime	Ciprofloxacin	Erythromycin
Cefotaxime/clavulanic acid	Cefotaxime/clavulanic acid	Colistin	Fusidic acid
Cefoxitin	Cefoxitin	Doripenem	Gentamicin
Ceftazidime	Ceftazidime	Gentamicin	Kanamycin
Ceftazidime/clavulanic acid	Ceftazidime/clavulanic acid	Imipenem	Linezolid
Chloramphenicol	Chloramphenicol	Levofloxacin	Penicillin
Ciprofloxacin	Ciprofloxacin	Meropenem	Quinupristin/dalfopristin
Colistin	Colistin	Piperacillin/tazobactam	Rifampin
Doripenem	Doripenem	Tobramycin	Sulfamethoxazole
Ertapenem	Ertapenem		Tetracycline
Gentamicin	Gentamicin		Trimethoprim
Imipenem	Imipenem		Vancomycin
Levofloxacin	Levofloxacin		
Meropenem	Meropenem		
Nalidixic acid	Nalidixic acid		
Piperacillin/tazobactam	Piperacillin/tazobactam		
Sulfamethoxazole	Sulfamethoxazole		
Tetracycline	Tetracycline		
Tigecycline	Tigecycline		
Tobramycin	Tobramycin		
Trimethoprim	Trimethoprim		
Trimethoprim/ sulfamethoxazole	Trimethoprim/ sulfamethoxazole		

2.4 Distribution

The bacterial strains were dispatched in March 2025 by CUVET to all participating laboratories. The strains were sent either lyophilized or on transport media swabs. 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 cultures as part of the EQA10 protocol (**Appendix 1**).

2.5 Procedure

Protocols and all relevant information were sent to the laboratories along with the shipment. The protocols were also sent via email and were available at the EQAsia website [7], allowing access to all the necessary information at any time. The participants were recommended to store the 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 perform testing for detection of ESBL-, AmpC-, and carbapenemase-producing *E. coli* and *K. pneumoniae*.

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 their obtained results into an informatics module developed as part of the EQAsia programme and adapted for this trial. The informatics module could be accessed through a secured individual login and password. After the results were released, the participants were invited to login and retrieve their 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. Taking into consideration this method limitation and the fact that the laboratories cannot test for lower antimicrobial concentrations, the informatics module was adapted to score these specific occurrences as '1' (correct).

3. Results – Human Health Laboratories

3.1 Overall participation

Among the 34 Human Health laboratories participating in the 10th EQA of the EQAsia programme, 24 laboratories submitted results for *E. coli* while 20 laboratories submitted results for *K. pneumoniae* panel. For *P. aeruginosa* panel, the number of laboratories submitting data was 28, while 31 laboratories submitted results for the *S. aureus* panel. The primary methodology applied 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 and MIC values were used as supplementary information. The majority of the participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results **(Figure 3)**.

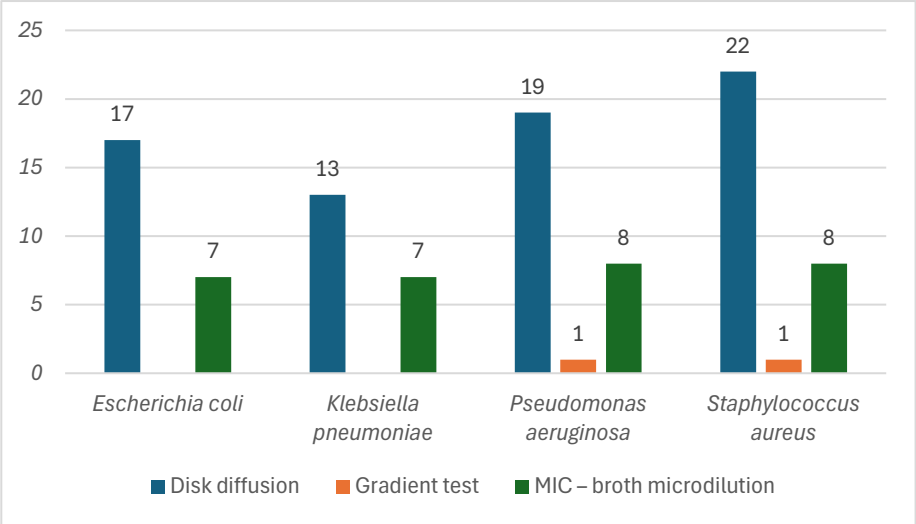

Figure 2. Methodologies used by the laboratories for antimicrobial susceptibility testing in each of the trials.

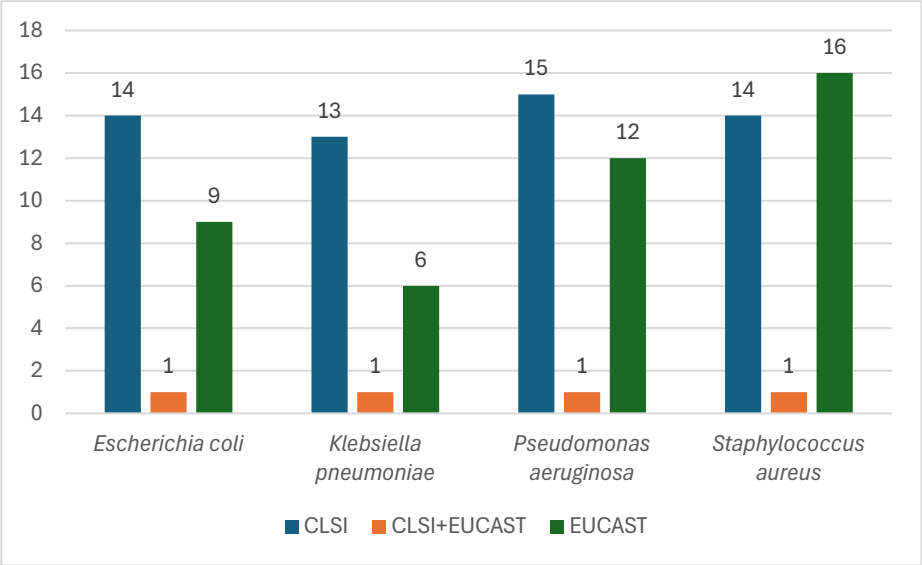

Figure 3. Use of international guidelines for interpretation of AST results by the participating laboratories.

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

The *E. coli* panel presented the highest number of total AST result submissions (n=2166) according to the recommended antimicrobials in CLSI (**Table 2**), followed by *S. aureus* panel (n=2002). For the Gram-negative bacteria, fewer laboratories tested the last resort antibiotics such as colistin and doripenem (**Table 2**). In contrast amikacin, cefepime. ceftazidime, meropenem, imipenem, ciprofloxacin and gentamicin were tested by most laboratories for the *E. coli*, *K. pneumoniae,* and *P. aeruginosa* panels, whereas cefoxitin, ciprofloxacin, erythromycin, gentamicin and tetracycline were tested by most laboratories for the *S. aureus* panel (**Table 2**).

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

	E. coli		*K. pneumoniae*		*P. aeruginosa*		*S. aureus*	
Amikacin	115	*5,31%*	100	*5,22%*	133	*8,82%*	--	--
Ampicillin	83	*3,83%*	79	*4,12%*	--	--	--	--
Azithromycin	48	*2,22%*	49	*2,56%*	--	--	--	--
Aztreonam	--	--	--	--	93	*6,17%*	--	--
Cefepime	106	*4,89%*	84	*4,38%*	128	*8,49%*	--	--
Cefotaxime	80	*3,69%*	69	*3,60%*	--	--	--	--
Cefotaxime and clavulanic acid	73	*3,37%*	62	*3,23%*	--	--	--	--
Cefoxitin	97	*4,48%*	78	*4,07%*	--	--	160	*7,99%*
Ceftazidime	119	*5,49%*	100	*5,22%*	138	*9,15%*	--	--
Ceftazidime and clavulanic acid	75	*3,46%*	68	*3,55%*	--	--	--	--
Chloramphenicol	81	*3,74%*	68	*3,55%*	--	--	114	*5,69%*
Ciprofloxacin	120	*5,54%*	100	*5,22%*	139	*9,22%*	165	*8,24%*
Clindamycin	--	--	--	--	--	--	124	*6,19%*
Colistin	62	*2,86%*	57	*2,97%*	87	*5,77%*	--	--
Doripenem	45	*2,08%*	44	*2,30%*	77	*5,11%*	--	--
Doxycycline	--	--	--	--	--	--	--	--
Ertapenem	73	*3,37%*	69	*3,60%*	--	--	--	--
Erythromycin	--	--	--	--	--	--	160	*7,99%*
Fusidic acid	--	--	--	--	--	--	103	*5,14%*
Gentamicin	119	*5,49%*	100	*5,22%*	104	*6,90%*	164	*8,19%*
Imipenem	100	*4,62%*	89	*4,64%*	128	*8,49%*	--	--
Kanamycin	--	--	--	--	--	--	81	*4,05%*
Levofloxacin	73	*3,37%*	74	*3,86%*	113	*7,49%*	--	--
Linezolid	--	--	--	--	--	--	118	*5,89%*
Meropenem	120	*5,54%*	100	*5,22%*	138	*9,15%*	--	--
Minocycline	--	--	--	--	--	--	--	--
Nalidixic acid	69	*3,19%*	64	*3,34%*	--	--	--	--
Penicillin	--	--	--	--	--	--	129	*6,44%*
Piperacillin and tazobactam	93	*4,29%*	85	*4,43%*	125	*8,29%*	--	--

Quinupristin and dalfopristin	--	--	--	--	--	--	91	*4,55%*
Rifampin	--	--	--	--	--	--	113	*5,64%*
Sulfamethoxazole	44	*2,03%*	39	*2,03%*	--	--	105	*5,24%*
Tetracycline	69	*3,19%*	64	*3,34%*	--	--	170	*8,49%*
Tigecycline	69	*3,19%*	66	*3,44%*	--	--	--	--
Tobramycin	68	*3,14%*	70	*3,65%*	105	*6,96%*	--	--
Trimethoprim	45	*2,08%*	39	*2,03%*	--	--	92	*4,60%*
Trimethoprim and sulfamethoxazole	120	*5,54%*	100	*5,22%*	--	--	--	--
Vancomycin	--	--	--	--	--	--	113	*5,64%*
Total	**2166**		**1917**		**1508**		**2002**	

Missing data or incomplete AST results entries were observed across all four EQA panels among the HH laboratories participating in EQA10. A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains.

Nine out of 24 laboratories submitted incomplete results for the *E. coli* panel (**Table 3**). The highest number of incomplete results in the *E. coli* panel were observed for laboratory #51.

Only two out of 20 laboratories that selected *K. pneumoniae* did not submit complete results of their own available antimicrobial agents (**Table 4**).

Four out of 28 laboratories that selected *P. aeruginosa* submitted incomplete results of their own available antimicrobial agents (**Table 5**). The highest number of incomplete results in the *P. aeruginosa* panel was seen for laboratory #06.

For S. aureus panel, only three out of 31 laboratories reported incomplete results of their own available antimicrobial agents (**Table 6**). The highest number of incomplete results in the *S. aureus* panel was seen for laboratory #51.

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *Escherichia coli* strains reported by human health laboratories (n=24) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Ec EQASIA 25.1	Ec EQASIA 25.2	Ec EQASIA 25.3	Ec EQASIA 25.4	Ec EQASIA 25.5
#01	--	--	--	--	--
#04	--	--	--	TAZ, CIP, MEM	--
#05	--	--	--	--	--
#06	NAL	--	--	--	--
#07	--	FEP	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	FEP	--	--	--	--
#14	--	--	--	--	--
#17	--	--	--	--	--
#32	--	--	TAZ, TOB	--	--
#34	--	--	--	--	--
#35	--	--	--	--	--
#40	--	--	--	--	--
#48	--	COL, GEN	--	--	--

#49	--	--	--	--	--
#50	--	--	--	--	--
#51	FOX, LVX	LVX	LVX	LVX	--
#52	--	--	--	--	--
#60	--	--	--	--	--
#66	--	--	--	--	--
#71	--	--	--	TET	FOT
#75	FOT	--	--	FOT	--
#77	--	--	--	--	--

Ec, *Escherichia coli;* CIP, ciprofloxacin; COL, colistin; FEP, cefepime; FOT, cefotaxime; FOX, cefoxitin; GEN, gentamicin; LVX, levofloxacin; MEM, meropenem; NAL, nalidixc acid; TAZ, tazobactam; TET, tetracycline; TOB, tobramycin
¨ Laboratory submitted complete dataset for the strain

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *Klebsiella pneumoniae* strains reported by human health laboratories (n=18) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Kp EQASIA 25.1	Kp EQASIA 25.2	Kp EQASIA 25.3	Kp EQASIA 25.4	Kp EQASIA 25.6
#01	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	--	--	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	FOX	--	--
#14	--	--	--	--	--
#17	-	--	--	--	--
#32	--	--	--	--	--
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	COL	--	COL
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--
#52	--	--	--	--	--
#66	--	--	--	--	--

Kp, *Klebsiella pneumoniae*; COL, colistin; FOX, cefoxitin
¨ Laboratory submitted complete dataset for the strain

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *Pseudomonas aeruginosa* strains reported by human health laboratories (n=28) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Pa EQASIA 25.1	Pa EQASIA 25.3	Pa EQASIA 25.4	Pa EQASIA 25.5	Pa EQASIA 25.7
#01	--	--	--	--	--
#04	--	--	TOB	--	--
#05	--	--	--	--	--

#06	--	PT4	PT4	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#10	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	MEM	--	--
#13	--	--	--	--	--
#14	--	--	--	--	--
#17	--	--	--	--	--
#32	AMK, TAZ, PT4, TOB	--	--	TOB	TOB
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--
#62	--	--	--	--	--
#64	--	--	--	--	--
#66	--	--	--	--	--
#70	--	--	--	--	--
#72	--	--	--	--	--
#73	--	--	--	--	--
#75	--	--	--	--	--
#76	--	--	--	--	--

Pa, *Pseudomonas aeruginosa*; AMK, amikacin; MEM, meropenem; PT4, piperacillin/tazobactam; TAZ, ceftazidime; TOB, tobramycin
-- Laboratory submitted complete dataset for the strain

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *Staphylococcus aureus* strains reported by human health laboratories (n=31) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Sa EQASIA 25.2	Sa EQASIA 25.2	Sa EQASIA 25.4	Sa EQASIA 25.4	Sa EQASIA 25.5
#01	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	--	--	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#14	--	--	--	--	--
#17	--	--	--	--	--
#32	--	CIP	CIP	CIP	CIP
#34	--	--	--	--	--
#35	--	--	--	--	--

#40	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--
#60	--	--	--	--	--
#62	--	--	--	--	--
#64	CIP, VAN	--	CIP, VAN	TET	CIP, VAN
#66	--	--	--	--	--
#70	--	--	--	--	--
#71	--	--	--	--	--
#72	--	--	GEN	GEN	--
#73	--	--	--	--	--
#74	--	--	--	--	--
#75	--	--	--	--	--
#76	--	--	--	--	--
#77	--	--	--	--	--

Sa, *Staphylococcus aureus;* CIP, ciprofloxacin; GEN, gentamicin; TET, tetracycline; VAN, vancomycin
¨ Laboratory submitted complete dataset for the strain

3.2 *Escherichia coli* panel

Twenty-four laboratories from 14 different countries submitted results for the *E. coli* panel.

3.2.1 Bacterial identification

laboratories submitted results for bacterial identification (**Table 7**). The five target *E. coli* strains were identified accurately by 20 laboratories.

Table 7. Number of correct bacterial identification submitted by human health laboratories for each of the 7 test strains included in the *Escherichia coli* panel.

Strain	Bacterial ID	No. correct
Ec EQASIA 25.1	*Escherichia coli*	24/24
Ec EQASIA 25.2	*Escherichia coli*	24/24
Ec EQASIA 25.3	*Escherichia coli*	24/24
Ec EQASIA 25.4	*Escherichia coli*	24/24
Ec EQASIA 25.5	*Escherichia coli*	20/24
Ec EQASIA 25.6	Non- *Escherichia coli*	23/24
Ec EQASIA 25.7	Non- *Escherichia coli*	21/24

Ec, Escherichia coli

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

Based on the data, the 24 human health laboratories demonstrated exceptionally high accuracy in the AST for the *Escherichia coli* panel. The percentage of correct results, which agree with the expected interpretation (Resistant/Intermediate/Susceptible) were from 78.4% (Ec EQASIA 25.1) to 94.6% (Ec EQASIA 25.3 (**Table 8**).

Table 8. Total number of AST performed and percentage of correct results in agreement with expected interpretation results (R/I/S). Results are from 24 human health laboratories for the *Escherichia coli* panel.

Strain	AST in total	% Correct
Ec EQASIA 25.1	333	87.7
Ec EQASIA 25.2	332	78.4

Ec EQASIA 25.3	334	94.6
Ec EQASIA 25.4	333	84.4
Ec EQASIA 25.5	283	93.1

Ec, Escherichia coli

Antimicrobial-based analysis

Chloramphenicol had the highest deviation rate at 30.2%. Several other agents, including Amikacin (25.0%), Cefepime (23.3%), Colistin (20.7%), and Azithromycin (20.0%), also demonstrated high deviation rates above 20%. In contrast, the carbapenems Imipenem (10.8%) and Meropenem (11.3%), along with Tobramycin (11.3%) and Ceftazidime (11.8%), had the lowest error rates. Only tetracycline and trimethoprim revealed no deviation from the expected results (**Figure 4**).

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed in laboratory #05, #12, #17 (**Figure 5**). One laboratory, #051, was a pronounced outlier with a deviation rate of 41.3%. Several others, such as #074 (19.0%) and #071 (18.8%), also demonstrated substantially high error rates. In contrast, only a few laboratories, notably #012 (1.7%) and #05 (4.4%), met or fell below the 5% benchmark, demonstrating a high level of proficiency. Overall, 21 laboratories did not meet the expected performance range for the *E. coli* panel.

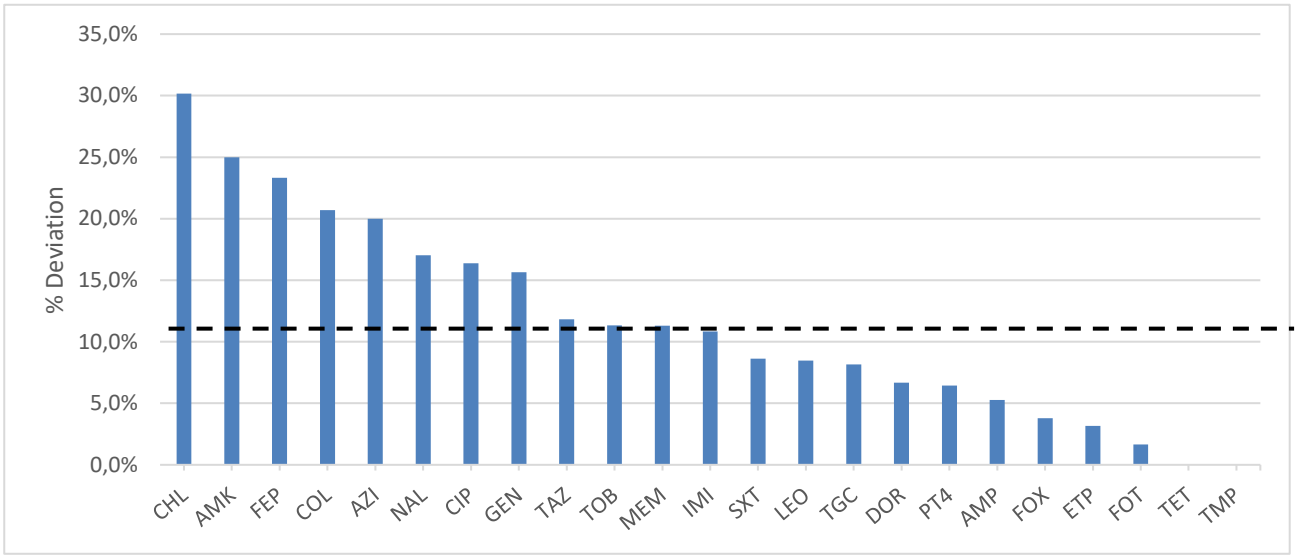

Figure 4. Percentage of deviation in the AST interpretation (R/I/S) from the expected results among *Escherichia coli* strains by human health laboratories (n=34) participating in the 10th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Dotted line represents the average of % deviations of all AST results for *Escherichia coli* panel (11.6%).

