DTU Fødevareinstituttet
DTU National Food Institute

8th EQAsia External Quality Assessment Trial:

Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp. and Staphylococcus aureus – 2024

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E. coli, K. pneumoniae, Acinetobacter spp., and *S. aureus* – 2024

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

This report summarizes the results of the 8th External Quality Assessment (EQA) trial of EQAsia, a Fleming Fund Regional Grant. It aims 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. The EQAsia project has entered a second phase (2023 to 2025) in which it continues to deliver the established EQA programme focussing on both Human Health (HH) sector and Food and Animal Health (AH) sector laboratories across the region.

The period of the trial was April – June 2024 and consisted of bacterial identification and antimicrobial susceptibility testing (AST) of four prominent WHO and FAO priority pathogens, namely: *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.* and *Staphylococcus aureus*.

A total of 20 HH and 18 AH laboratories participated in this EQA trial. Four AH laboratories did not submit any results. Similarly to the previous EQAsia EQAs, participating laboratories could choose one or more panels among the ones offered in the current EQA round. In total, data were submitted by 32 laboratories for the *E. coli* panel, 25 laboratories for the *K. pneumoniae* panel, 21 – for Acinetobacter spp., and 33 – 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).

The bacterial identification component consisted of identification of the five strains of the organism in question (target organism) among a total of seven strains. Half of the laboratories that submitted data for the *E. coli* panel (n=16) identified all isolates in this panel correctly. While in the other three panels, there were only a few laboratories that had difficulties in determining the correct bacterial identification of the target isolates.

Overall, laboratories had a very good performance score throughout all four panels. The success rate in the *S. aureus* and *E. coli* panel was the highest (95.6% and 94.8% average score, respectively), followed by *K. pneumoniae* – 91.4% and *Acinetobacter spp.* – 90.0%.

Laboratories were ranked based on their average score across the panels in which they participated. The average score varied between 69.6% (rank #34) and 98.9% (rank #1). The total average score among all 31 laboratories that submitted results was 92.9%, while the median was 95.3%.

As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (7 in the *Acinetobacter spp.* panel and 5 in each of the *S. aureus, E. coli* and *K. pneumoniae* panels) did not submit results from reference strain testing. For the *E. coli* EQA round, there were 11 laboratories (9 HH and 2 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=16) presented deviations between 5.9% and 78.6%. Since the same quality control strains were used also for the *K. pneumoniae* panel, the submitted results were similar. Nine laboratories (8 HH and 1 AH) showed no deviations, while the results from the other 11 laboratories deviated ranging between 6.7% to 37.5%. There was much less heterogeneity in the *Acinetobacter spp.* panel where the deviations were between 8.3% and 25.0%. The results from the quality control testing also for *S. aureus* varied substantially between the different laboratories with deviations from the QC ranges between 9.1% and 90.9%.

Not all laboratories from both HH and AH sectors submitted results for ESBL-, AmpC-, or

carbapenemase-production for the *E. coli* and *K. pneumoniae* isolates. 17 HH (53%) and 5 AH (16%) out of 32 laboratories tested and submitted results for *E. coli*, while 17 HH (68%) and 3 AH (12%) out of 25 laboratories tested and submitted results for *K. pneumoniae.*

Overall, the results from this EQAsia show improvement since the last similar EQA trial, EQA6. However, continuous participation in EQA programmes is crucial to maintain quality in a microbiology laboratory, and is a requirement for submission to the World Health Organization (WHO) Global Antimicrobial Resistance and Use Surveillance System (GLASS). Laboratories from both HH and AH sector should continue to participate in training and capacity building activities. Participating laboratories need to make sure they have a good quality management system in place that allows for constant improvement in their routine practice. Providing and maintaining a standardized level of credible diagnostic services would allow laboratories to generate reliable results.

Therefore, 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.

A special emphasis needs to be placed also on introducing methods that enable or reinforce the detection of multidrug-resistant pathogens, such as ESBL- and carbapenemase-producing Gram-negatives.

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 eight EQA trials have taken place since 2021, all of which focused on the WHO GLASS [1] and FAO priority pathogens [2]: *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Campylobacter* (*C. coli* and *C. jejuni*), *Enterococcus* (*E. faecium* and *E. faecalis*), *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. In addition, a Matrix EQA trial was offered 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 eight EQA trial of the EQAsia project (EQA8) carried out in April – June 2024. 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-[organism], 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 the two organisms different from the expected, no additional testing was needed. For the remaining five test strains of the target organism, AST results were requested.

This eight EQA trial includes identification and AST of *E. coli, K. pneumoniae, Acinetobacter spp.* 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 EQA8 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 EQA8 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 HH or AH sectors are presented separately. The laboratories are identified by codes and each code is known only by the corresponding laboratory and the organizers. The full list of laboratory codes is confidential and known only by the EQAsia consortium.

This report, 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 EQA8

A total of 38 laboratories participated in the eight EQA trial of the EQAsia project: 21 laboratories belonging to the HH Sector and 17 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 included in EQA8, one of the test strains is used as an internal control strain that will also be included in future EQAs with varying strain code.

Candidate strains for this EQA were tested at DTU Food and additionally verified by the external partner (The Peter Doherty Institute for Infection and Immunity, Australia). Expected MIC values (**Appendix 2a-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 M100-33rd 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 EQA8 protocol (**Appendix 1**) and also 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, 33rd Ed. and VET06, 1st Ed.) [3, 4]. When not available, EUCAST clinical breakpoints (Tables v. 13.0, 2023) [4] or epidemiological cut off values [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 EQA8 protocol.