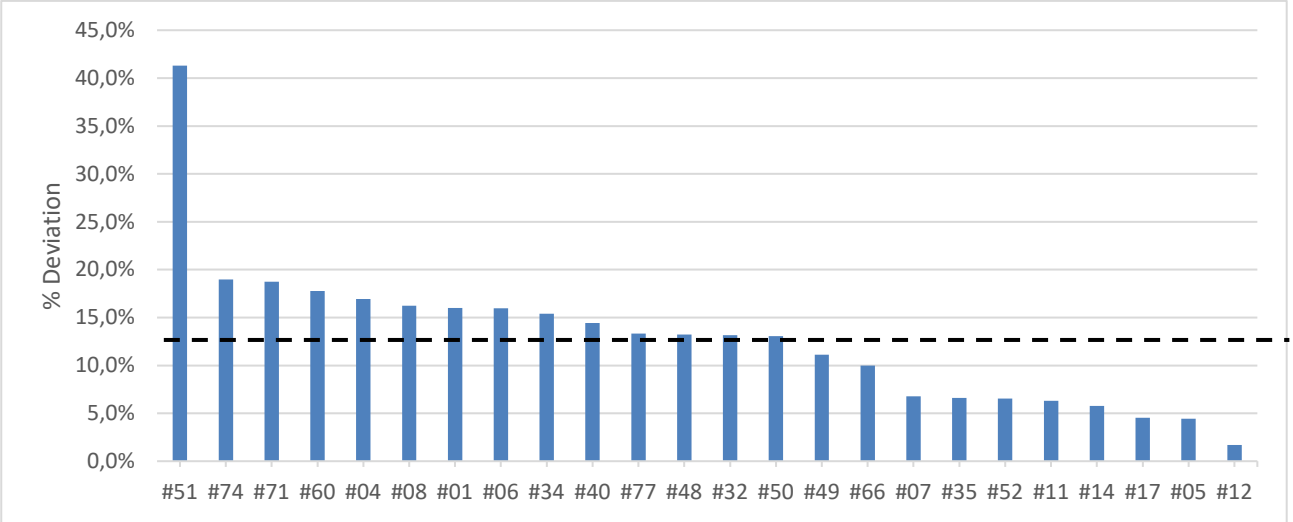

Figure 5. Percentage of deviation in the AST interpretation (R/I/S) from the expected results among *Escherichia coli* strains by human health laboratories (n=34) participating in the 10th EQA in the EQAsia project. Results are categorized according to the laboratory ID number. Dotted line represents the average of % deviations of all AST results for *Escherichia coli* panel (12.8%).

3.2.3 β-lactamase producing *E. coli*

In total, 23 laboratories tested for ESBL/AmpC/carbapenemase production in the *E. coli* panel (**Table 9**). Most of the laboratories (83.3%) correctly identified ESBL production in EC EQAsia 25.5. For the carbapenemase-producing strains (Ec EQASIA 25.2 and 25.3), laboratory performance was satisfactory, with correct identification rates of 71.4% and 80.6%, respectively. The ESBL + AmpC strain Ec EQASIA 25.1 was correctly identified by only 15% of laboratories, with most (50%) misidentifying it as a carbapenemase producer.

Table 9. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 23 human health laboratories.

Strain code		Ec EQASIA 25.1	Ec EQASIA 25.2	Ec EQASIA 25.3	Ec EQASIA 25.4	Ec EQASIA 25.5
Expected results		ESBL + AmpC	Carbapenemase	Carbapenemase	Susceptible*	ESBL
Obtained results (n/N)	ESBL	4/20 (20%)	2/21 (9.5%)	1/21 (4.8%)	--	**15/18 (83.3%)**
	Carbapenemase	10/20 (50%)	**15/21 (71.4%)**	**17/21 (80.6%%)**	--	1/18 (5.6%)
	ESBL + AmpC	**3/20 (15%)**	1/21 (4.8%)	**--**	--	--
	AmpC	--	--	--	1/17 (5.9%)	--
	Other	--	--	--	1/17 (5.9%)	1/18 (5.6%)
	Susceptible*	3/20 (15%)	3/21 (14.3%)	3/21 (14.3%)	**15/17 (88.2%)**	1/18 (5.6%)

Ec, *Escherichia coli* , *no ampC, ESBL, and carbapenemase

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

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

In total, 22 human health laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only five laboratories performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922. Inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test or broth microdilution (incl. automated methods) (**Table 10**). For testing *E. coli* NCTC 13846, MIC was determined by standard method by broth microdilution. The overall error rate, indicated by results outside the expected range, was notably high for several agents. The combination drug trimethoprim/sulfamethoxazole had the highest number of errors (8/22). In contrast, some antimicrobials like colistin, piperacillin/tazobactam, and nalidixic acid had no errors reported across all methodologies **(Table 10).**

Table 10. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *Escherichia coli* panel. A proportion of test results outside of the expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk diffusion	Gradient*	MIC	**Total**
Amikacin	1/16		2/5	3/21
Ampicillin	0/10		1/5	1/15
Cefepime	1/10		4/6	5/16
Cefotaxime	1/8	0/1	1/1	2/10
Cefoxitin	0/14		/1	0/15
Ceftazidime	2/16		3/5	5/21
Chloramphenicol	1/11			1/11
Ciprofloxacin	1/15	0/1	5/5	6/21
Colistin			0/5	0/5
Doripenem	0/3			0/3
Ertapenem	2/6	0/1	3/4	5/11
Gentamicin	2/16		2/6	4/22
Imipenem	1/8	0/1	1/5	2/14
Levofloxacin	1/6		3/4	4/10
Meropenem	2/15	0/2	3/3	5/20
Nalidixic acid	0/8	0/1	0/1	0/10
Piperacillin and tazobactam	0/11	0/1	0/4	0/16
Tetracycline	1/8		0/1	1/9
Tigecycline	0/3		4/4	4/7
Tobramycin	1/8		0/2	1/10
Trimethoprim	0/2			0/2
Trimethoprim and sulfamethoxazole	3/16		5/6	8/22

Disk diffusion – Inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution.
*Gradient test is not recommended for colistin testing

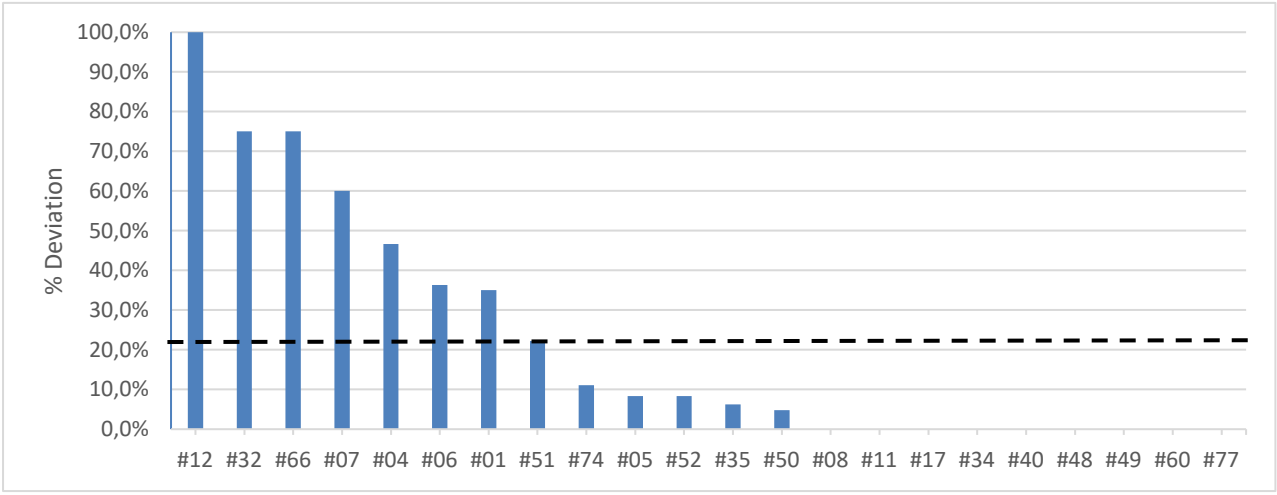

Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Escherichia coli* panel submitted by human health laboratories. Dotted line indicated the average of % deviations across all the laboratories (22.2%).

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 6).** A significant number of laboratories (09 out of 22 listed) achieved a perfect score with a 0% deviation rate. However, the results also reveal critical failures in a portion of the participating labs. Four laboratories, notably #012 with a 100% deviation rate, had error rates exceeding 60%. (**Figure 6**).

These overall deviations indicate poor performance of individual laboratories, highlighting the need for improvement particularly on disk diffusion, a widely recognized and routinely used method.

3.3 *Klebsiella pneumoniae* panel

Twenty human health laboratories submitted results for the *K. pneumoniae* panel.

3.3.1 Bacterial identification

Twenty participating laboratories submitted results for bacterial identification (**Table 11**). The five target *K. pneumoniae* strains were identified accurately by all 20 laboratories.

Table 11. Bacterial identification of each of the 7 test strains provided within the *Klebsiella pneumoniae panel*. Results are from 20 human health laboratories for the *Klebsiella pneumoniae* panel.

Strain	Bacterial ID	No. Correct
Kp EQASIA 25.1	*Klebsiella pneumoniae*	20/20
Kp EQASIA 25.2	*Klebsiella pneumoniae*	20/20
Kp EQASIA 25.3	*Klebsiella pneumoniae*	20/20
Kp EQASIA 25.4	*Klebsiella pneumoniae*	20/20
Kp EQASIA 25.5	Non- *K. pneumoniae*	20/20
Kp EQASIA 25.6	*Klebsiella pneumoniae*	20/20
Kp EQASIA 25.7	Non- *K. pneumoniae*	18/18

Kp, *Klebsiella pneumoniae*

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/I/S) ranged from 85.1% (strain Kp EQASIA 25.2) to 96.6% (strain Kp EQASIA 25.4) (**Table 12**).

Antimicrobial-based analysis

The results revealed significant challenges in AST for *K. pneumoniae*, with an overall average deviation rate of 12.3%. The most substantial errors were concentrated among specific drug classes. Carbapenems showed high deviation rates, with Doripenem (40.0%), Meropenem (28.0%), and Imipenem (22.4%) being particularly problematic. Tigecycline and Trimethoprim also showed high deviation rate of 30.0%. In contrast, many other agents, including Cefotaxime and Cefoxitin (0.0%), as well as Chloramphenicol, Gentamicin and Ertapenem (below 2.0%), were tested with near-perfect accuracy. (**Figure 7**).

Laboratory-based analysis

Only three laboratories (#05, #11, #66) had a deviation at or below 5% in their performance in terms of interpretation of the results (R/I/S) (**Figure 8**). One lab, #63, had the highest error rate at 20%. On average, the deviation was 10.4% (ranging from 3.2% to 20%).

Table 12. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 20 human health laboratories for the *Klebsiella pneumoniae* panel.

Strain	AST in total	% Correct
Kp EQAsia 25.1	290	95.5
Kp EQAsia 25.2	289	85.1
Kp EQAsia 25.3	287	85.4
Kp EQAsia 25.4	292	96.6
Kp EQASIA 25.6	289	95.5

Kp, *Klebsiella pneumoniae*

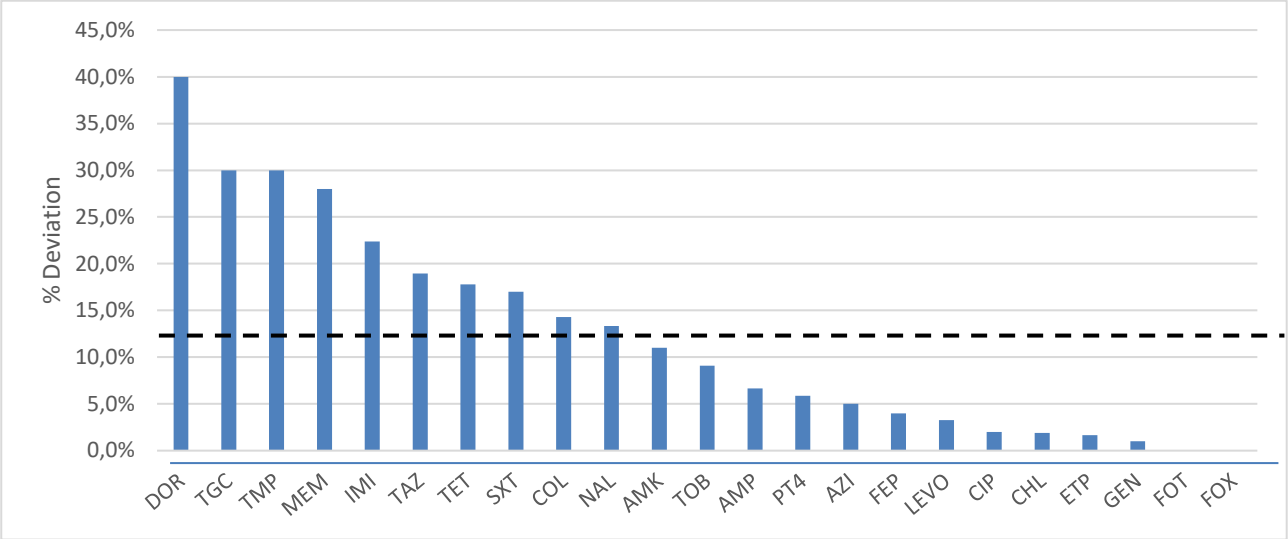

Figure 7. Percentage of deviation in the AST interpretation (R/I/S) from the expected results among *Klebsiella pneumoniae* strains by human health laboratories (n=20) participating in the 10th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Dotted line represents the average of % deviations of all AST results for *Klebsiella pneumoniae* panel (12.3%).

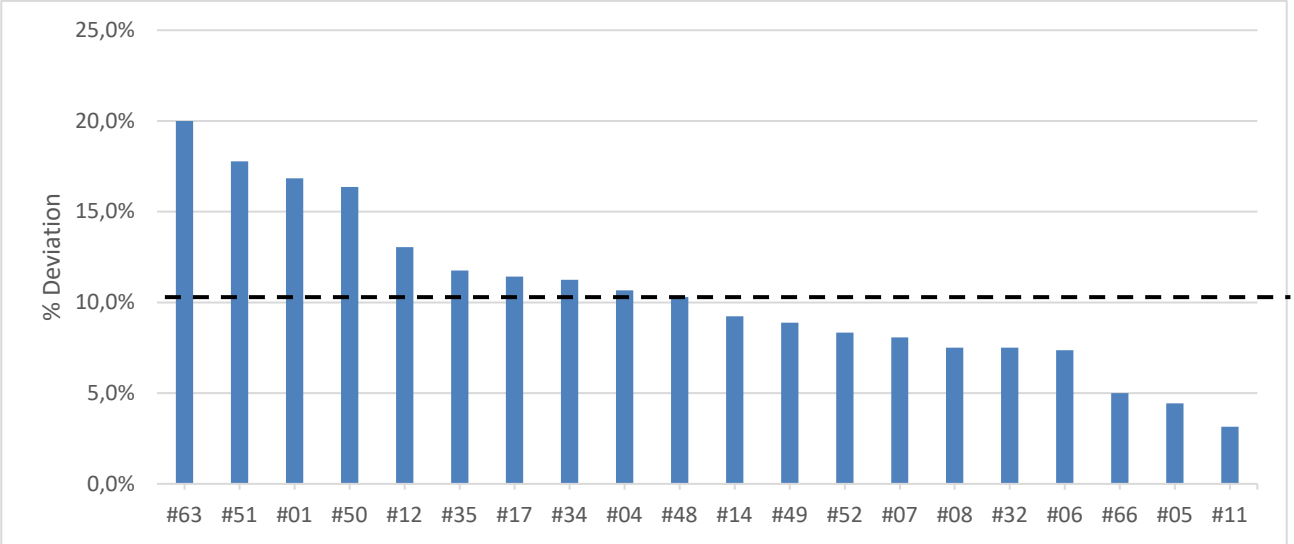

Figure 8. Percentage of deviation in the AST interpretation (R/I/S) from the expected results among *Klebsiella pneumoniae* strains by human health laboratories (n=20) participating in the 10th EQA in the EQAsia project. Results are categorized according to the laboratory ID number. Dotted line represents the average of % deviations of all AST results for *Klebsiella pneumoniae* panel (10.4%).

3.3.3 β-lactamase producing *K. pneumoniae*

19 out of the 20 participating laboratories tested for ESBL/AmpC/carbapenemase production (**Table 13**). Only two laboratories accurately identified the resistant phenotype of all five *K. pneumoniae* strains. Performance was strong for two carbapenemase-producing strains (Kp EQASIA 25.1 and 25.4), with correct identification rates of 89.5% and 84.2%, respectively **(Table 13)**. However, accuracy was poor for the other strains. For Kp EQASIA 25.2, nearly half of the labs (47.4%) correctly identified it as a carbapenemase producer, with many (42.1%) misidentifying it as an ESBL. Most critically, for the complex ESBL + AmpC strain (Kp EQASIA 25.6), labs struggled significantly, with only 27.8% reporting the correct result.

Table 13. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *Klebsiella pneumoniae* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 19 human health laboratories.

Strain code		**Kp EQASIA 25.1**	**Kp EQASIA 25.2**	**Kp EQASIA 25.3**	**Kp EQASIA 25.4**	**Kp EQASIA 25.6**
Expected results		Carbapenemase	Carbapenemase	Susceptible*	Carbapenemase	ESBL + AmpC
Obtained results (n/N)	ESBL	2/19 (10.5%)	8/19 (42.1%)	--	2/19 (10.5%)	5/18 (27.8%)
	Carbapenemase	**17/19 (89.5%)**	**9/19 (47.4%)**	--	**16/19 (84.2%)**	7/18 (38.9%)
	ESBL + AmpC	--	--	--	**--**	**5/18 (27.8%)**
	AmpC	**--**	**--**	1/16 (6.3%)	--	--
	Other	--	1/19 (5.3%)	1/16 (6.3%)	--	**--**
	Susceptible*	--	1/19 (5.3%)	**13/16 (81.3%)**	1/19 (5.3%)	1/18 (5.6%)

Kp, *Klebsiella pneumoniae* , *no ampC, ESBL, and carbapaenmase

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

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent at no cost to all participating laboratories as part of previous EQAsia EQA trials to be used as reference strains for *K. pneumoniae*. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials. All 20 laboratories that signed up for K. pneumoniae panel submitted results for the reference strain *E. coli* ATCC 25922 and performed colistin testing for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922. Inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test or broth microdilution (**Table 14**). For testing *E. coli* NCTC 13846, MIC was determined by standard method broth microdilution. The combination drug trimethoprim/sulfamethoxazole had the most errors (10/20), and the broth microdilution (MIC) method consistently showed more out-of-range results than disk diffusion for ciprofloxacin and ertapenem. In contrast, some antimicrobials like colistin, doripenem, and trimethoprim had no errors. (**Table 14**).

Table 14. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *Klebsiella pneumoniae* panel. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk diffusion	Gradient*	MIC	**Total**
Amikacin	2/14	--	2/5	**4/20**
Ampicillin	0/9	--	1/6	**1/15**
Cefepime	2/10	--	4/5	**6/15**
Cefotaxime	2/7	0/1	2/2	**4/10**
Cefoxitin	1/11	--	1/2	**2/13**
Ceftazidime	2/14	--	4/5	**6/19**
Chloramphenicol	2/10	--	1/1	**3/11**
Ciprofloxacin	1/12	0/1	6/6	**7/19**
Colistin	--	--	0/6	**0/6**
Doripenem	0/3	--	--	**0/3**
Ertapenem	1/4	1/1	5/6	**7/11**
Gentamicin	2/13	--	2/7	**4/20**
Imipenem	2/9	1/1	1/5	**4/15**
Levofloxacin	1/7	--	3/4	**4/11**
Meropenem	2/12	1/2	4/4	**7/18**
Nalidixic acid	0/7	0/1	1/2	**1/10**
Piperacillin and tazobactam	1/10	0/1	1/5	**2/16**
Tetracycline	1/7	0/1	1/1	**2/9**
Tigecycline	0/2	--	4/4	**4/6**
Tobramycin	2/8	--	0/2	**2/10**
Trimethoprim	0/2	--	--	**0/2**
Trimethoprim and sulfamethoxazole	4/13	--	6/7	**10/20**

Disk diffusion – Inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution.
*Gradient test is not recommended for colistin testing

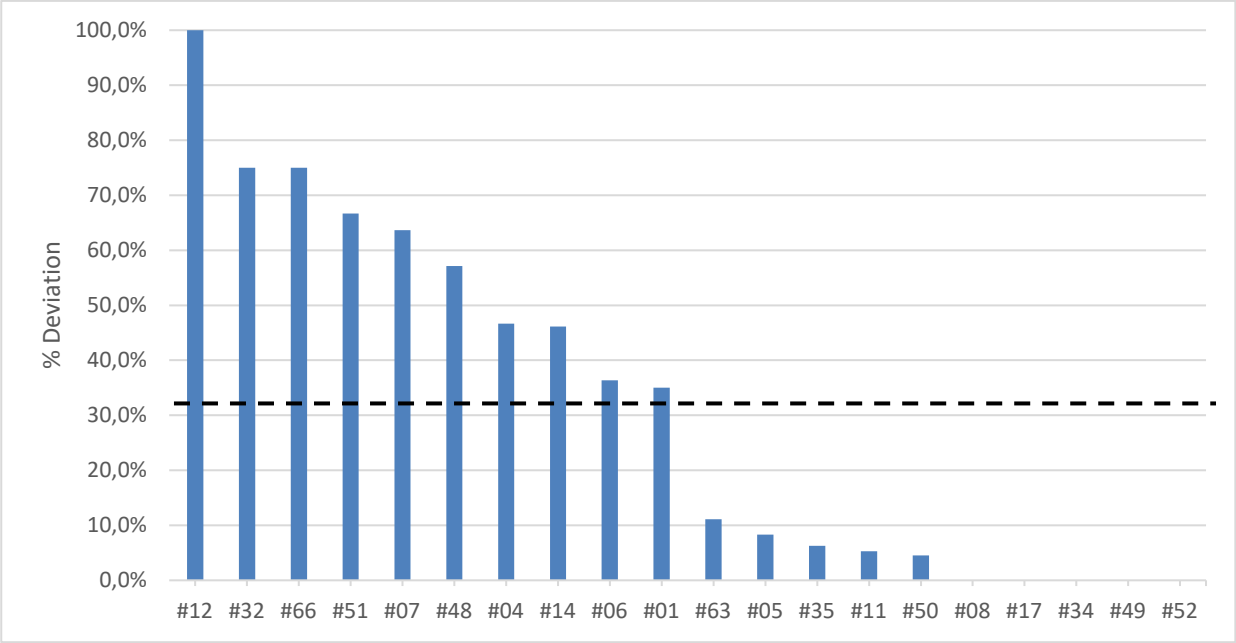

Figure 9. Percentage of deviation in the AST of *Escherichia coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Klebsiella pneumoniae* panel by human health laboratories. Dotted line represents the average of % deviations of all AST results for *K. pneumoniae* panel (31,9%).