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

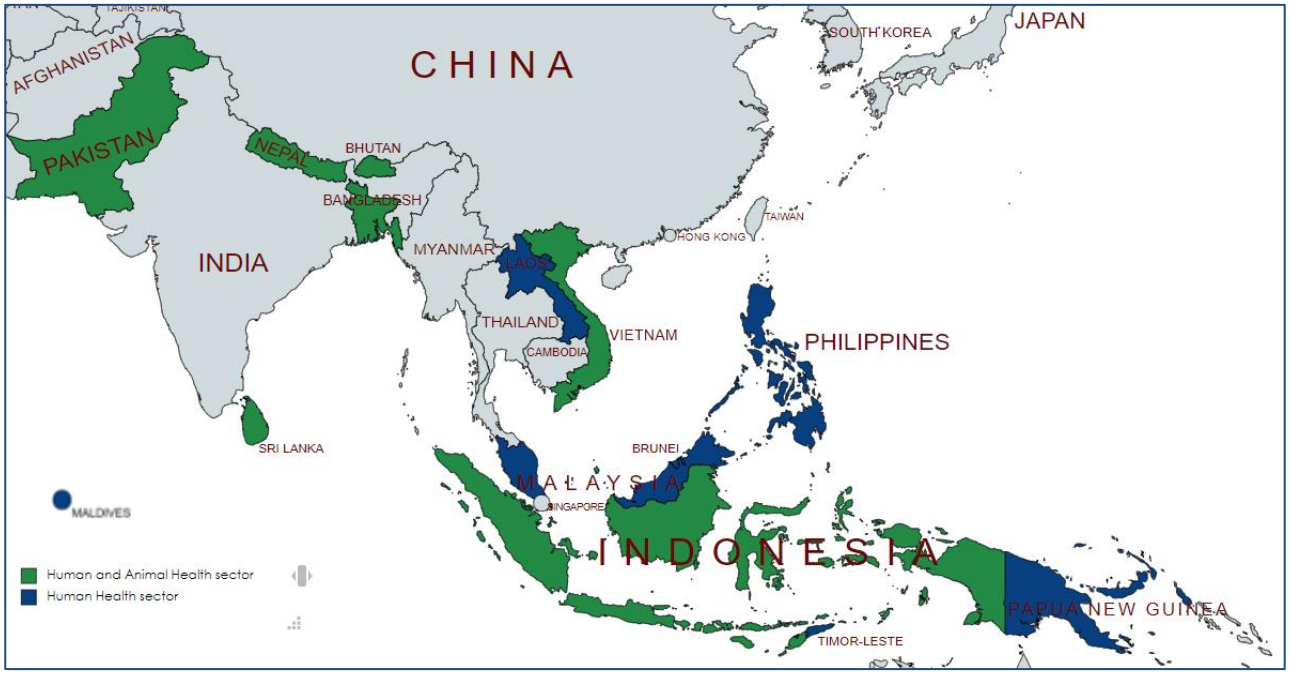


Figure 1: Countries participating in the 8th EQA of the EQAsia project. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA8 2024. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

Escherichia coli	*Klebsiella pneumoniae*	*Acinetobacter spp.*	*Staphylococcus aureus*
Amikacin	Amikacin	Amikacin	Cefoxitin
Ampicillin	Ampicillin	Cefepime	Chloramphenicol
Azithromycin	Azithromycin	Cefotaxime	Ciprofloxacin
Cefepime	Cefepime	Ceftazidime	Clindamycin
Cefotaxime	Cefotaxime	Ciprofloxacin	Erythromycin
Cefotaxime/clavulanic acid	Cefotaxime/clavulanic acid	Colistin	Fusidic acid
Cefoxitin	Cefoxitin	Doripenem	Gentamicin
Ceftazidime	Ceftazidime	Doxycycline	Kanamycin
Ceftazidime/clavulanic acid	Ceftazidime/clavulanic acid	Gentamicin	Linezolid
Chloramphenicol	Chloramphenicol	Imipenem	Penicillin
Ciprofloxacin	Ciprofloxacin	Levofloxacin	Quinupristin/dalfopristin
Colistin	Colistin	Meropenem	Rifampin
Doripenem	Doripenem	Minocycline	Sulfamethoxazole
Ertapenem	Ertapenem	Piperacillin/tazobactam	Tetracycline
Gentamicin	Gentamicin	Tigecycline	Trimethoprim
Imipenem	Imipenem	Tobramycin	Vancomycin
Levofloxacin	Levofloxacin	Trimethoprim/ sulfamethoxazole	
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 lyophilized either in ampoules or vials in March 2024 by CUVET to all participating laboratories. The shipments (UN3373, biological substances category B) were sent according to the International Air Transport Association (IATA) regulations. Participating laboratories received detailed information on how to open, revive and store these lyophilized cultures as part of the EQA8 protocol (**Appendix 1**).

2.5 Procedure

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

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

All 20 Human Health laboratories participating in the 8th EQA of the EQAsia project submitted results. Among these, 18 laboratories submitted results for *E. coli* and *K. pneumoniae* panel each. For the *Acinetobacter spp.* panel, the number of laboratories submitting data was 16, while for the *S. aureus* panel it was 19. The methodologies applied primarily by the laboratories varied and are summarized in **Figure 2**. The participants were invited to report inhibition zone diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each drug-bug combination. Only the categorisation was evaluated, whereas the inhibition zone diameters 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)**.

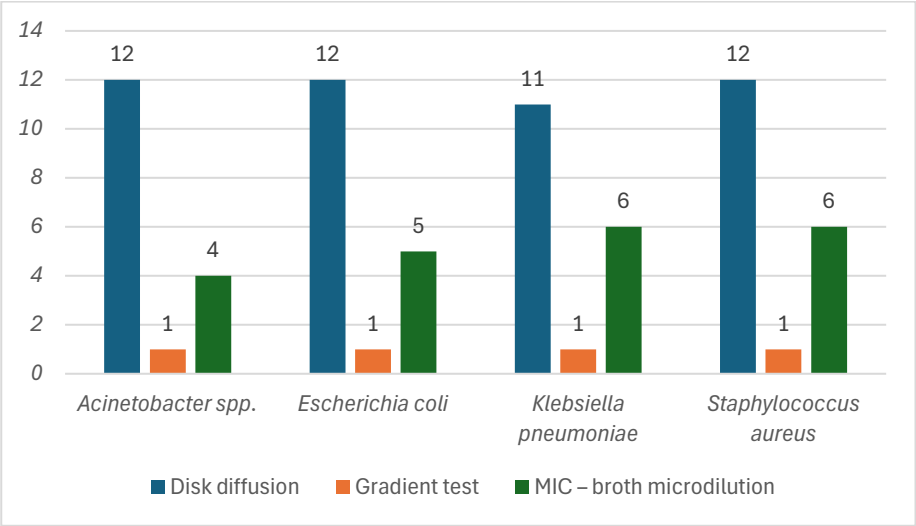


Figure 2. Methodologies used by the laboratories for antimicrobial susceptibility testing in each of the trials.

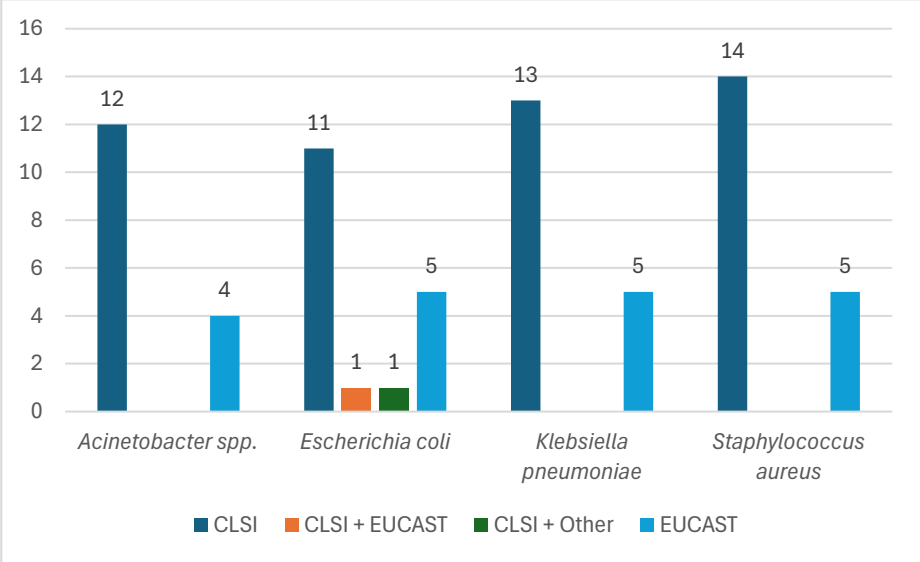


Figure 3. Use of international guidelines for interpretation of AST results by the participating laboratories.

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

The *K. pneumoniae* panel presented the highest number of total AST results (n=1271) according to the recommended antimicrobials in CLSI (**Table 2**). For the Gram-negative bacteria, fewer laboratories tested the last resort antibiotics such as colistin, doripenem and ertapenem (**Table 2**). In contrast amikacin, ampicillin, ceftazidime, ciprofloxacin, gentamicin, meropenem, and trimethoprim/ sulfamethoxazole were tested by most laboratories for the *E. coli* and *K. pneumoniae* panels, whereas amikacin, gentamicin and ciprofloxacin were tested by most laboratories for the *Acinetobacter spp.* panel.

For Gram-positive bacteria, cefoxitin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin, and tetracycline were tested by most laboratories in the *S. aureus* panel (**Table 2**).