Given the deviations, the laboratories' performance appeared independent of the methodology used for AST of the quality control strains (**Figure 9**). While five laboratories achieved a perfect score of 0% deviation, six laboratories had error rates above 50%, with one laboratory (#12) with 100% deviation. Laboratories #08, #17, #34, #49 and #52 presented no deviation (**Figure 9**).

These overall deviations indicate that while some labs maintain excellent quality control, many others have severe, fundamental errors in their testing procedures. The poor performance of individual laboratories highlights the need for improvement, particularly on disk diffusion, a widely recognized and routinely used method.

3.4 *Pseudomonas aeruginosa* panel

Twenty-eight laboratories submitted results for the *P. aeruginosa* panel.

3.4.1 Bacterial identification

All 28 participating laboratories submitted results for bacterial identification (**Table 15**). The five target *P. aeruginosa* strains were identified correctly by 27 laboratories.

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.

Table 15. Bacterial identification of each of the 7 test strains provided within the *Pseudomonas aeruginosa* panel. Number of correct results out of the total of human heath participating laboratories is presented.

Strain	Bacterial ID	No. correct
Pa EQASIA 25.1	*P. aeruginosa*	28/28
Pa EQASIA 25.2	Non-*P. aeruginosa*	28/28
Pa EQASIA 25.3	*P. aeruginosa*	28/28
Pa EQASIA 25.4	*P. aeruginosa*	28/28
Pa EQASIA 25.5	*P. aeruginosa*	27/28
Pa EQASIA 25.6	Non-*P. aeruginosa.*	24/24
Pa EQASIA 25.7	*P. aeruginosa*	28/28

Pa, Pseudomonas aeruginosa.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 62% (strain Pa EQASIA 25.4) to 96.5% (strain Pa EQASIA 25.1) (**Table 16**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected results higher than 10% were Ceftazidime (47.8%), Piperacillin and tazobactam (33.0%), Meropenem (28.3%), Colistin (23.3%), Aztreonam (20.0%), Ciprofloxacin (17.3%), Cefepime (16.7%), Doripenem (13.3%), Levofloxacin (12.9%), Imipenem (10.7%), (**Figure 10**). Ceftazidime was the most problematic agent with a 47.8% deviation rate. In contrast, labs demonstrated significantly better accuracy for the aminoglycosides like gentamicin (3.3%) and tobramycin (3.6%).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was not observed for any laboratory (**Figure 11**). In average, the deviation was 22.9% (ranging from 5.5 to 44.4%).

Table 16. Total number of AST performed and percentage of correct results in agreement with the expected interpretive results (R/I/S). Results are from 28 human health laboratories that submitted results for the *Pseudomonas aeruginosa* panel.

Strain	AST in total	% Correct
Pa EQASIA 25.1	228	96.5
Pa EQASIA 25.3	229	75.1
Pa EQASIA 25.4	229	62.0
Pa EQASIA 25.5	226	79.6
Pa EQASIA 25.7	231	77.9

Pa, *Pseudomonas aeruginosa.*

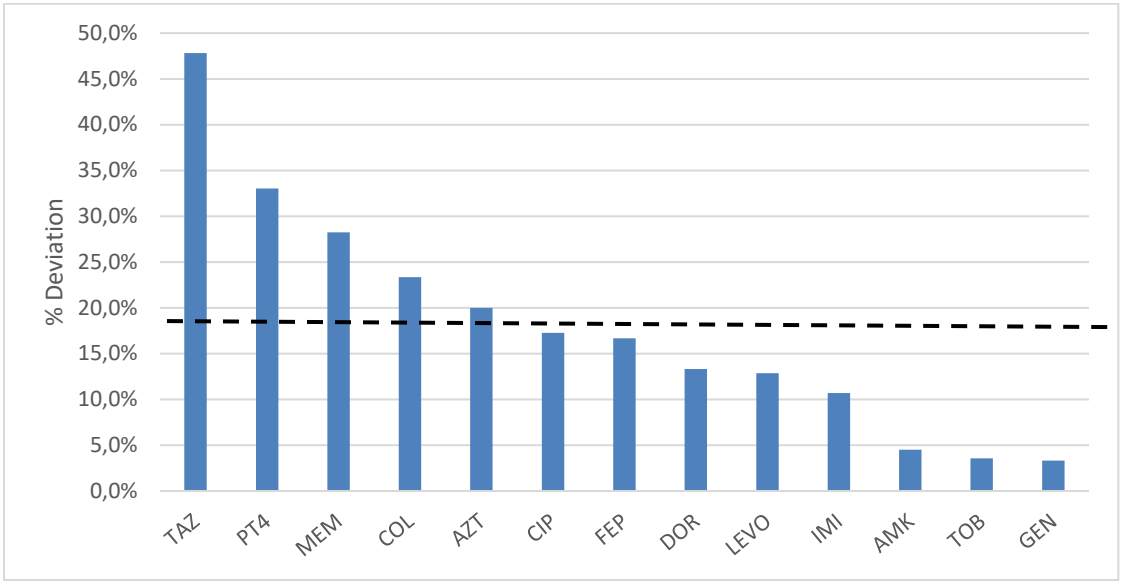

Figure 10. Percentage of deviation in the AST interpretation (R/I/S) among *Pseudomonas aeruginosa* strains by human health laboratories (n=28) participating in the 10th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Dotted line represents the average of % deviations of all AST results for *P. aeruginosa* panel (18.1%).

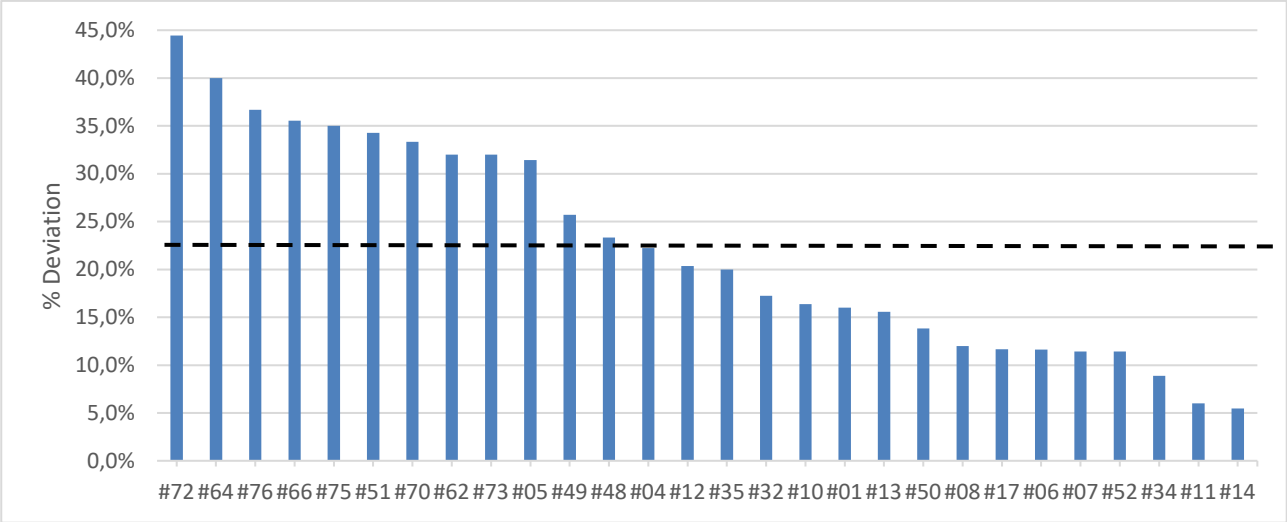

Figure 11. Percentage of deviation in the AST interpretation (R/I/S) among *Pseudomonas aeruginosa* strains by human health laboratories (n=28) participating in the 10th EQA in the EQAsia project. Results are categorized by laboratory ID numbers. Dotted line represents the average of % deviations of all AST results for *P. aeruginosa* panel (22.9%).

3.4.3 Quality control strains *P. aeruginosa* ATCC 27853

The quality control strains *P. aeruginosa* ATCC 27853 were sent at no cost to all participating laboratories within previous EQAsia EQA trials to be used as reference strains also for subsequent *P. aeruginosa* panels. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the 28 participating laboratories, 16 submitted results for the reference strain *P. aeruginosa* ATCC 27853. The laboratories used different methodologies for testing the reference strain *P. aeruginosa* ATCC 27853. Inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test or broth microdilution (**table 17**). The highest number of out-of-range results occurred with piperacillin/tazobactam and ceftazidime, each with 3 errors. There were no errors observed for ciprofloxacin, imipenem, and levofloxacin. The disk diffusion method was the source of all recorded errors, while gradient tests showed no deviations where they were applied

Ten laboratories (#05, #08, #10, #11, #13, #14, #17, #34, #48, and #50) presented no deviations. Two laboratories (#32 and #01) reported high deviations 83.3% and 60%, respectively (**Figure 12**).

Table 17. AST of the reference strains *Pseudomonas aeruginosa* ATCC 27853 in the *P. aeruginosa* panel. Proportion of test results outside of the expected range is presented by methodology used.

	Proportion outside of range			
Antimicrobial	Disk diffusion	Gradient*	MIC	**Total**
Amikacin	2/13	0/1	--	2/14
Aztreonam	1/7	--	0/1	1/8
Cefepime	2/9	--	--	2/9
Ceftazidime	3/12	0/1	--	3/13
Ciprofloxacin	0/12	0/1	--	0/13
Doripenem	1/3	--	--	1/3
Imipenem	0/10	0/1	--	0/11
Levofloxacin	0/8	--	--	0/8
Meropenem	2/12	0/1	--	2/13

Piperacillin and tazobactam	3/10	0/1	--	3/11
Tobramycin	1/7	0/1	--	1/7

Disk diffusion – Inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution.
*Gradient test is not recommended for colistin testing

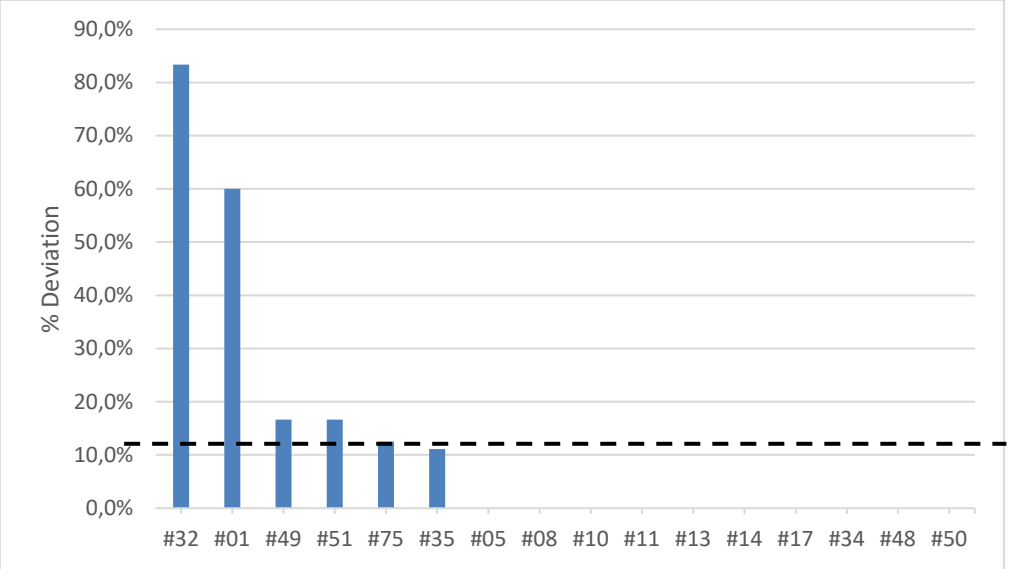

Figure 12. Percentage of deviation in the AST of *Pseudomonas aeruginosa* ATCC 27853 in the *P. aeruginosa panel* by the human health laboratories. Dotted line represents the average of % deviations of all AST results for *P. aeruginosa* panel (12.5%).

3.5 *Staphylococcus aureus* panel

31 laboratories from 14 countries submitted results for the *S. aureus* panel.

3.5.1 Bacterial identification

All 31 laboratories that selected the *S. aureus* panel submitted results for bacterial identification. Among these, 22 laboratories accurately identified the five *S. aureus* strains (**Table 18**).

Table 18. Bacterial identification of each of the 7 test strains provided within the *Staphylococcus aureus* panel. Number of correct results out of the total of human health participating laboratories is presented.

Strain	Bacterial ID	No. correct
Sa EQASIA 25.1	*Staphylococcus aureus*	31/31
Sa EQASIA 25.2	*Staphylococcus aureus*	31/31
Sa EQASIA 25.3	*Staphylococcus aureus*	31/31
Sa EQASIA 25.4	*Staphylococcus aureus*	31/31
Sa EQASIA 25.5	*Staphylococcus aureus*	31/31
Sa EQASIA 25.6	Non-*Staphylococcus aureus*	9/31
Sa EQASIA 25.7	Non-*Staphylococcus aureus*	27/31

Sa, *Staphylococcus aureus*

3.5.2 AST performance

The AST performance for the *S. aureus* 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/I/S) ranged from 90.3% (strain Sa EQASIA 25.5) to 93.5% (strain Sa EQASIA 25.1) for each strain (**Table 19**).

Table 19. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/S). Results are from 19 HH laboratories for the *S. aureus* panel.

Strain	AST in total	% Correct
Sa EQASIA 25.1	246	93.5
Sa EQASIA 25.2	226	91.2
Sa EQASIA 25.3	235	91.9
Sa EQASIA 25.4	216	91.2
Sa EQASIA 25.5	226	90.3

Sa, *Staphylococcus aureus*

Antimicrobial-based analysis

Three antimicrobials resulted in percentage of deviations higher than 10% were ciprofloxacin (45.5%) clindamycin (11.3%), and vancomycin (10.2%). whereas gentamicin, kanamycin, rifampin, quinupristin/dalfopristin, and trimethoprim revealed no deviation from the expected results (**Figure 13**).

Laboratory-based analysis

For the *S. aureus* panel, 15 out of the 31 HH laboratories presented a deviation below 5% (laboratories #04, #06, #07, #08, #11, #12, #14, #17, #34, #35, #49, #50, #52, #62, #70). The average deviation was 9.4% (ranging from 0% to 36.7%) (**Figure 14**).

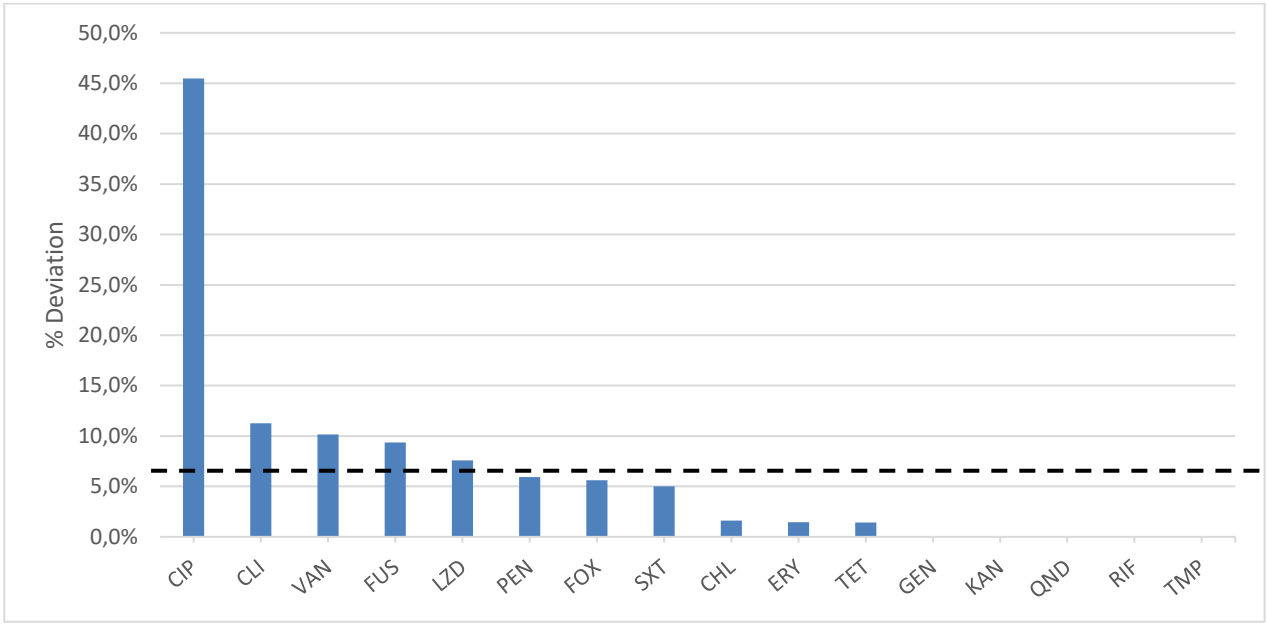

Figure 13. Percentage of deviation in the AST interpretation (R/I/S) among *Staphylococcus aureus* strains by human health laboratories (n=31) participating in the 10th EQA of the EQAsia project. Results are categorized by antimicrobial agent. Dotted line represents the average % deviations of all AST results for *S. aureus* panel (6.6%).

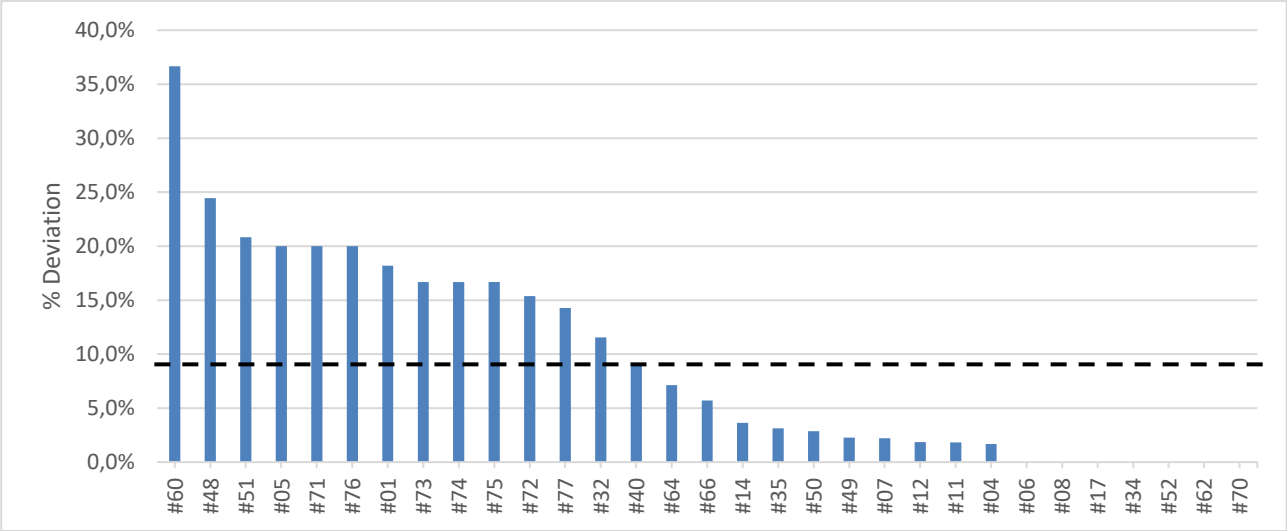

Figure 14. Percentage of deviation in the AST interpretation (R/I/S) among *Staphylococcus aureus* strains by human health laboratories (n=31) participating in the 10th EQA of the EQAsia project. Results are categorized by laboratory ID numbers. Results are categorized by antimicrobial agent. Dotted line represents the average % deviations of all AST results for *S. aureus* panel (9.4%).

3.5.3 Quality control strains *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC)

The quality control strains *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC) that were sent to participating laboratories as part of previous EQAsia EQA trials. Antimicrobial susceptibility test results for the quality control strains were assessed individually for each of the trials.

Among the 31 participating laboratories, 18 laboratories submitted results for the reference strain *S. aureus* ATCC 25923 (for disk diffusion) and/or *S. aureus* ATCC 29213 (for MIC). The different methodologies were applied for testing the quality control strain *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC).

The highest proportion of test results outside of the expected range were observed for fusidic acid (4 out of 7 tests) followed by rifampin (5 out of 9 tests) (**Table 20**).

Laboratories #05, #06, #08, #11, #17, #34, #35, #48, #60, #75 had no deviations. The other eight laboratories had deviations ranging from 11.1% to 27.3% (**Figure 15)**. In this panel, all the reported deviations were above the acceptance interval of 5%.

Table 20. AST of the reference strains *Staphylococcus aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC) in the *S. aureus* panel. The test results outside of the expected range are presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk diffusion	Gradient	MIC	**Total**
Cefoxitin	1/12	--	0/2	**1/14**
Chloramphenicol	1/8	--	0/1	**1/9**
Ciprofloxacin	0/10	0/1	0/6	**0/17**
Clindamycin	0/6	--	0/7	**0/13**
Erythromycin	0/10	0/1	0/6	**0/17**
Fusidic acid	1/4	--	3/3	**4/7**
Gentamicin	1/11	0/1	0/6	**1/18**
Kanamycin	0/1	--	--	**0/1**

Linezolid	1/5	0/1	0/6	**1/12**
Penicillin	1/6	0/1	0/3	**1/10**
Quinupristin and dalfopristin	--	--	0/2	**0/2**
Rifampin	1/3	0/1	4/5	**5/9**
Sulfamethoxazole	0/3	--	--	**0/3**
Tetracycline	1/10	0/1	0/6	**1/17**
Trimethoprim	0/2	--	0/1	**0/3**
Vancomycin	0/2	--	0/8	**0/10**

Disk diffusion – Inhibition zone diameter determination by disk diffusion; Gradient – MIC determination by gradient test; MIC – MIC determination by broth microdilution.

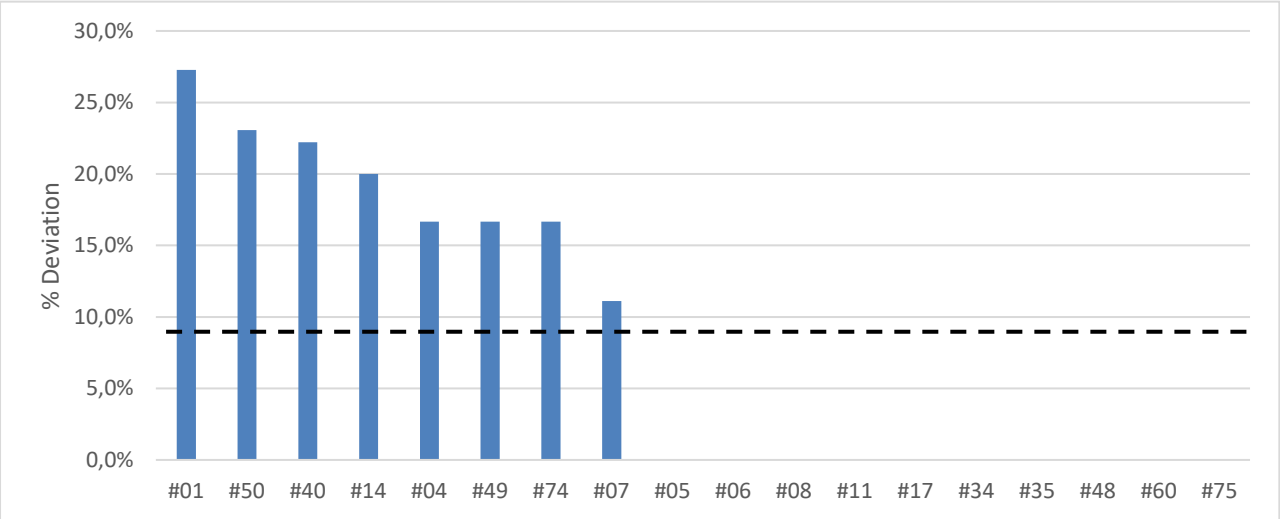

Figure 15. Percentage of deviation in the AST of *Staphylococcus aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC) in the *S. aureus* panel by the human health laboratories. Dotted line represents the average % deviations of all AST results for S. aureus panel (9.4%).

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 25 Animal Health laboratories participating in the 10th EQA of the EQAsia Programme, 24 laboratories submitted results for the *E. coli* panel, 10 for the *K. pneumoniae* panel, 9 for the *P. aeruginosa* panel, and 19 for the *S. aureus* panel **(Figure 16)**.

The applied AST methodologies across the four trials are summarized in **Figure 17**. Disk diffusion was the most widely used and was the sole method applied by most laboratories. Three laboratories (#20, #38, and #47) reported results using only broth microdilution (automated). Four laboratories (18, 28, #36, and #42) used a combination of disk diffusion and automated broth microdilution. Laboratory #53 applied disk diffusion alongside conventional broth microdilution, while Laboratory #19 was the only participant using agar dilution method.

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each strain/antimicrobial combination. Only the categorisation was evaluated, whereas the Inhibition Zone Diameters/MIC values were used as supplementary information. The majority of participants used the Clinical Laboratory Standards Institute (CLSI) guidelines when interpreting antimicrobial susceptibility testing (AST) results **(Figure 17).**

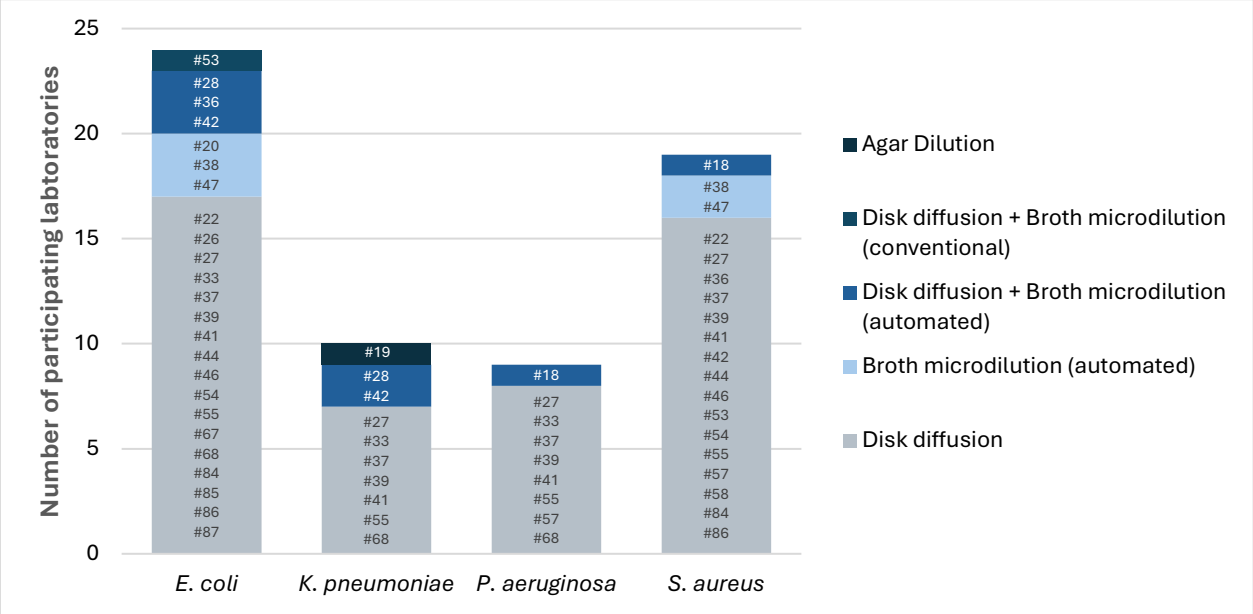

Figure 16. Methodologies applied by the AH laboratories participating for antimicrobial susceptibility testing in each of the panels.

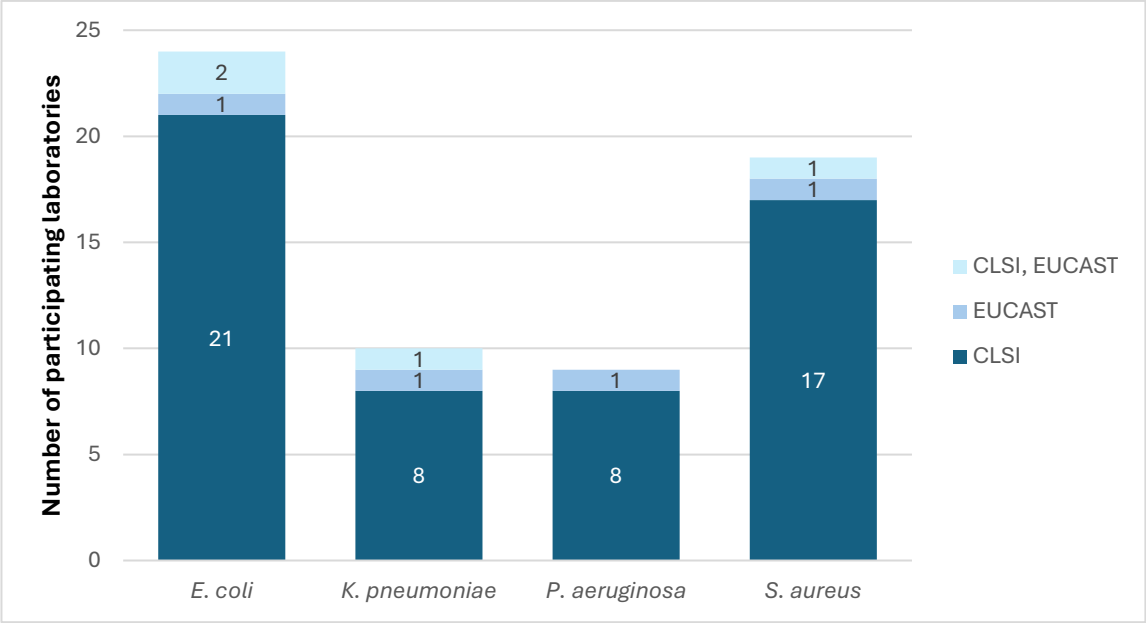

Figure 17. Use of international guidelines for interpretation of AST results by the participating laboratories.

The EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested antimicrobials **(Table 21).**

The *E. coli* panel generated the highest number of AST results (n = 1,371) from 24 participating laboratories, based on the recommended antimicrobials in CLSI **(Table 21)**. The most frequently tested antibiotics included ampicillin, chloramphenicol, gentamicin, tetracycline, and trimethoprim/sulfamethoxazole. For the *K. pneumoniae* panel, the antibiotics most commonly tested and reported were ciprofloxacin, gentamicin, nalidixic acid, and tetracycline. In the *P. aeruginosa* panel, laboratories most frequently tested amikacin, ceftazidime, ciprofloxacin, gentamicin, and meropenem. For the Gram-positive *S. aureus* panel, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, and tetracycline were the most frequently tested.

Table 21. Total of ASTs performed for each antimicrobial and in total for each of the panels by animal health laboratories

	E. coli		*K. pneumoniae*		*P. aeruginosa*		*S. aureus*	
Amikacin	57	4.2%	26	5.0%	35	11.0%	-	-
Ampicillin	105	7.7%	31	5.9%	-	-	-	-
Azithromycin	52	3.8%	11	2.1%	-	-	-	-
Aztreonam	-	-	-	-	25	7.8%	-	-
Cefepime	43	3.1%	16	3.1%	20	6.3%	-	-
Cefotaxime	93	6.8%	33	6.3%	-	-	-	-
Cefoxitin	48	3.5%	24	4.6%	-	-	53	7.8%
Ceftazidime	76	5.5%	30	5.7%	45	14.1%	-	-
Chloramphenicol	96	7.0%	36	6.9%	-	-	74	10.8%
Ciprofloxacin	91	6.6%	41	7.8%	40	12.5%	70	10.2%
Clindamycin	-	-	-	-	-	-	41	6.0%

Colistin	25	1.8%	10	1.9%	-	-	-	-
Doripenem	15	1.1%	1	0.2%	10	3.1%	-	-
Doxycycline	-	-	-	-	-	-	-	-
Ertapenem	21	1.5%	6	1.1%	-	-	-	-
Erythromycin	-	-	-	-	-	-	70	10.2%
Fusidic acid	-	-	-	-	-	-	19	2.8%
Gentamicin	106	7.7%	41	7.8%	35	11.0%	79	11.6%
Imipenem	58	4.2%	21	4.0%	30	9.4%	-	-
Kanamycin	-	-	-	-	-	-	23	3.4%
Levofloxacin	38	2.8%	15	2.9%	19	6.0%	-	-
Linezolid	-	-	-	-	-	-	37	5.4%
Meropenem	71	5.2%	26	5.0%	35	11.0%	-	-
Minocycline	-	-	-	-	-	-	-	-
Nalidixic acid	61	4.4%	36	6.9%	-	-	-	-
Penicillin	-	-	-	-	-	-	50	7.3%
Piperacillin/tazobactam	29	2.1%	6	1.1%	20	6.3%	-	-
Quinupristin/dalfopristin	-	-	-	-	-	-	13	1.9%
Rifampin	-	-	-	-	-	-	23	3.4%
Sulfamethoxazole	14	1.0%	15	2.9%	-	-	15	2.2%
Tetracycline	103	7.5%	46	8.8%	-	-	79	11.6%
Tigecycline	25	1.8%	1	0.2%	-	-	-	-
Tobramycin	10	0.7%	5	1.0%	5	1.6%	-	-
Trimethoprim	38	2.8%	15	2.9%	-	-	23	3.4%
Trimethoprim/sulfamethoxazole	96	7.0%	31	5.9%	-	-	-	-
Vancomycin	-	-	-	-	-	-	14	2.0%
Total	1371		523		319		683	

Scattering of missing data or incomplete AST results entries were observed in the four trials **(Tables 22, 23, 24, and 25).** Two of the 24 laboratories selecting *E. coli* did not submit complete results. Regarding the *K. pneumoniae* trial, three out of the ten participating laboratories revealed incomplete results of their own available antimicrobial agents **(Table 23)**. One out of nine laboratories that submitted AST data for *P. aeruginosa* had incomplete results of their own available antimicrobial agents **(Table 24).** Three out of 19 laboratories selecting *S. aureus* revealed incomplete results of their own available antimicrobial agents **(Table 25).** 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 22. Distribution of incomplete or missing data of antimicrobial agents among *E. coli* strains reported by AH laboratories (n=24) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Ec EQAsia 25.1	Ec EQAsia 25.2	Ec EQAsia 25.3	Ec EQAsia 25.4	Ec EQAsia 25.5
#26	ERT	ERT	-	ERT	ERT
#39	TGC	TET	TGC	TGC	TGC

Ec, *Escherichia. coli*

Table 23. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by AH laboratories (n=10) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Kp EQAsia 25.1	Kp EQAsia 25.2	Kp EQAsia 25.3	Kp EQAsia 25.4	Kp EQAsia 25.6
#33	-	FOT	FOT	FOT	FOT
#55	-	-	-	-	FOX
#68	TAZ	-	-	-	-

Kp, *Klebsiella pneumoniae*

Table 24. Distribution of incomplete or missing data of antimicrobial agents among *P. aeruginosa* strains reported by AH laboratories (n=9) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Pa EQAsia 25.1	Pa EQAsia 25.3	Pa EQAsia 25.4	Pa EQAsia 25.5	Pa EQAsia 25.7
#33	LEVO	-	-	-	-

Pa, *Pseudomonas aeruginosa*

Table 25. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by AH laboratories (n=19) participating in the 10th EQA of the EQAsia project.

Lab ID No.	Sa EQAsia 25.1	Sa EQAsia 25.2	Sa EQAsia 25.3	Sa EQAsia 25.4	Sa EQAsia 25.5
#22	-	-	-	-	FOX
#57	-	CIP	-	-	-
#86	-	-	-	-	GEN

Sa, *Staphylococcus aureus*

4.2 *Escherichia coli* panel

Twenty-four laboratories from ten countries uploaded results for the *E. coli* trial.

4.2.1 Bacterial identification

24 laboratories submitted results for bacterial identification **(Table 26)**. The five target *E. coli* strains were identified correctly by 20 laboratories. Two non-*E. coli* strains (strain Ec EQAsia 25.6 and Ec EQAsia 25.7) were misidentified as *E. coli* by laboratory #41.

4.2.2 AST performance

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

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

Strain	Bacterial ID	No. correct
Ec EQAsia 25.1	*Escherichia coli*	22/23
Ec EQAsia 25.2	*Escherichia coli*	23/24
Ec EQAsia 25.3	*Escherichia coli*	24/24
Ec EQAsia 25.4	*Escherichia coli*	24/24
Ec EQAsia 25.5	*Escherichia coli*	21/23
Ec EQAsia 25.6	Non-*Escherichia coli*	20/24
Ec EQAsia 25.7	Non-*Escherichia coli*	19/24

Ec, *Escherichia coli*

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 90.7% (strain Ec EQASIA 25.1) to 95.8%

(strain Ec EQASIA 25.2) 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 24 AH laboratories for the *Escherichia coli* panel.

Strain	AST in total	% Correct
Ec EQAsia 25.1	1100	90.7
Ec EQAsia 25.2	1096	95.8
Ec EQAsia 25.3	1148	94.3
Ec EQAsia 25.4	1144	94.1
Ec EQAsia 25.5	996	95.0

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were doripenem (21.7%), followed by azithromycin (18.3%), and colistin (16.0%). In reverse, ampicillin and cefoxitin revealed no deviation from the expected results **(Figure 18).**

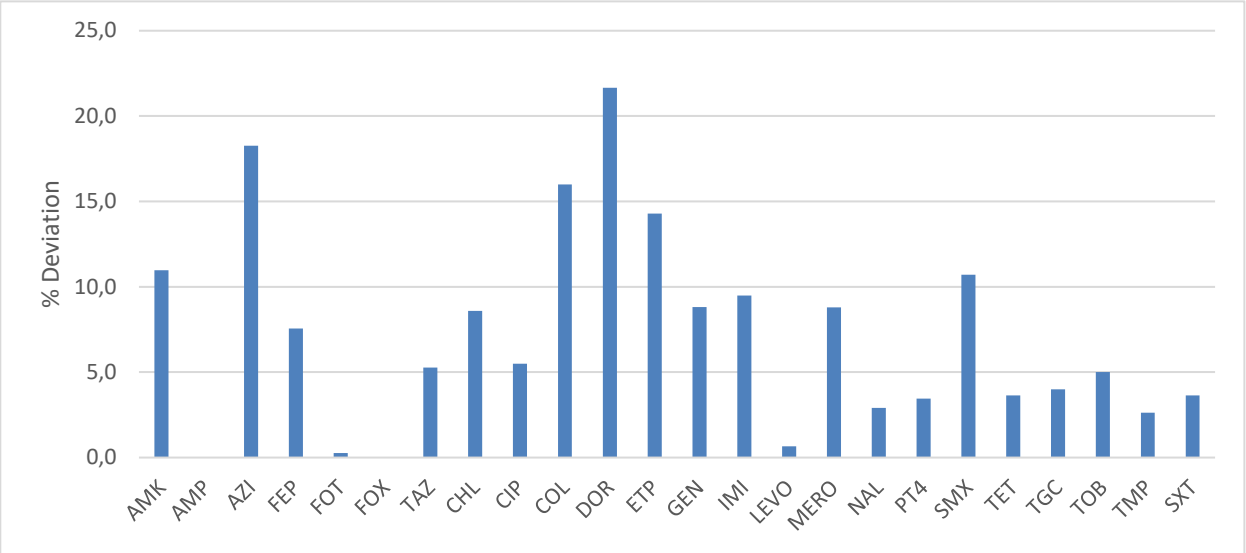

Figure 18. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=24) participating in the 10th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

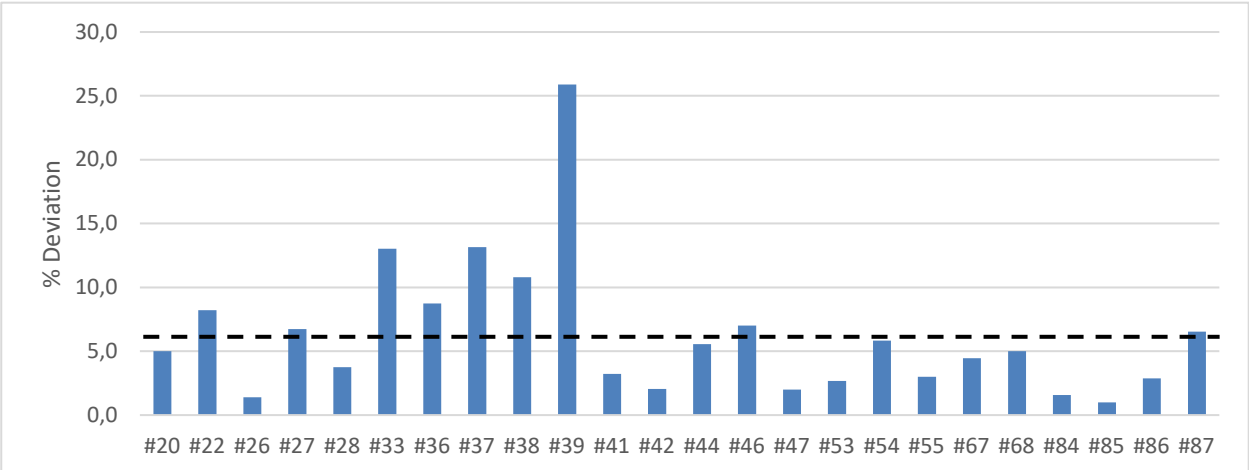

Figure 19. Percentage of deviation in the AST interpretation (R/S) among *Escherichia coli* strains by AH laboratories (n=24) participating in the 10th EQA of the EQAsia project. Results are categorized by laboratory ID numbers. Dotted line represents the average % deviations of all AST results for *E coli* panel (6.2%).

Laboratory-based analysis

A deviation equal or below to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for 13 out of the 24 participants **(Figure 19).** In average, the deviation was 6.2% (ranging from 1.0 to 25.9%). As the acceptance level was set to 5% deviation, 11 laboratories did not perform within the expected range for the trial.

4.2.3 β-lactamase-producing *E. coli*

Nine out of the twenty-four participating laboratories uploaded results for this component of the *E. coli* trial (laboratories #22, #33 #37, #39, #42, #44, #46, #47 and #67). Results were reported for Ec EQASIA 25.3 and Ec EQASIA 25.5 by nine laboratories, for Ec EQASIA 25.1 and Ec EQASIA 25.2 by eight laboratories, and for Ec EQASIA 25.4 by six laboratories. Discrepancies from the expected results are summarized in **Table 28**. None of laboratories correctly identified all phenotypes among the five *E. coli* strains.

Table 28. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *E. coli* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 9 AH laboratories.