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

	Acinetobacter spp.		*E. coli*		*K. pneumoniae*		*S. aureus*	
Amikacin	76	*8,7%*	72	*6,8%*	82	*6,5%*	--	--
Ampicillin	--	--	57	*5,4%*	71	*5,6%*	--	--
Azithromycin	--	--	31	*2,9%*	36	*2,8%*	--	--
Cefepime	58	*6,6%*	54	*5,1%*	60	*4,7%*	--	--
Cefotaxime	37	*4,2%*	38	*3,6%*	50	*3,9%*	--	--
Cefotaxime and clavulanic acid	--	--	--	--	--	--	--	--
Cefoxitin	--	--	41	*3,9%*	55	*4,3%*	89	*9,7%*
Ceftazidime	74	*8,5%*	68	*6,4%*	86	*6,8%*	--	--
Ceftazidime and clavulanic acid	--	--	--	--	--	--	--	--
Chloramphenicol	--	--	46	*4,3%*	58	*4,6%*	83	*9,0%*
Ciprofloxacin	75	*8,6%*	70	*6,6%*	84	*6,6%*	84	*9,1%*
Clindamycin	--	--	--	--	--	--	61	*6,6%*
Colistin	34	*3,9%*	27	*2,5%*	35	*2,8%*	--	--
Doripenem	18	*2,1%*	8	*0,8%*	8	*0,6%*	--	--
Doxycycline	34	*3,9%*	--	--	--	--	--	--
Ertapenem	--	--	34	*3,2%*	48	*3,8%*	--	--
Erythromycin	--	--	--	--	--	--	87	*9,4%*
Fusidic acid	--	--	--	--	--	--	30	*3,3%*
Gentamicin	76	*8,7%*	70	*6,6%*	80	*6,3%*	84	*9,1%*
Imipenem	62	*7,1%*	51	*4,8%*	68	*5,4%*	--	--
Kanamycin	--	--	--	--	--	--	9	*1,0%*
Levofloxacin	44	*5,0%*	35	*3,3%*	46	*3,6%*	--	--
Linezolid	--	--	--	--	--	--	64	*6,9%*
Meropenem	67	*7,7%*	74	*7,0%*	87	*6,8%*	--	--
Minocycline	19	*2,2%*	--	--	--	--	--	--
Nalidixic acid	--	--	33	*3,1%*	39	*3,1%*	--	--
Penicillin	--	--	--	--	--	--	83	*9,0%*
Piperacillin and tazobactam	67	*7,7%*	62	*5,8%*	74	*5,8%*	--	--
Quinupristin and dalfopristin	--	--	--	--	--	--	22	*2,4%*
Rifampin	--	--	--	--	--	--	49	*5,3%*
Sulfamethoxazole	--	--	1	*0,1%*	--	--	10	*1,1%*
Tetracycline	--	--	32	*3,0%*	46	*3,6%*	74	*8,0%*
Tigecycline	15	*1,7%*	34	*3,2%*	30	*2,4%*	--	--
Tobramycin	46	*5,3%*	44	*4,1%*	39	*3,1%*	--	--
Trimethoprim	--	--	10	*0,9%*	11	*0,9%*	29	*3,1%*
Trimethoprim and sulfamethoxazole	71	*8,1%*	69	*6,5%*	78	*6,1%*	--	--
Vancomycin	--	--	--	--	--	--	63	*6,8%*
Total	**873**		**1061**		**1271**		**921**	

Missing data or incomplete AST results entries were observed across all four EQA panels among the HH laboratories participating in EQA8. A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains.

Eleven out of 20 laboratories submitted partially incomplete results for the *E. coli* panel (**Table 3**). The highest number of incomplete results in the *E. coli* panel were observed for laboratories #02, #05, #32, #35 and #49.

Six out of 18 laboratories that selected *K. pneumoniae* did not submit complete results of their own available antimicrobial agents (**Table 4**). The highest number of incomplete results in the *K. pneumoniae* panel were seen for laboratories #32 and #35.

Only three out of 16 laboratories that selected *Acinetobacter spp.* submitted incomplete results of their own available antimicrobial agents (**Table 5**). The highest number of incomplete results in the *Acinetobacter spp.* panel was seen for laboratories #06 and #35.

Only four out of 19 laboratories selecting *S. aureus* revealed 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 #10.

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *E. coli* strains reported by HH laboratories (n=18) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Ec EQASIA 24.1	Ec EQASIA 24.3	Ec EQASIA 24.5	Ec EQASIA 24.6	Ec EQASIA 24.7
#01	--	--	--	--	--
#02	CAZ, NAL	--	--	NAL	CAZ, NAL
#04	COL	--	--	--	COL
#05	AMK, AZI	--	--	CHL	FEP, FOX, CAZ
#06	--	TOB	--	TOB	TOB
#07	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	CHL	--
#13	--	--	--	--	--
#17	TGC	--	--	--	--
#32	CHL, PT/4, TOB	CHL, PT/4	CHL, PT/4	TOB	CHL, PT/4
#34	--	--	--	--	--
#35	SMT	SMT	SMT	SMT	--
#40	--	--	--	--	--
#48	--	AMK	--	--	--
#49	--	SMT, TET	SMT, TET	SMT, TET	SMT, TET
#50	--	--	--	--	--
#51	TET	--	--	--	--

Ec, *E. coli*

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

Lab ID No.	Kp EQASIA 24.1	Kp EQASIA 24.3	Kp EQASIA 24.5	Kp EQASIA 24.6	Kp EQASIA 24.7
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	NAL
#06	--	CIP	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#13	NAL	--	--	--	--
#17	--	--	--	--	--
#32	CHL, CIP, TOB	CHL	AMP, CHL, PT/4, TOB	AMP, PT/4	AMP, CHL, PT/4
#34	--	FOX	--	--	--
#35	PT/4	TGC	TGC	TGC	MEM, TGC
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--

Kp, *K. pneumoniae*

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter spp.* strains reported by HH laboratories (n=16) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Ac EQASIA 24.1	Ac EQASIA 24.2	Ac EQASIA 24.4	Ac EQASIA 24.5	Ac EQASIA 24.7
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	COL	DOX	DOX	DOX	DOX, MIN
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#17	--	--	--	--	--
#32	CIP	--	--	--	--
#34	--	--	--	--	--
#35	CAZ	AMK, CAZ	--	--	CAZ
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--

Ac, *Acinetobacter spp.*

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by HH laboratories (n=19) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Sa EQASIA 24.2	Sa EQASIA 24.3	Sa EQASIA 24.4	Sa EQASIA 24.5	Sa EQASIA 24.7
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#05	CIP	--	--	--	PEN
#06	--	--	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#10	QND	--	QND	QND	--
#11	--	--	--	CHL	--
#12	--	--	--	--	--
#13	--	--	--	--	--
#17	--	--	--	--	--
#32	--	--	--	--	VAN
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	--	--	--
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--

Sa, *S. aureus*

3.2 *Escherichia coli* panel

18 laboratories from 14 different countries submitted results for the *E. coli* panel.

3.2.1 Bacterial identification

18 laboratories submitted results for bacterial identification (**Table 7**). The five target *E. coli* strains were identified accurately by 6 laboratories.

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

Strain	Bacterial ID	No. correct
Ec EQASIA 24.1	*Escherichia coli*	18/18
Ec EQASIA 24.2	Non- *E. coli*	13/17
Ec EQASIA 24.3	*Escherichia coli*	16/17
Ec EQASIA 24.4	Non- *E. coli*	7/15
Ec EQASIA 24.5	*Escherichia coli*	8/17
Ec EQASIA 24.6	*Escherichia coli*	16/17
Ec EQASIA 24.7	*Escherichia coli*	17/18