Strain code		Ec EQASIA 25.1	Ec EQASIA 25.2	Ec EQASIA 25.3	Ec EQASIA 25.4	Ec EQASIA 25.5
Expected results		ESBLs +AmpC	Carbapenemase	Carbapenemase	Susceptible	ESBLs
Obtained results (n/N)	ESBLs	2/8 (25.0%)	--	1/9 (11.1%)	--	**7/9 (77.8%)**
	ESBLs + AmpC	**3/8 (37.5%)**	1/8 (12.5%)	**--**	--	--
	Carbapenemase	1/8 (12.5%)	**3/8 (37.5%)**	**5/9 (55.6%)**	--	--
	AmpC	2/8 (25.0%)	**--**	--	**--**	--
	Other	--	2/8 (25.0%)	2/9 (22.2%)	1/6 (16.7%)	--
	Susceptible*	--	2/8 (25.0%)	1/9 (11.1%)	**5/6 (83.3%)**	2/9 (22.2%)

Ec, *Escherichia coli*. *no AmpC, ESBL and carbapenemase.

4.2.4 Quality control strains *E. coli* ATCC 25922

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

Among the 24 participating laboratories, 23 laboratories submitted results for the reference strain *E. coli* ATCC 25922 and none of laboratories reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli ATCC* 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated) **(Table 29).** The highest proportion of test results outside of the expected range was observed for cefepime (3 out of 8) and trimethoprim (3 out of 8) **(Table 29).**

Regarding the laboratories' **performance (Figure 20)**, 23 laboratories presented deviations that ranged from 0.0% to 100.0%. Overall, the average deviation for this part of the panel was 20.0%. Laboratories #20, #38 and #47 applied broth microdilution (automated), while the other 20 laboratories used disk diffusion method. These overall deviations imply poor performance of individual laboratories, which needs to be strengthened particularly on disk diffusion, a well-known and routinely used method.

Table 29. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *E. coli* trial. A proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMK	1/9	0/2	**1/11**
AMP	7/19	0/2	**7/21**
FEP	3/7	0/1	**3/8**
FOT	5/18	0/1	**5/19**
FOX	1/10	-	**1/10**
TAZ	2/14	1/2	**3/16**
CHL	4/18	0/1	**4/19**
CIP	1/17	1/2	**3/19**
COL	-	-	**-**
DOR	0/3	-	**0/3**
ETP	0/3	-	**0/3**
GEN	4/19	0/3	**4/22**
IMI	3/9	0/1	**3/10**
LEVO	1/6	1/1	**2/7**
MERO	2/12	1/2	**3/14**
NAL	3/11	0/1	**3/12**
PT4	0/4	0/1	**0/5**
SMX	1/3	-	**1/3**
TET	3/19	0/2	**3/21**
TGC	0/3	1/2	**1/5**
TOB	0/1	-	**0/1**
TMP	3/7	0/1	**3/8**
SXT	2/17	2/2	**4/19**

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

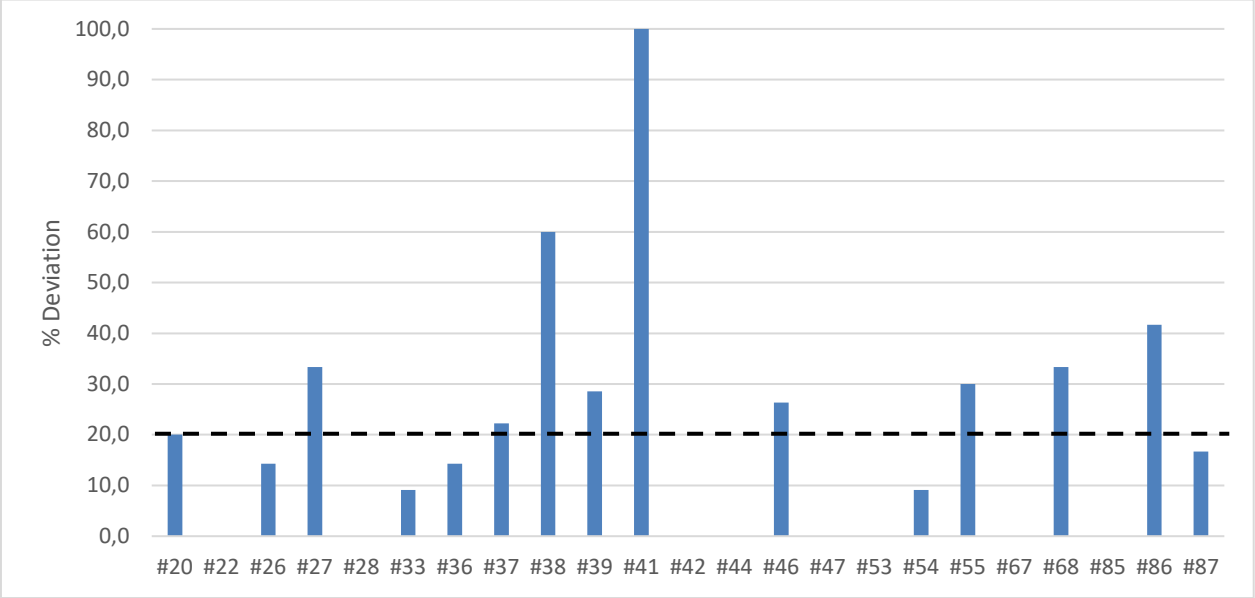

Figure 20. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *E. coli* trial by the AH laboratories Dotted line indicated the average of % deviations across all the laboratories (20%).

4.3 *Klebsiella pneumoniae* panel

A total of ten laboratories from six countries uploaded results for the *K. pneumoniae* trial.

4.3.1 Bacterial identification

All ten participating laboratories submitted results for bacterial identification **(Table 29).** All ten laboratories correctly identified 5 target strains provided. Two non- *K. pneumoniae* strains (strain Kp EQAsia 25.5 and Kp EQAsia 25.7) were misidentified as *E. coli* by laboratory #41.

Table 29. Bacterial identification of each of the seven test strains provided related to the *K. pneumoniae* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Kp EQAsia 25.1	*Klebsiella pneumoniae*	10/10
Kp EQAsia 25.2	*Klebsiella pneumoniae*	10/10
Kp EQAsia 25.3	*Klebsiella pneumoniae*	10/10
Kp EQAsia 25.4	*Klebsiella pneumoniae*	10/10
Kp EQAsia 25.5	Non-*Klebsiella pneumoniae*	9/10
Kp EQAsia 25.6	*Klebsiella pneumoniae*	10/10
Kp EQAsia 25.7	Non-*Klebsiella pneumoniae*	9/10

Kp, *Klebsiella pneumoniae*

4.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 90.1% (strain Kp EQASIA 25.3) to 99.0% (strain Kp EQASIA 25.4) for each strain **(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 10 AH laboratories for the *K. pneumoniae* trial.

Strain	AST in total	% Correct
Kp EQAsia 25.1	480	97.9
Kp EQAsia 25.2	404	93.6
Kp EQAsia 25.3	404	90.1
Kp EQAsia 25.4	404	99.0
Kp EQAsia 25.6	400	92.8

Kp, *Klebsiella pneumoniae*

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were ceftazidime (16.7%), colistin (12.5%), meropenem (11.5%), tetracycline (13.0%) and tigecycline (75.0%), whereas ampicillin, cefepime, chloramphenicol, doripenem, ertapenem, piperacillin/tazobactam and tobramycin revealed no deviation from the expected results **(Figure 21).** Tigecycline was tested by only one laboratory. Despite the low number of incorrect results, it caused the high deviation observed.

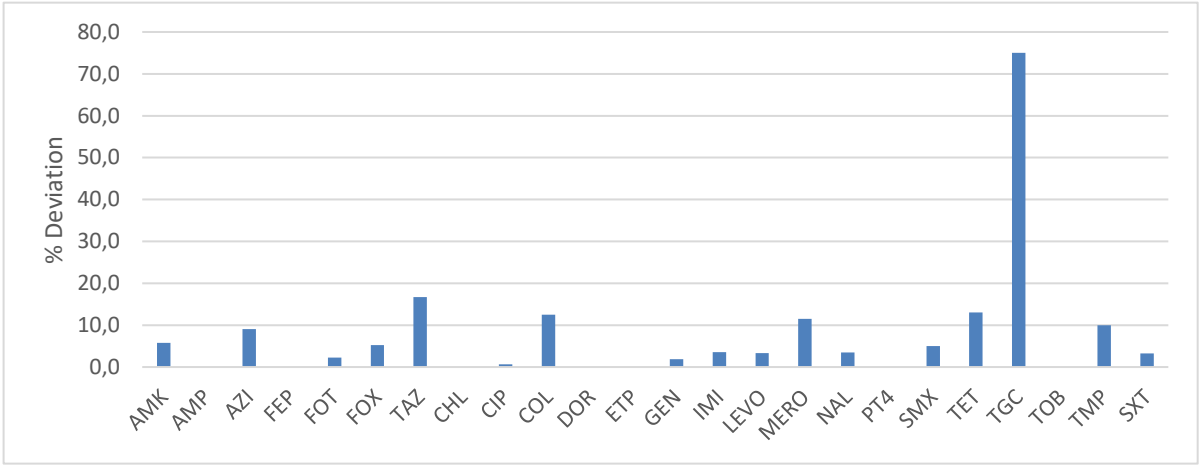

Figure 21. Percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by AH laboratories (n=10) participating in the 10th EQA of the EQAsia project. Results are categorized according toantimicrobial agent.

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 six participants (#19, #27, #28, #33, #41 and #42) **(Figure 22)**. On average, the deviation was 5.6% (ranging from 1.3 to 15.8%). For laboratories #55 and #68, the deviations were only a bit above the acceptance level.

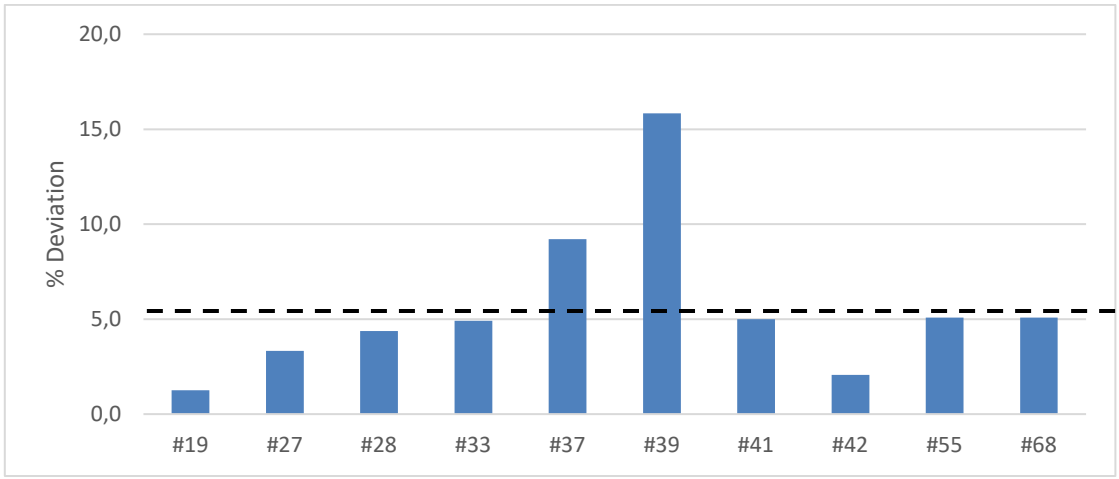

Figure 22. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=10) participating in the 10th EQA of the EQAsia project. Results are categorized by laboratory ID numbers. Dotted line indicated the average of % deviations across all the laboratories (5.6%).

4.3.3 β-lactamase-producing K. pneumoniae

Three of the ten participating laboratories uploaded results for this component of the *K. pneumoniae* trial. Discrepancies from the expected results are summarized in **Table 31**.

Firstly, laboratories identified the strains that produced ESBL/AmpC/carbapenemase and then reported the specific phenotype. Strain Kp EQASIA 25.6 was expected to be an ESBL+AmpC producer. However, laboratories #37 and #42 misclassified the strain as a carbapenem producer, while laboratory #33 misclassified it as an AmpC producer. Strains Kp EQAsia 25.1 and Kp EQAsia 25.4 were correctly identified by three laboratories.

Table 31. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *K. pneumoniae* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 3 AH laboratories.

Strain code		Kp EQASIA 25.1	Kp EQASIA 25.2	Kp EQASIA 25.3	Kp EQASIA 25.4	Kp EQASIA 25.6
Expected results		Carbapenemase	Carbapenemase	Susceptible	Carbapenemase	ESBLs+AmpC
Obtained results (n/N)	ESBLs	--	1/3 (33.3%)	--	--	--
	ESBLs + AmpC	--	--	--	--	**--**
	Carbapenemase	**3/3 (100.0%)**	**2/3 (66.7%)**	--	**3/3 (100.0%)**	2/3 (66.7%)
	AmpC	--	**--**	--	--	1/3 (33.3%)
	Other	--	--	--	--	--
	Susceptible*	--	--	**2/2 (100%)**	--	--

Kp, K. pneumoniae; *no AmpC, ESBL and carbapenemase.

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

The quality control strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were sent free of charge (in previous trials) to all

participating laboratories to be used as reference strains for the *K. pneumoniae* trial.

Among the ten participating laboratories, nine submitted results for the reference strain *E. coli* ATCC 25922 and none of laboratories reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli ATCC* 25922. Inhibition zone diameters were determined using the disk diffusion method, while MIC was determined using agar dilution **(Table 32).** The highest proportion of test results outside of the expected range was observed for imipenem (2 out of 3), trimethoprim (2 out of 3) and ampicillin (4 out of 7) **(Table 32).**

Regarding the laboratories' performance, nine laboratories presented deviations that ranged from 0.0% to 100.0% **(Figure 23)**. Overall, the average deviation for this part of the panel was 31.5%. Laboratory #19 and presented no deviation. Contrarily, laboratory #41 presented a 100.0% deviation, corresponding to incorrect results for 14 out of 14 tested antimicrobials.

Table 32. AST of the reference strains *E. coli* ATCC 25922 in the *K. pneumoniae* trial. A proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMK	1/4		1/4
AMP	4/7		4/7
FEP	1/2		1/2
FOT	2/6		2/6
FOX	1/5		1/5
TAZ	1/6		1/6
CHL	2/7		2/7
CIP	1/7	0/1	1/8
GEN	3/7	0/1	3/8
IMI	2/3		2/3
LEVO	0/2	0/1	0/3
MERO	1/4		1/4
NAL	1/6	0/1	1/7
SMX	1/2	0/1	1/3
TET	1/8	0/1	1/9
TMP	2/2	0/1	2/3
SXT	2/6		2/6

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

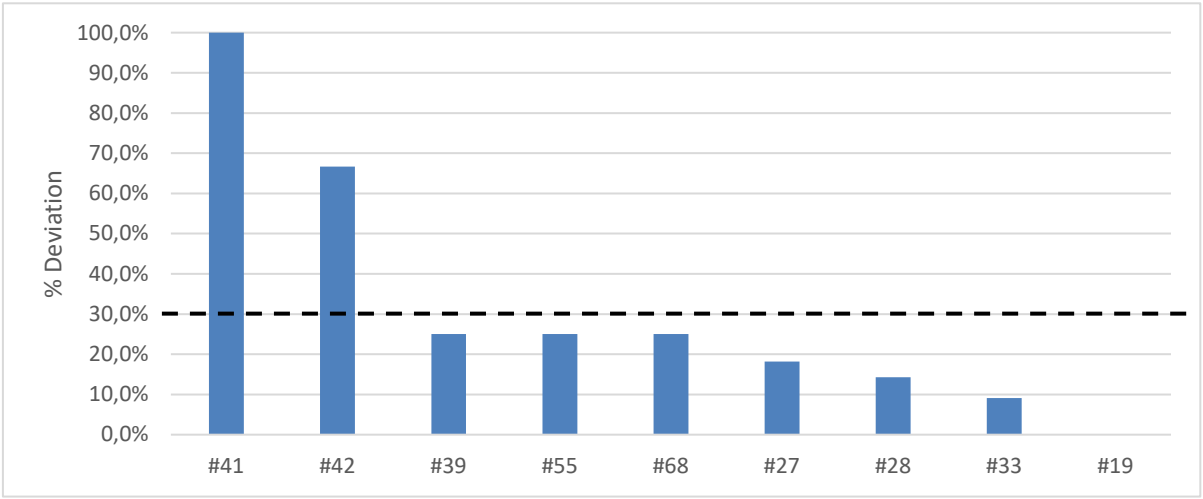

Figure 23. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *K. pneumoniae* trial by the AH laboratories. Dotted line represents the average of % deviations for all the laboratories. Dotted line indicated the average of % deviations across all the laboratories (31.5%).

4.4 *Pseudomonas aeruginosa* panel

Nine laboratories from six countries uploaded results for the *P. aeruginosa* trial.

4.4.1 Bacterial identification

All nine participating laboratories submitted results for bacterial identification **(Table 33).** Eight out of nine laboratories correctly identified all seven test strains provided. Two non- *P. aeruginosa* strains (strain Pa EQAsia 25.2 and Pa EQAsia 25.6) were misidentified as *P. aeruginosa* by laboratory #41.

Table 33. Bacterial identification of each of the seven test strains provided related to the *P. aeruginosa* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Pa EQAsia 25.1	*Pseudomonas aeruginosa*	9/9
Pa EQAsia 25.2	Non-*Pseudomonas aeruginosa*	8/9
Pa EQAsia 25.3	*Pseudomonas aeruginosa*	9/9
Pa EQAsia 25.4	*Pseudomonas aeruginosa*	9/9
Pa EQAsia 25.5	*Pseudomonas aeruginosa*	9/9
Pa EQAsia 25.6	Non-*Pseudomonas aeruginosa*	8/9
Pa EQAsia 25.7	*Pseudomonas aeruginosa*	9/9

Pa, *Pseudomonas aeruginosa*

4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 78.5% (strain Pa EQASIA 25.5) to 91.8% (strain Pa EQASIA 25.3) for each strain **(Table 34).**

Table 34. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 9 AH laboratories for the *P. aeruginosa* trial.

Strain	AST in total	% Correct
Pa EQAsia 25.1	252	90.1
Pa EQAsia 25.3	256	91.8
Pa EQAsia 25.4	256	87.5
Pa EQAsia 25.5	256	78.5
Pa EQAsia 25.7	256	89.1

Pa, *Pseudomonas aeruginosa*

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were cefepime (23.8%), ceftazidime (31.7%), doripenem (22.5%), imipenem (12.5%), meropenem (12.9%), piperacillin/tazobactam (12.5%) and tobramycin (25.0%) **(Figure 24).** Tobramycin was tested by only one laboratory. Despite the low number of incorrect results, it caused the high deviation observed.

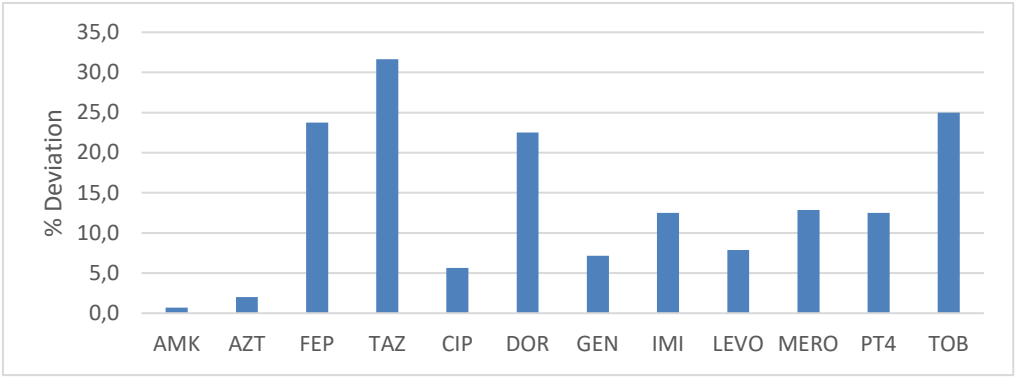

Figure 24. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=9) participating in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed only 2 participants (#18 and #68) **(Figure 25)**. On average, the deviation was 14.1% (ranging from 4.5 to 38.3%). Laboratory #39 presented the highest deviation observed for this panel.

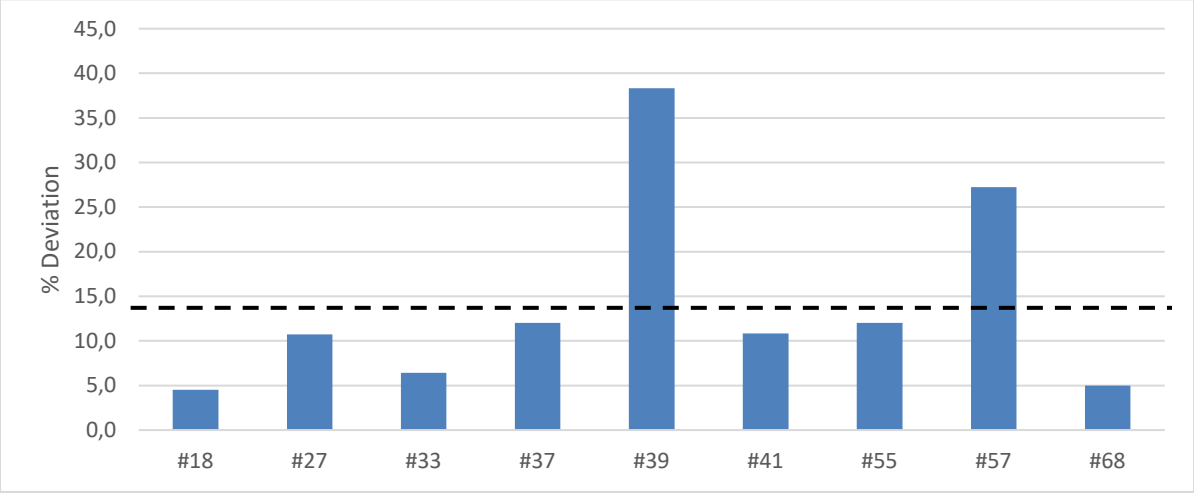

Figure 25. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=9) participating in the 10th EQA of the EQAsia project. Results are categorized by laboratory ID number. Dotted line indicated the average of % deviations across all the laboratories (14.1%).