Ec, E. 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

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 71.2% (strain Ec EQASIA 24.5) to 94.5% (strains Ec EQASIA 24.1) (**Table 8**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were tigecycline (29.4%), chloramphenicol (29.2%), colistin (25.9%), ceftazidime (25.0%), piperacillin/tazobactam (18.8%), azithromycin (17.1%), amikacin (12.3%), and tobramycin (11.1%) whereas ampicillin, cefotaxime, doripenem, imipenem,

sulfamethoxazole, and trimethoprim revealed no deviation from the expected results (**Figure 4**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in laboratory #01, #05, #06, #07, #11, #12, #32, and #34 (**Figure 5**). In average, the deviation was 9.1% (ranging from 1.9 to 39.0%). With the acceptance level set to 5% deviation, 10 laboratories (#02, #04, #13, #17, #35, #40, #48, #49, #50, and #51) did not meet the expected performance range for the *E. coli* panel.

Table 8. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 18 HH laboratories for the *E. coli* panel.

Strain	AST in total	% Correct
Ec EQASIA 24.1	275	94.5
Ec EQASIA 24.3	248	89.9
Ec EQASIA 24.5	111	71.2
Ec EQASIA 24.6	249	93.2
Ec EQASIA 24.7	255	92.9

Ec, E. coli

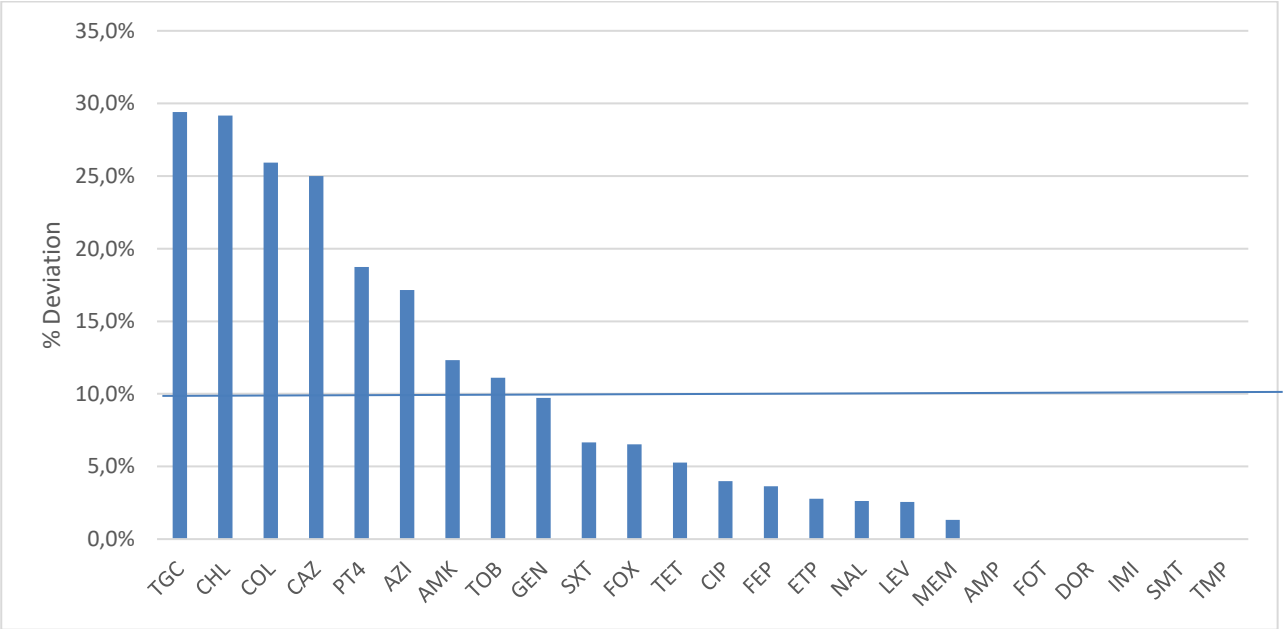


Figure 4. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=18) participating in the 8th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