4.4.3 Quality control strains *Pseudomonas aeruginosa* ATCC 27853

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

Among the nine participating laboratories, two submitted AST results for the reference strain *P. aeruginosa* ATCC 27853 using the disk diffusion method **(Table 35).** Regarding the laboratories' performance **(Figure 26).** Laboratory #39 presented no deviation. Inversely, laboratory #44 presented a 100.0% deviation, corresponding to incorrect results for 5 out of 5 tested antimicrobials.

Table 35. AST of the reference strain *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diffusion	Total
AZT	1/1	1/1
TAZ	1/2	1/2
CIP	1/2	1/2
LEVO	1/1	1/1
MERO	1/1	1/1

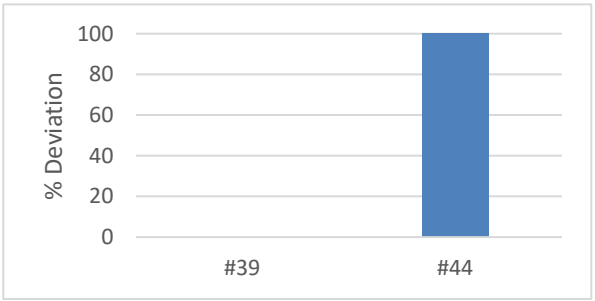

Figure 26. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* trial by the AH laboratories.

4.5 *Staphylococcus aureus* trial

19 laboratories from ten countries uploaded results for the *S. aureus* trial.

4.5.1 Bacterial identification

All 19 participating laboratories submitted results for bacterial identification **(Table 35)**. Eight laboratories correctly identified the five *S. aureus* strains.

Table 35. Bacterial identification of each of the seven test strains provided related to the *S. aureus* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	*Bacterial ID*	No. correct
Sa EQAsia 25.1	*Staphylococcus aureus*	19/19
Sa EQAsia 25.2	*Staphylococcus aureus*	12/19
Sa EQAsia 25.3	*Staphylococcus aureus*	18/19
Sa EQAsia 25.4	*Staphylococcus aureus*	17/19
Sa EQAsia 25.5	*Staphylococcus aureus*	18/19
Sa EQAsia 25.6	Non-*Staphylococcus aureus*	6/19
Sa EQAsia 25.7	Non-*Staphylococcus aureus*	17/19

Sa, *Staphylococcus aureus*

4.5.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 91.4% (strain Sa EQASIA 25.5) to 98.8% (strain Sa EQASIA 25.3) for each strain **(Table 36).**

Table 36. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 19 AH laboratories for the *S. aureus* trial.

Strain	AST in total	% Correct
Sa EQAsia 25.1	612	97.5
Sa EQAsia 25.2	436	97.7
Sa EQAsia 25.3	572	98.8
Sa EQAsia 25.4	540	97.2
Sa EQAsia 25.5	572	91.4

Sa, *Staphylococcus aureus*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result was quinupristin/dalfopristin (28.8%), whereas rifampin, sulfamethoxazole and vancomycin revealed no deviation from the expected results **(Figure 27).**

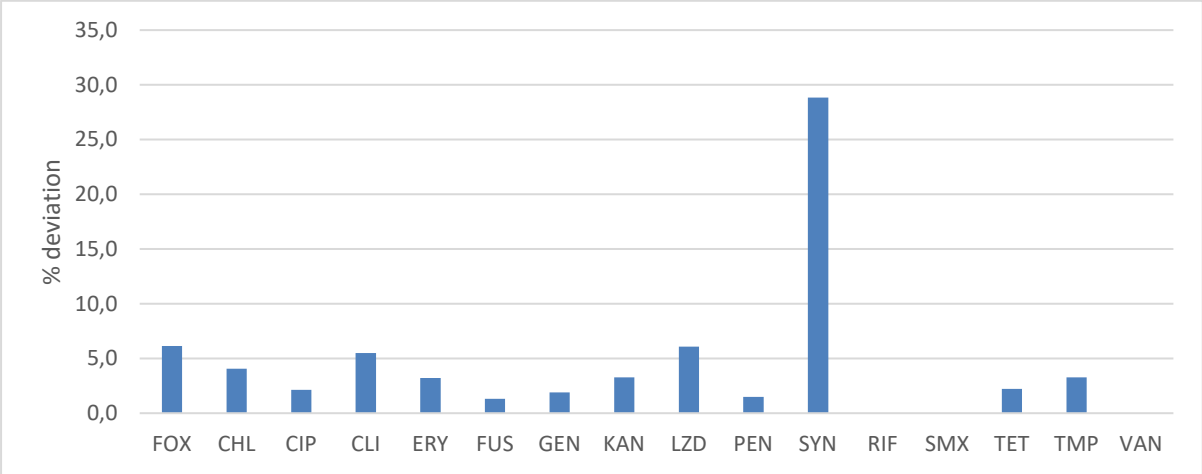

Figure 27. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=19) participating in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below or equal 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for 13 out of the 19 participants **(Figure 28)**. On average, the deviation was 4.0% (ranging from 0.0 to 15.8%). Laboratory #86 presented the highest deviation. As the acceptance level was set to 5% deviation, 6 laboratories (#27, #36, #39, #46, #84 and #86) did not perform within the expected range for the *S. aureus* panel.

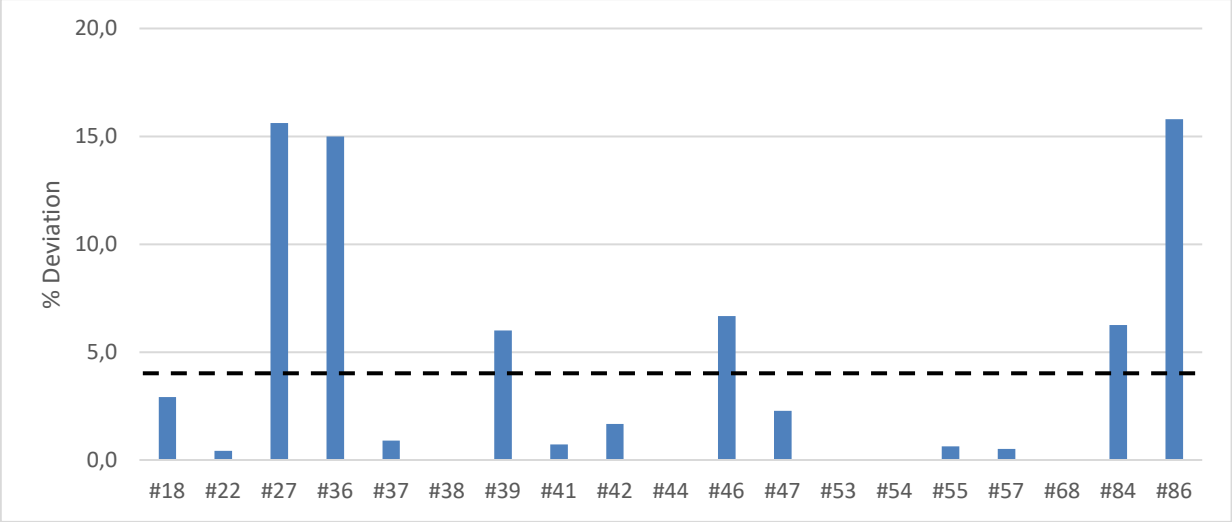

Figure 28. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=19) participating in the 10th EQA of the EQAsia project. Results are categorized by laboratory ID number. Dotted line indicated the average of % deviations across all the laboratories (4%).

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4.5.3 Quality control strains S. aureus ATCC 25923 and S. aureus ATCC 29213

The quality control strains *S. aureus* ATCC 25923 and *S. aureus* 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 *S. aureus* trial.

Among the 19 participating laboratories, 16 submitted results for the reference strains: 14 laboratories reported data for *S. aureus* ATCC 25923 reference strain as disk diffusion was the methodology applied **(Table 37, *).** Laboratories #18, #38 and #47 submitted AST results for *S. aureus* ATCC 29213 reference strain as broth microdilution was the methodology applied **(Table 37, **).**

The highest proportion of test results outside of the expected range was observed for quinupristin/dalfopristin (1 out of 3) and sulfamethoxazole (1 out of 3) **(Table 37).**

A closer look at the laboratories' performance **(Figure 29)** shows that seven laboratories had no deviation from the expected range (#38, #44, #53, #54, #55, #68 and #86). Inversely, laboratory #36 presented a 100.0% deviation, corresponding to incorrect results for 5 out of 5 tested antimicrobials.

Table 37. AST of the reference strain *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the S. aureus trial. Proportion of test results outside of expected range is presented by methodology used

Antimicrobial	Proportion outside of range		
	Disk Diff. *	MIC **	Total
FOX	2/10	--	**2/10**
CHL	3/13	0/1	**3/14**
CIP	1/12	0/3	**1/15**
CLI	0/4	1/3	**1/7**
ERY	1/10	0/3	**1/13**
FUS	0/2	--	**0/2**
GEN	2/13	0/3	**2/16**
KAN	0/3	--	**0/3**
LZD	1/4	0/2	**1/6**
PEN	2/7	0/2	**2/9**
SYN	1/2	0/1	**1/3**
RIF	0/2	2/2	**2/4**
SMX	0/2	1/1	**1/3**
TET	2/13	0/3	**2/16**
TMP	0/4	--	**0/4**
VAN	1/2	0/3	**1/5**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion;
MIC –MIC determination by broth microdilution
**S. aureus* ATCC 25923 for disk diffusion
** *S. aureus* ATCC 29213 for MIC

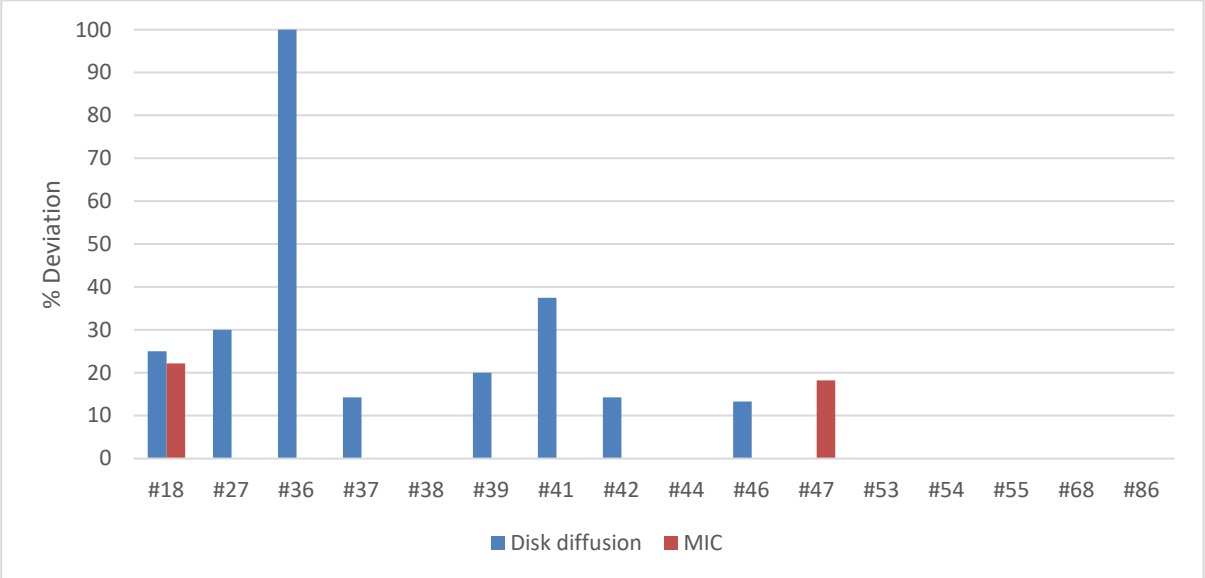


Figure 29. Percentage of deviation in the AST of S. aureus ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial by the AH laboratories.

5. Results – Overall

5.1 Bacterial identification

A total of 34 HH and 27 AH laboratories participated in this EQA trial. As during the previous EQAsia EQAs, participating laboratories could choose one or more panels among the ones offered in the current EQA round. In total, data was submitted by 48 laboratories for the *E. coli* panel, 30 laboratories for the *K. pneumoniae* panel, 37 for *P. aeruginosa.*, and 50 for *S. aureus*. 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).

Considering the test strains tested by each laboratory in each of the panels, it is possible to calculate the percentage of incorrectly identified isolates. **Figure 30** 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 in all panels, mostly in the *E. coli* panel.

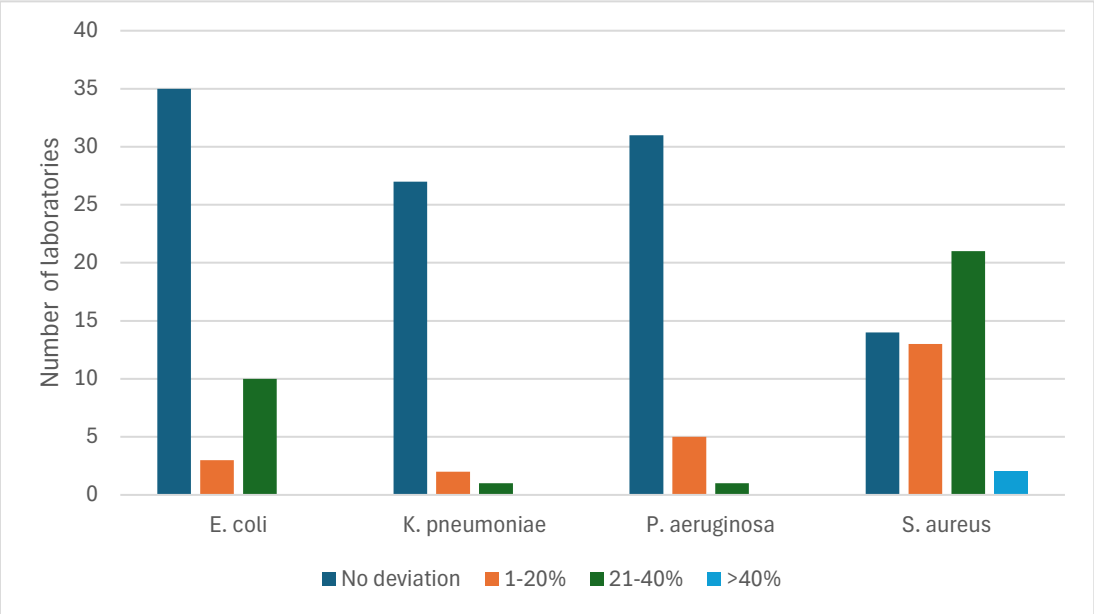


Figure 30. Percentage of deviation in the bacterial identification of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates by the participating laboratories.

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 panels, and for each panel 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 31-34** 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 *E. coli* panel mainly attributed to colistin, chloramphenicol, amikacin, cefepime (40.7.0%, 31.4%, 27.2%, 23.3%, respectively) (**Figure 31**). All other antimicrobials showed deviations below 20%.

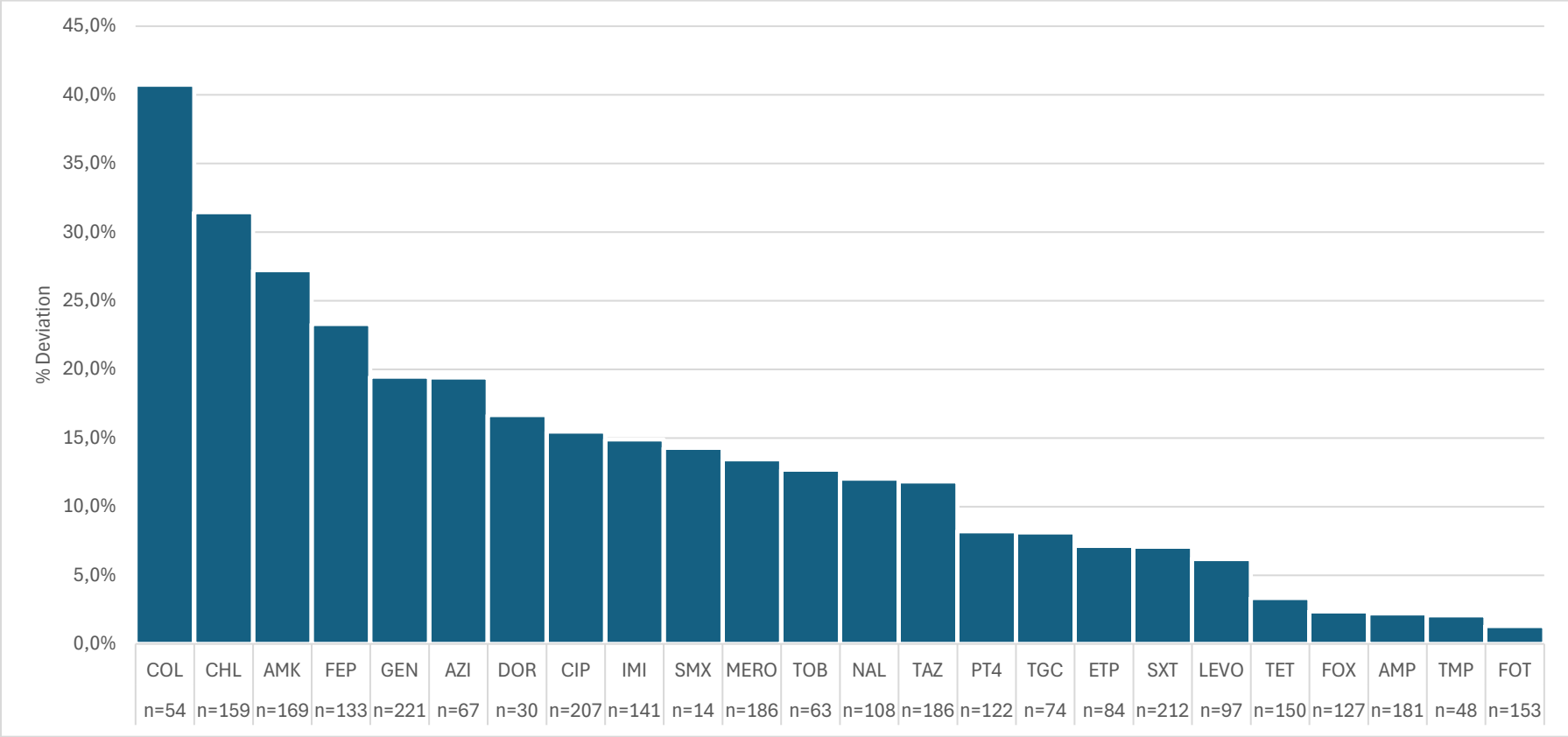


Figure 31. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Escherichia coli* strains of the laboratories that submitted results (n=48) in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The "n" represents the total number of tests evaluated for each antimicrobial.

The results submitted for the *K. pneumoniae* panel showed most deviations for doripenem (37.5%), followed by tigecycline (32.3%), meropenem (28.6%), colistin (23.7%), and tetracycline (20.9%) (**Figure 32**). The remaining 19 antimicrobials showed equal to or less than 20% deviations.

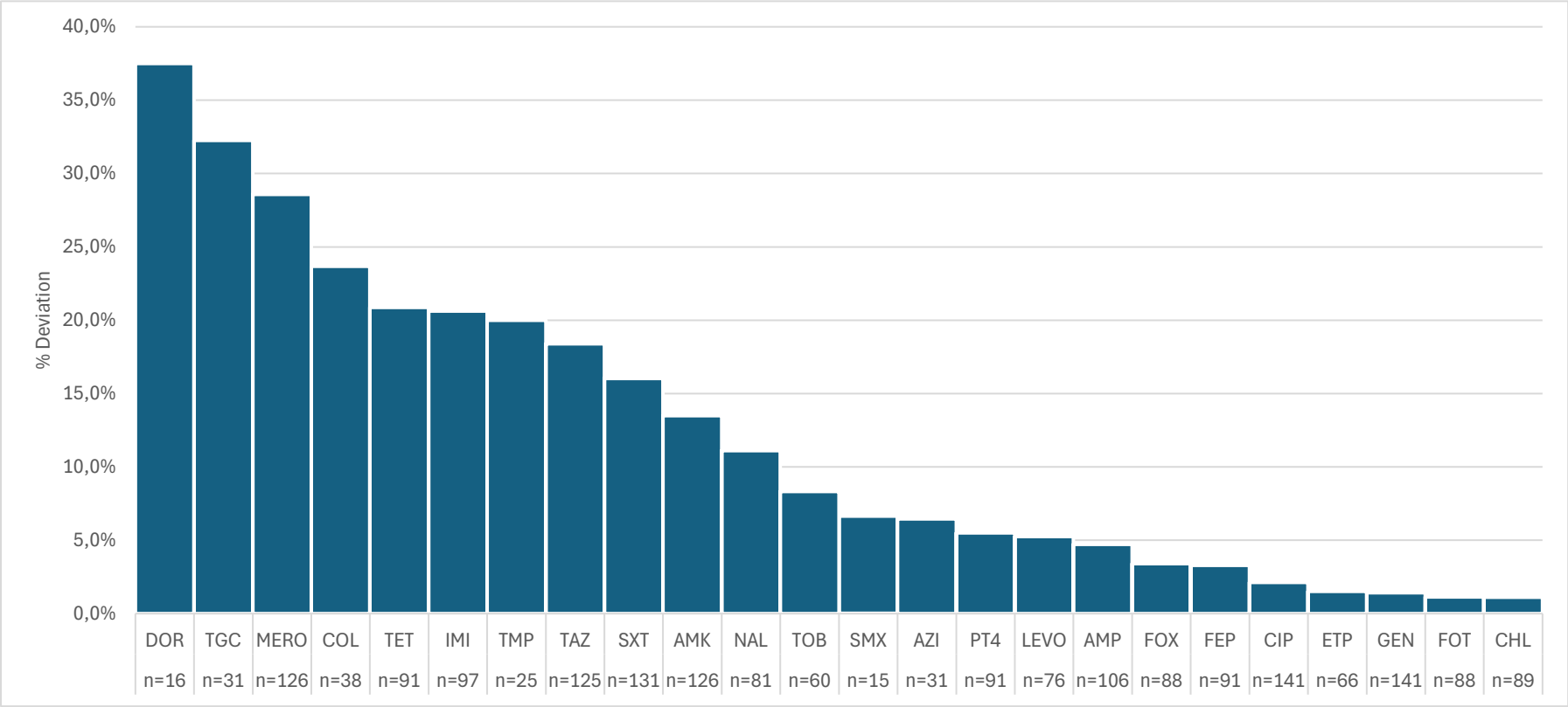


Figure 32. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Klebsiella pneumoniae* strains of the laboratories that submitted results (n=30) in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The "n" represents the total number of tests evaluated for each antimicrobial.

The results submitted for the *P. aeruginosa* panel showed deviations for all reported antimicrobials, mostly for ceftazidime (53.3%) and Piperacillin/tazobactam (33.3%) (**Figure 33)**. All other results showed deviations of less than 30%.

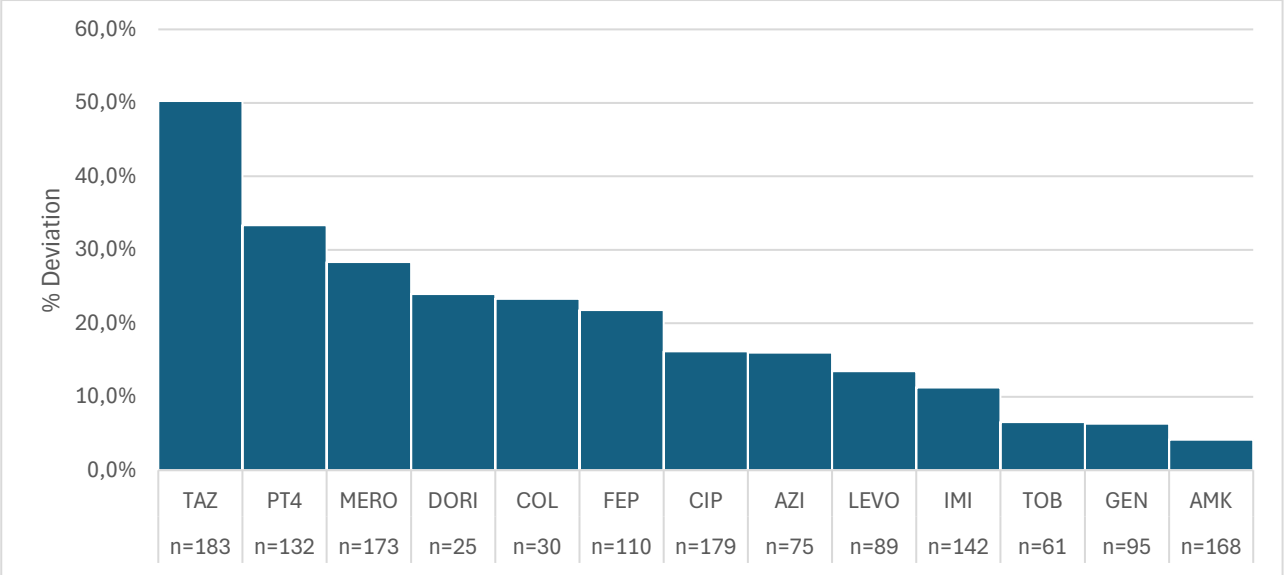


Figure 33. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Pseudomonas aeruginosa* strains of the laboratories that submitted results (n=37) in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The "n" represents the total number of tests evaluated for each antimicrobial.

The results submitted in the *S. aureus* panel showed deviations for all reported antimicrobials. Most of the deviations occurred in the results of cefoxitin (37.5%) followed by chloramphenicol (32.3%) (**Figure 34)**. All the other tested antibiotics showed deviations of less than 30%.

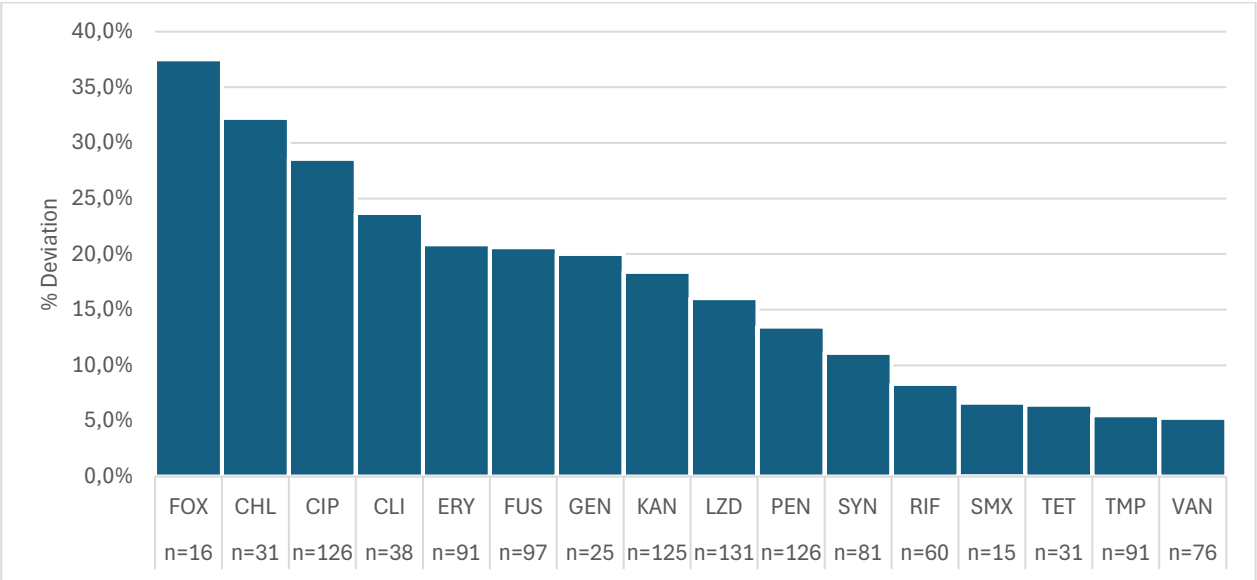


Figure 34. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Staphylococcus aureus* strains of the laboratories that submitted results (n=50) in the 10th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The "n" represents the total number of tests evaluated for each antimicrobial.

5.2.2 Laboratories performance

In each of the panels, the laboratories' performance score varied based on their performance in the AST component of the EQA.

Out of the four panels included in this trial, laboratories achieved higher scores for AST for the *S. aureus* panel (average score 96%) followed by *E. coli* and *K. pneumoniae* panels (94% each). The performance score of the participating laboratories in the *P. aeruginosa* panel was mostly clustered between 62% and 99%, with four laboratories having a score below 80%. The average score for this panel was 90%.

Laboratories were ranked (#1 to #50) based on their average score across the panels in which they participated. The average score varied between 78.5% (rank #50) and 99% (rank #1). The total average score among all 61 laboratories that submitted results was 94%, while the median was 95%.

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. However, the performance rate was not substantially different between the four panels included in this EQA round (**Figure 35**).

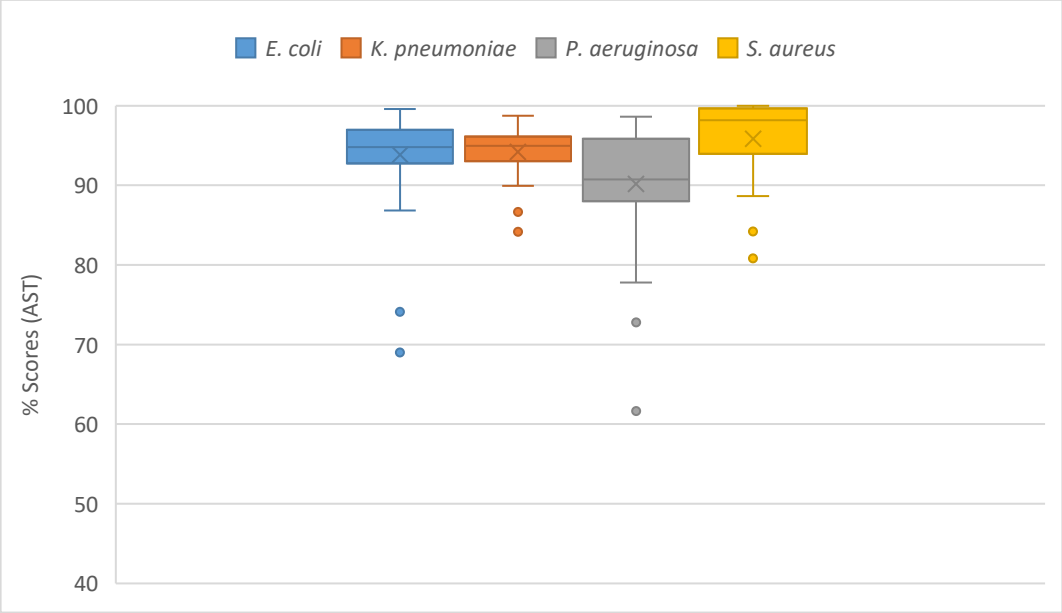


Figure 35. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 10th EQA of the EQAsia project. Most laboratories' performance rate was clustered between 85% and 100%, with a few outliers in each of the four panels.

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 *E. coli* and *K. pneumoniae* panels, *P. aeruginosa* ATCC 27853 for the *P. aeruginosa* panel, and *S. aureus* ATCC 25923 and *S. aureus* ATCC 29212 for testing when disk diffusion or MIC determination methodologies were applied, respectively, for the *S. aureus* panel. Similar to the previous EQAsia EQAs, many laboratories reported deviations in the results obtained when testing quality control strains. Moreover, 19 laboratories (51.4%) did not submit the results from the quality control strains for *P. aeruginosa* panel, while 16 laboratories (32%) did not report reference strain results for *S. aureus* panel. Majority of the laboratories reported results for quality control strain for *E. coli* and *K. pneumoniae* panels and only a few laboratories did not submit the results (n=3 (6.3%) and n=1 (3.3%), respectively). For the *E. coli* panel, 15 laboratories (9 HH and 6 AH) that did not have deviation in their quality control results. However, all the other laboratories

(n=30) presented deviations between 4.8% and 100%. For *K. pneumoniae* panel, six laboratories (5 HH and 1 AH) showed no deviations, while the results from the other 23 laboratories deviated between 4.5% and 100%. For P. aeruginosa panel, the deviations were between 11.1% and 100%. More than half of the laboratories (n=11) that reported results did not have any deviations. Among the 34 laboratories that submitted the results from the quality control testing for *S. aureus* panel, 16 laboratories did not report any deviations. The remaining laboratories (n=18) showed deviations ranging from 11.1% to 100%.

Compared to the submitted AST results of the target strains, the results from the testing of the quality control strains showed significant heterogeneity, which led to a wide dispersion of performance scores in this EQA trial component. Higher variability was evident in both the *E. coli* and *K. pneumoniae* panels **(Figure 36)**. For *E. coli*, a significant number of laboratories (18 out of 45, or 40%) recorded scores at or below 80%. The *K. pneumoniae* panel demonstrated even greater inconsistency, with over a third of laboratories (15 out of 29, or 52%) scoring at or below 80%.

In contrast, majority of the laboratories achieved high scores for the *P. aeruginosa* and *S. aureus* quality control strains **(Figure 36)**. The *P. aeruginosa* panel was the most consistent, with most laboratories (11 out of 18, or 61%) achieving a perfect 100%. The *S. aureus* panel also showed better performance, with most results clustering between 80% and 100% (28 out of 34, 82.3%). A small number of outliers were present, such as scores of 62.5% and 70%.

Overall, the data indicates the highest average performance was seen in the *S. aureus* panel, followed by *P. aeruginosa*. The lowest average performance was observed in the *K. pneumoniae* panel, which also had the lowest median score. The *E. coli* panel similarly demonstrated a depressed median compared to the other organisms.

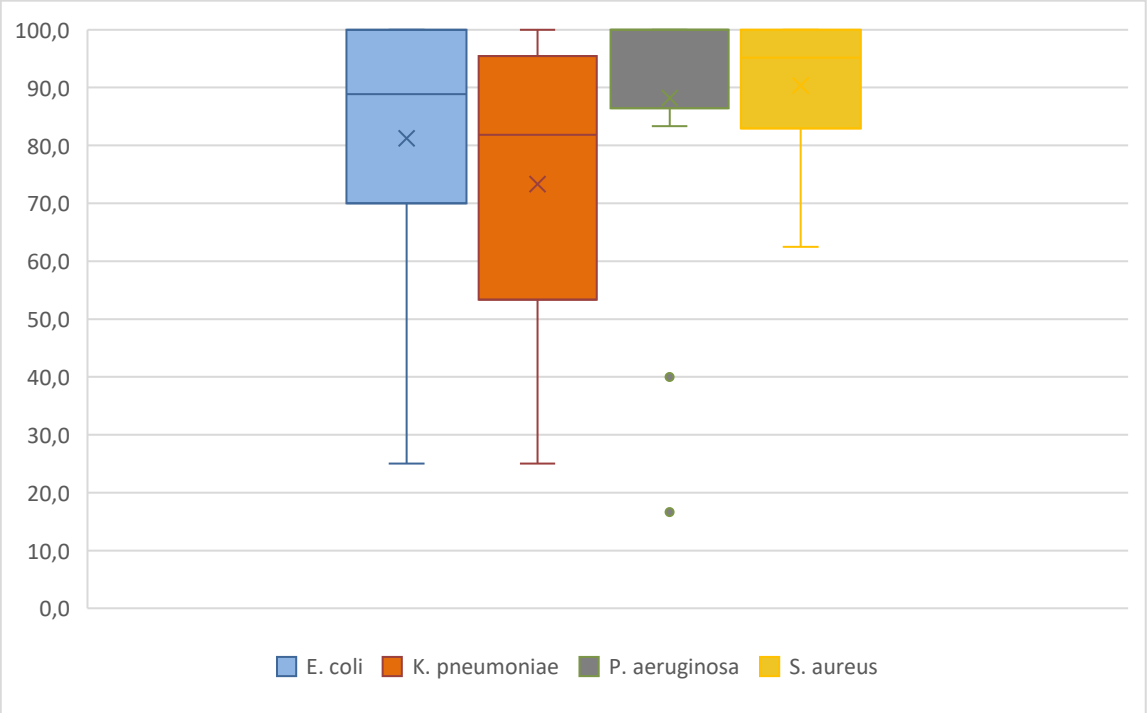


Figure 36. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 10th EQA of the EQAsia project. Extreme outliers (0% scores) were removed from *E. coli* (n=2), *K. pneumoniae* (n=2), *P. aeruginosa* (n=1), and *S. aureus* (n=1)

6. Discussion

6.1 Human Health Laboratories

All 34 Human Health laboratories participating in the 10th EQA of the EQAsia programme submitted results for at least one bacterial panel, with participation varying from 20 laboratories for the *K. pneumoniae* panel to 31 for the *S. aureus* panel. Disk diffusion was the predominant methodology employed by most participants, with most laboratories utilizing Clinical and Laboratory Standards Institute (CLSI) guidelines for interpretation. While the EQA setup allowed flexibility in antimicrobial selection, a core set of agents including aminoglycosides, fluoroquinolones, and key beta-lactams were tested consistently across the Gram-negative panels.

A notable issue observed across all four panels was the submission of incomplete data, where laboratories did not report results for all antimicrobials in their own repertoire for every strain. This was most pronounced in the *E. coli* and *P. aeruginosa* panels. For a robust assessment of laboratory capability, it is essential that all isolates within a panel are tested against a consistent set of antimicrobials.

Bacterial identification was generally proficient, with near-perfect accuracy for *K. pneumoniae* and *P. aeruginosa* strains. However, some misidentifications occurred with non-*E. coli* and non-*S. aureus* strains included in the respective panels, indicating a need for reinforcement of identification protocols, particularly for differentiating closely related species.

The analysis of antimicrobial susceptibility testing revealed significant concerns, particularly for Gram-negative pathogens. Performance was highly variable and depended on the specific antimicrobial agent, the bacterial species, and the individual laboratory. For *E. coli*, the overall deviation was 11.6%, but agents like chloramphenicol (30.2%) and amikacin (25.0%) showed alarmingly high error rates. The *K. pneumoniae* panel performed slightly worse on average (12.3% deviation), with critically high error rates for carbapenems like doripenem (40.0%) and meropenem (28.0%). Most alarming was the performance for *P. aeruginosa*, which had an average deviation of 18.1%, with essential drugs like ceftazidime (47.8%) and piperacillin-tazobactam (33.0%) being particularly problematic. In stark contrast, the *S. aureus* panel demonstrated the highest overall proficiency, with a low average deviation of 6.6%.

The detection of resistance mechanisms in *E. coli* and *K. pneumoniae* proved challenging. While carbapenemase-producing strains were generally well-identified, laboratories struggled significantly with strains possessing complex resistance profiles, such as ESBL+AmpC. A common error was the misclassification of these strains as carbapenemase producers, suggesting a tendency to overcall this resistance mechanism and a need for improved phenotypic confirmation methods.

The testing of quality control strains uncovered critical deficiencies in fundamental laboratory procedures. The performance for reference strains *E. coli* ATCC 25922 and NCTC 13846 was poor, with average deviation rates of 22.2% and 31.9% within the *E. coli* and *K. pneumoniae* panels, respectively. The results showed a stark divide: while several laboratories achieved a 0% deviation rate, others failed completely, with one laboratory reporting a 100% error rate. This indicates severe, systemic issues in the application of basic AST methodologies in a significant number of participating labs. The fact that these errors persisted even with quality control strains, which should yield predictable results, underscores an urgent need for widespread remedial training, particularly in the execution and interpretation of disk diffusion testing.

In conclusion, while a subset of laboratories demonstrates high proficiency, the EQA results reveal substantial and widespread inconsistencies in AST performance across the

participating HH laboratories. The high error rates for last-resort antibiotics against Gram-negative pathogens and the fundamental failures in quality control testing are major concerns for patient care and antimicrobial resistance containment. These findings highlight the indispensable role of ongoing EQA programmes in identifying gaps and mandating targeted interventions to improve the reliability of laboratory data guiding antimicrobial therapy.

6.2 Animal Health Laboratories

For the Animal Health sector, 27 laboratories participated in the 10th 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 broth microdilution (conventional) or broth microdilution (automated).

Participants were instructed to first perform bacterial identification followed by antimicrobial susceptibility testing (AST) of the target strains. However, incomplete AST result entries were observed across all panels, meaning that the participating laboratories did not submit complete results of their own available antimicrobial agents. Ideally, isolates in each panel should be tested against a consistent set of antimicrobials to ensure a reliable assessment of laboratory performance and testing capacity. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance.

In the bacterial identification component of this EQA, the participants showed high proficiency in correctly identifying the K. pneumoniae and P. aeruginosa species among the test strains. However, in the *E. coli* and *S. aureus* panels, several laboratories showed limited capacity for accurate species identification, with instances of misidentification observed. Nevertheless, proper pathogen identification is crucial, especially in a clinical setting. There is a clear need to assess the causes for bacterial misidentification and provide guidance and training to strengthen laboratory capacity.

The antimicrobial susceptibility testing performance was assessed from different angles to better identify deviations from the expected results. In the *E. coli* panel, several antimicrobials showed high deviation rates, including amikacin (11.0%), azithromycin (18.3%), colistin (16.0%), doripenem (21.7%), ertapenem (14.3%) and sulfamethoxazole (10.7%). The K. pneumoniae panel also showed discrepancies, particularly for ceftazidime (16.7%), colistin (12.5%, meropenem (11.5%), tetracycline (13%) and tigecycline (75.0%). In the P. aeruginosa panel, the high deviations were observed for cefepime (23.8%), ceftazidime (31.7%), doripenem (22.5%), imipenem (12.5%), meropenem (12.9%), and tobramycin (25.0%). For the Gram-positive bacteria panel (S. aureus), quinupristin/dalfopristin revealed a rather high deviation (28.8%).

On average, the AST performance of participating laboratories was the best in the S. aureus (96.5%), followed by *K. pneumoniae* panel (94.8%), *E. coli* panel (94.0%) and P. aeruginosa (87.4%).

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 in 13 of 24 for E. coli panel, 6 of 10 for *K. pneumoniae* panel, 2 out of 8 for *P. aeruginosa* panel, and 13 out of 19 for *S. aureus* participants.

Detection and confirmation of presumptive beta-lactamase producing *E. coli* and *K. pneumoniae* was an optional component of this EQA but highly encouraged due to its importance. Nine

and three participating laboratories submitted results for *E. coli* and *K. pneumoniae*, respectively. However, none of laboratories correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the *E. coli* and *K. pneumoniae* strains. The observations suggest a need for further clarification and support on capacity building.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the panels. Several laboratories did not submit results for the reference strains. Notably, laboratories #57 and #84 did not submit results for any of the reference strains, while the other nine laboratories did not submit results for some of the reference strains. Testing the recommended reference strains is required in terms of quality control and reliability of AST results and performance. Lack of AST results for the reference strain would invalidate the results for the test strains.

7. Conclusions

This report summarizes the findings from the 10th External Quality Assessment (EQA) trial conducted by the EQAsia project in March – May 2025, focusing on bacterial identification and antimicrobial susceptibility testing (AST) of four priority pathogens: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The trial involved 61 laboratories from the Human Health (HH) and Animal Health (AH) sectors across 14 countries in South and Southeast Asia.

The primary objective of EQAsia remains to enhance laboratory capacity and ensure the reliability of diagnostic and surveillance data through regular EQA participation. While many laboratories demonstrated satisfactory performance, significant variability was observed across sectors, pathogens, and antimicrobial agents. Bacterial identification was generally accurate, though errors persisted with non-target strains, highlighting the need for continued reinforcement of identification protocols.

AST performance revealed critical gaps, particularly for Gram-negative pathogens and last-resort antimicrobials such as carbapenems and colistin. High deviation rates were noted for several key drugs, and the detection of complex resistance mechanisms (e.g., ESBL, AmpC, carbapenemase) proved challenging for many laboratories. Furthermore, quality control testing using reference strains uncovered fundamental procedural deficiencies, with some laboratories reporting unacceptable error rates.

These results underscore the ongoing need for targeted training, capacity building, and adherence to standardized methodologies. EQAsia will continue to provide individualized feedback, technical support, and training resources to help underperforming laboratories improve their accuracy and reliability. Ensuring robust quality management systems and routine quality control practices is essential for generating credible data that supports both clinical decision-making and antimicrobial resistance surveillance efforts in the region.

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*. 35th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2025.

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

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

[6] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018.

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

9. Appendices

Appendix 1: EQA10 Protocol

EQAsia EQA10 trial
Protocol
Identification and antimicrobial susceptibility testing (AST) of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* 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 EQA10 trial** includes four EQA panels each composed of seven test strains – *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa.,* and *Staphylococcus aureus*, 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, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC).

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 *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3 EQA10 OUTLINE

3.1 Shipping and receipt of strains

Your laboratory is one of the 61 human health and animal health laboratories from South and Southeast Asia participating in EQA10. In March 2025, you are expected to receive a parcel containing one or more of the following panels:

- ***Escherichia coli* panel** - seven test strains of which five are *E. coli* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) are the recommended reference strains for QC of AST.
- ***Klebsiella pneumoniae* panel** - seven test strains of which five are *K. pneumoniae* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) are the recommended reference strains for QC of AST.
- ***Pseudomonas aeruginosa* panel** - seven test strains of which five are *P. aeruginosa.* and two are non-target species. The reference strain to be used for this panel is *Pseudomonas aeruginosa* ATCC 27853/CCM 3955.
- ***Staphylococcus aureus* panel** - seven test strains of which five are *S. aureus* and two are non-target species. The *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC) are the recommended reference strains for QC of AST.

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

N.B.!!!. The *E. coli* and *S. aureus* isolates are sent in ampoules. The *K. pneumoniae* and *P. aeruginosa* strains are shipped on media in transport tubes (swabs).

Transport swabs


Lyophilized cultures in ampoules

3.2 Reviving and storage of strains

- Reviving K. pneumoniae and P. aeruginosa 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). 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 (for the ampoules)
- Tweezers (for the vials)
- 70% alcohol
- Sterile Luria Bertani (LB) broth
- Agar plates (5 to 6 plates per one strain)
- Autopipette with tips or Pasteur pipettes
- Inoculating loop
- Sterile syringe and needle (optional)

To open and reconstitute the **ampoules**:

1. Carefully take the ampoule out of the wrap.

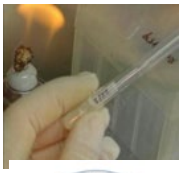
   Note: To maintain the vacuum condition, do not break the tip of the ampoule. Otherwise, the air will enter the ampoule and the cotton wool plug will be pushed down and in contact with dried bacterial culture. If it happens, please simply remove the cotton plug with forceps.

   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.



6. Pipette 0.5 ml of sterile LB broth into the dried cells. Mix gently and carefully to avoid creating aerosols.
7. Transfer one drop of each strain onto one LB agar plate 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.

8. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

To open and reconstitute the **vials:**

1. Flip up the round part of the metal cap using tweezers.
2. The entire metal ring and rubber stopper can be removed.
3. Pipette 0.5 ml of sterile LB broth into the vial with dried cells. Mix gently and carefully to avoid creating aerosols.
4. Incubate the vial for 10-15 minutes at 37°C with the rubber stopper on. Be careful to avoid contamination.
5. Transfer one drop of each strain onto one LB agar plate 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.
6. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

OR

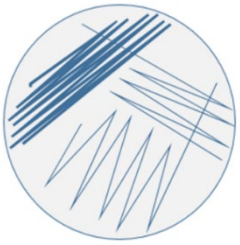
2. Alternatively, after step 1 you can keep the metal ring and rubber stopper on the vial. Sterilize the exposed part of the rubber stopper with 70% alcohol.
3. Using a syringe and needle, aseptically take 0.5 ml of sterile LB broth.
4. Insert the needle through the rubber stopper into the vial and inject the content of the syringe.
5. Incubate the vial for 10-15 minutes at 37°C.
6. Take a few drops of the content of the vial using a sterile syringe and needle and inoculate media appropriate for the strain type. 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. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

- **Reviving *K. pneumoniae* and *P. aeruginosa* strains**

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.

It is furthermore recommended that the strains are stored in your strain collection (e.g., in a -80°C freezer), at least until you have reviewed your results from this EQA trial. 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.

- **Safety precautions**:

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 *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa,* and *Staphylococcus aureus* test strains

Each of the four panels in this EQA round contains five target species. i.e. five *E. coli* isolates in the *E. coli* panel. The remaining two isolates in each panel are non-target species – their identification differs from the five target species.

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

3.4 Antimicrobial susceptibility testing of *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa,* and *Staphylococcus aureus* test strains, and reference strains

The strains identified as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* (five isolates from each panel), as well as the appropriate reference strains, should be tested for susceptibility towards as many as possible of the antimicrobials 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, 35th Edition). When not available, breakpoints were based on previous CLSI M100 editions or EUCAST (Clinical Breakpoints Tables v.15.0, 2025), or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* 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 *E. coli*

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

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

Reference values are based on Enterobacterales breakpoints from CLSI M100, 35th Edition.

[a] Reference values are based on Enterobacterales breakpoints from CLSI M100, 33rd Edition.

[b] Reference values are based on Enterobacterales clinical breakpoints from www.eucast.org (Tables v. 15.0, 2025)

NA; Not Applicable

Table 2. Breakpoints for interpretation of MICs and zone diameters for *K. pneumoniae*

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

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

Reference values are based on Enterobacterales breakpoints from CLSI M100, 35th Edition.

[a] Reference values are based on Enterobacterales breakpoints from CLSI M100, 33rd Edition.

[b] Reference values are based on epidemiological cut-off values from www.eucast.org (February 2025)

NA; Not Applicable

Beta-lactam and carbapenem resistance

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

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

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

    - It is defined as a ≥ 3 two-fold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (Gradient Test 3 dilution steps difference; MIC FOT: FOT/Cl or TAZ: TAZ/Cl ratio ≥ 8).

    - A positive synergy testing for Disk Diffusion is defined as ≥ 5 mm increase of diameter of FOT or TAZ in combination with clavulanic acid (FOT/Cl or TAZ/Cl) compared to testing them alone. The presence of synergy indicates ESBL production.

- Detection of AmpC-type beta-lactamases: it can be performed by testing the bacterial culture for susceptibility to cefoxitin (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

- Confirmatory test for carbapenemase production: it requires the testing of meropenem (MERO) and combination disk test method incl. meropenem ± various inhibitors, i.e. boronic acid, dipicolinic acid or EDTA, cloxacillin.

It should be noted that some resistance mechanisms do not always confer clinical resistance. Therefore, the classification of the phenotypic results (**Figure 1** below) should be based on the "EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance", Version 2.0, July 2017, and the most recent EFSA recommendations – The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA Journal 2020;18 (3) https://doi.org/10.2903/j.efsa.2020.6007

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

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

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

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

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

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

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

Genotypic testing by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either ESBL-, AmpC, and/or carbapenemase-producer, but it is **not** required as part of this EQA.

Table 3. Breakpoints for interpretation of MICs and zone diameters for *P. aeruginosa*

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

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

Reference values are based on *P. aeruginosa* breakpoints from CLSI M100, 35th Edition.

[a] Reference values are based on *P. aeruginosa* breakpoints from CLSI M100, 33rd Edition.

Table 4. Breakpoints for interpretation of MICs and zone diameters for *S. aureus*

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
Cefoxitin, FOX	≤ 4	-	≥ 8	≥ 22	-	≤ 21
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Clindamycin, CLI	≤ 0.5	1-2	≥ 4	≥ 21	15-20	≤ 14
Erythromycin, ERY	≤ 0.5	1-4	≥ 8	≥ 23	14-22	≤ 13
Fusidic acid, FUS[a]	≤ 1	-	> 1	≥ 24	-	≤ 23
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Kanamycin, KAN[b]	≤ 8	-	≥ 16	≥ 18	-	≤ 17
Linezolid, LZD	≤ 4	-	≥ 8	≥ 26	23-25	≤ 22
Penicillin, PEN	≤ 0.12	-	≥ 0.25	≥ 29	-	≤ 28
Quinupristin/dalfopristin, SYN	≤ 1	2	≥ 4	≥ 19	16-18	≤ 15
Rifampin, RIF	≤ 1	2	≥ 4	≥ 20	17-19	≤ 16
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 19	15-18	≤ 14
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10
Vancomycin, VAN	≤ 2	4-8	≥ 16	NA	NA	NA

Reference values are based on *Staphylococcus aureus* breakpoints from CLSI M100, 35th Edition.

[a] Reference values are based on *Staphylococcus aureus* clinical breakpoints from www.eucast.org (Tables v. 15.0, 2025).

[b] Reference values are based on epidemiological cut-off values from www.eucast.org (February 2025)

4 SUBMISSION OF RESULTS VIA THE INFORMATICS MODULE

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

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

Login to the Informatics Module:

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

When first given access to login to the Informatics Module, your **personal loginID and password** is sent to you by email.

Note that the primary contact person for a participating institution is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact eqasia@food.dtu.dk

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

Results must be submitted no later than May 05th, 2025.

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

Before submitting your final input for all the organisms, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once**! 'Final submit' blocks further data entry.

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

5 EVALUATION OF RESULTS

The scores for the submitted results will be released after the submission deadline has passed. Then, you will be able to access the evaluation of your results. Results in agreement with the expected interpretation are categorised as ‘4’ (correct), while results deviating from the expected interpretation are categorised as ‘3’ (incorrect, minor), ‘1’ (incorrect, major) or ‘0’ (incorrect, very major).

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

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

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

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

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

Appendix 2a: Reference values (MIC values and interpretation) – *Escherichia coli* panel

	Ec EQASIA 25.1		Ec EQASIA 25.2		Ec EQASIA 25.3		Ec EQASIA 25.4		Ec EQASIA 25.5	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Amikacin	<=4	S	>128	R	=8	I	<=4	S	<=4	S
Ampicillin	>32	R	>32	R	>32	R	>32	R	>32	R
Azithromycin	>64	R	=16	S	=8	S	=32	R	=8	S
Cefepime	=8	I	>32	R	>32	R	=0.12	S	=16	R
Cefotaxime	>64	R	>64	R	>64	R	<=0.25	S	=64	R
Cefotaxime and clavulanic acid	=4/4		>64/4		>64/4		=0.12/4		<=0.06/4	
Cefoxitin	>64	R	>64	R	>64	R	=8	S	=4	S
Ceftazidime	=32	R	>128	R	>128	R	<=0.25	S	=4	S
Ceftazidime and clavulanic acid	=16/4		>128/4		>128/4		<=0.12/4		=0.25/4	
Chloramphenicol	=16	I	<=8	S	=16	I	<=8	S	<=8	S
Ciprofloxacin	=0.5	I	>8	R	>8	R	<=0.015	S	<=0.015	S
Colistin	<=0.25	I	<=0.25	I	<=0.25	I	<=0.25	I	<=0.25	I
Doripenem	<=0.12	S	>2	R	>2	R	<=0.12	S	<=0.12	S
Ertapenem	=2	R	>4	R	>4	R	<=0.015	S	<=0.015	S
Gentamicin	=1	S	>16	R	=1	S	<=0.5	S	<=0.5	S
Imipenem	=0.5	S	=8	R	=8	R	<=0.25	S	<=0.12	S
Levofloxacin	<=1	S	>8	R	>8	R	<=1	S	<=1	S
Meropenem	=0.25	S	=8	R	=8	R	<=0.03	S	<=0.03	S
Nalidixic acid	=8	S	>64	R	>64	R	<=4	S	<=4	S
Piperacillin and tazobactam	=32	R	>64	R	>64	R	<=8	S	<=8	S
Sulfamethoxazole	>512	R	>512	R	>512	R	<=8	S	<=8	S
Tetracycline	>32	R	<=2	S	>32	R	<=2	S	<=2	S
Tigecycline	<=0.25	S	<=0.25	S	=0.5	S	<=0.25	S	<=0.25	S
Tobramycin	<=1	S	>8	R	>8	R	<=1	S	<=1	S
Trimethoprim	>16	R	<=0.25	S	>16	R	>16	R	<=0.25	S
Trimethoprim and sulfamethoxazole	>4/76	R	<=0.5/9.5	S	>4/76	R	<=0.5/9.5	S	<=0.5/9.5	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2b: Reference values (MIC values and interpretation) – *Klebsiella pneumoniae* panel

	Kp EQASIA 25.1		Kp EQASIA 25.2		Kp EQASIA 25.3		Kp EQASIA 25.4		Kp EQASIA 25.6	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Amikacin	<=4	S	<=4	S	<=4	S	<=4	S	>128	R
Ampicillin	>32	R	>32	R	>32	R	>32	R	>32	R
Azithromycin	=32	R	=32	R	=16	S	>64	R	>64	R
Cefepime	>32	R	=16	R	=0.12	S	>32	R	>32	R
Cefotaxime	>64	R	=64	R	<=0.25	S	>64	R	>64	R
Cefotaxime and clavulanic acid	>64/4		=1/4		<=0.06/4		>64/4		=1/4	
Cefoxitin	>64	R	=4	S	=8	S	>64	R	>64	R
Ceftazidime	>128	R	=64	R	=0.5	R	>128	R	>128	R
Ceftazidime and clavulanic acid	>128/4		=1/4		<=0.25/4		>128/4		=2/4	
Chloramphenicol	>64	R	>64	R	>64	R	<=8	S	>64	R
Ciprofloxacin	>8	R	=4	R	>8	R	>8	R	>8	R
Colistin	<=0.25	I	<=0.25	I	<=0.25	I	<=0.25	I	<=0.25	I
Doripenem	>4	R	=0.5	S	<=0.12	S	>2	R	=1	S
Ertapenem	>4	R	=2	R	<=0.25	S	>4	R	>4	R
Gentamicin	>16	R	>16	R	<=0.5	S	>16	R	>16	R
Imipenem	=16	R	=1	S	<=0.12	S	=16	R	<=0.25	S
Levofloxacin	>8	R	=1	I	=4	R	>8	R	>8	R
Meropenem	=16	R	=1	S	<=0.03	S	>16	R	=2	I
Nalidixic acid	>64	R	=8	S	>64	R	>64	R	>64	R
Piperacillin and tazobactam	>64	R	>64	R	<=8	S	>64	R	>64	R
Sulfamethoxazole	>512	R	>512	R	<=8	S	>512	R	>512	R
Tetracycline	>32	R	=4	S	=4	S	<=2	S	=16	R
Tigecycline	=2	S	<=0.25	S	=0.5	S	<=0.25	S	=1	S
Tobramycin	>8	R	>8	R	<=1	S	=8	R	>8	R
Trimethoprim	>16	R	>16	R	=8	S	>16	R	=2	S
Trimethoprim and sulfamethoxazole	>4/76	R	>4/76	R	<=0.5/9.5	S	>4/76	R	=1/19	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2c: Reference values (MIC values and interpretation) – *Pseudomonas aeruginosa* panel

	Pa EQASIA 25.1		Pa EQASIA 25.3		Pa EQASIA 25.4		Pa EQASIA 25.5		Pa EQASIA 25.7	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Amikacin	=8	S	>32	R	<=4	S	<=4	S	=32	R
Aztreonam	>32	R	>32	R	=4	S	=16	I	=4	S
Cefepime	>16	R	>16	R	<=2	S	=4	S	=8	S
Ceftazidime	=128	R	=16	I	<=1	S	=4	S	=8	S
Ciprofloxacin	>2	R	>2	R	<=0.25	S	=0.5	S	>2	R
Colistin	=1	I	=1	I	=1	I	=1	I	=1	I
Doripenem	>4	R	>4	R	=4	I	=1	S	>4	R
Gentamicin	=4	S	>8	R	<=1	S	<=1	S	>8	R
Imipenem	>8	R	>8	R	>8	R	<=1	S	>8	R
Levofloxacin	>8	R	>8	R	<=1	S	=2	I	>8	R
Meropenem	>16	R	>16	R	=4	I	=2	S	>16	R
Piperacillin and tazobactam	>128	R	=128	R	<=8	S	=32	I	=32	I
Tobramycin	<=1	S	>8	R	<=1	S	<=1	S	>8	R

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2d: Reference values (MIC values and interpretation) – *Staphylococcus aureus* panel

	Sa EQASIA 25.1		Sa EQASIA 25.2		Sa EQASIA 25.3		Sa EQASIA 25.4		Kp EQASIA 25.6	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Cefoxitin	=4	S	=16	R	=4	S	>256	R	=4	S
Chloramphenicol	=8	S	=8	S	=8	S	=8	S	=8	S
Ciprofloxacin	<=0.25	S	=0.5	S	<=0.25	S	=0.25	S	<=0.5	S
Clindamycin	<=0.12	S	<=0.12	S	<=0.12	S	<=0.12	S	<=0.12	S
Erythromycin	=0.5	S	=0.5	S	=0.5	S	=0.5	S	>8	R
Fusidic acid	<=0.25	S	<=0.25	S	<=0.25	S	<=0.25	S	<=0.25	S
Gentamicin	<=0.5	S	<=0.5	S	<=0.5	S	<=0.5	S	<=0.5	S
Kanamycin	<=4	S	<=4	S	<=4	S	<=4	S	<=4	S
Linezolid	=2	S	=2	S	=2	S	=2	S	=2	S
Penicillin	<=0.06	S	>8	R	=1	R	>8	R	>1	R
Quinupristin and dalfopristin	<=0.5	S	<=0.5	S	=0.5	S	=0.25	S	<=0.5	S
Rifampin	<=0.015	S	<=0.015	S	<=0.015	S	<=0.015	S	<=0.015	S
Sulfamethoxazole	<=64	S	<=64	S	<=64	S	<=64	S	<=64	S
Tetracycline	<=0.5	S	<=0.5	S	<=0.5	S	<=0.5	S	<=0.5	S
Trimethoprim	<=1	S	<=1	S	=1	S	<=1	S	<=1	S
Vancomycin	<=1	S	<=1	S	<=1	S	<=1	S	<=1	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 3: Quality control ranges for the reference strains

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

E. coli ATCC 25922		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amikacin, AMK	0.5-4	19-26
Ampicillin, AMP	2-8	15-22
Azithromycin, AZI	--	--
Cefepime, FEP	0.016-0.12	31-37
Cefotaxime, FOT	0.03-0.12	29-35
Cefotaxime and clavulanic acid, F/C	--	--
Cefoxitin, FOX	2-8	23-29
Ceftazidime, TAZ	0.06-0.5	25-32
Ceftazidime and clavulanic acid, T/C	--	--
Chloramphenicol, CHL	2-8	21-27
Ciprofloxacin, CIP	0.004-0.016	29-38
Doripenem, DOR	0.016-0.06	27-35
Ertapenem, ETP	0.004-0.016	29-36
Gentamicin, GEN	0.25-1	19-26
Imipenem, IMI	0.06-0.5	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	1-8	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	0.0010-0.5	23-29

MIC ranges and disk diffusion ranges are according to CLSI M100 35th edition, Tables 4A-1 and 5A-1. When not available, breakpoints were based on previous CLSI M100 editions or EUCAST (Clinical QC Tables v.15.0, 2025), or epidemiological cut off values (https://mic.eucast.org/) were used instead.

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

MIC ranges are according to CLSI M100 35th edition, Table 5A-1, Supplemental QC ranges.

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

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

MIC ranges and disk diffusion ranges are according to CLSI M100 35th edition, Tables 4A-1 and 5A-1

Appendix 3c: Quality control ranges for *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213

	S. aureus ATCC 29213	*S. aureus* ATCC 25923
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Cefoxitin, FOX	1-4	23-29
Chloramphenicol, CHL	2-16	19-26
Ciprofloxacin, CIP	0.12-0.5	22-30
Clindamycin, CLI	0.06-0.25	24-30
Erythromycin, ERY	0.25-1	22-30
Fusidic acid, FUS	0.06-0.25	24-32
Gentamicin, GEN	0.12-1	19-27
Kanamycin, KAN	1-4	19-26
Linezolid, LZD	1-4	25-32
Penicillin, PEN	0.25-2	26-37
Quinupristin and dalfopristin, SYN	0.25-1	21-28
Rifampin, RIF	0.004-0.016	26-34
Sulfamethoxazole, SMX	32-128	24-34
Tetracycline, TET	0.12-1	24-30
Trimethoprim, TMP	1-4	19-26
Vancomycin, VAN	0.5-2	17-21

MIC ranges and disk diffusion ranges are according to CLSI M100 35th edition, Tables 4A-1 and 5A-1