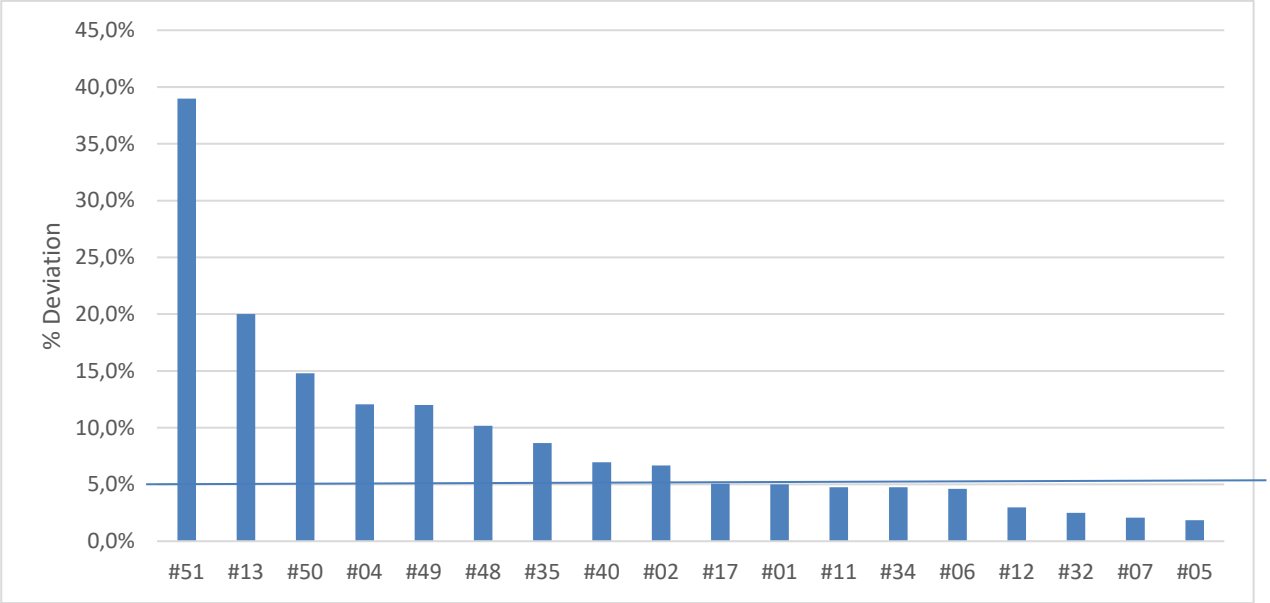


Figure 5. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=18) participating in the 8th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 β-lactamase producing *E. coli*

In total, 17 laboratories tested for ESBL/AmpC/carbapenemase production in the *E. coli* panel (**Table 9**). All laboratories that tested strain Ec EQASIA 24.3 for ESBL/AmpC/carbapenemase production have confirmed it as a carbapenemase-producer. None of the laboratories reported isolate Ec EQASIA 24.5 correctly as an ESBL + AmpC-producer.

Table 9. 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 17 HH laboratories.

Strain code		Ec EQASIA 24.1	Ec EQASIA 24.3	Ec EQASIA 24.5	Ec EQASIA 24.6	Ec EQASIA 24.7
Expected results		Carbapenemase	Carbapenemase	ESBL + AmpC	AmpC	ESBL
Obtained results (n/N)	ESBL	1/14 (7.1%)	--	5/6 (82.3%)	2/12 (16.7%)	**14/15 (93.3%)**
	Carbapenemase	**13/14 (92.9%)**	**12/12 (100.0%)**	--	--	--
	ESBL + AmpC	--	--	**--**	2/12 (16.7%)	1/15 (6.7%)
	AmpC	--	--	1/6 (16.7%)	**8/12 (66.7%)**	--

Ec, *E. coli*
(n/N) number of responses (n) out of the total of reported results (N)

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.

17 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only four 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.

Table 10. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *E. 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**
AMK	1/12	0/1	0/5	**1/18**
AMP	1/12	0/1	0/4	**1/17**
CAZ	2/12	0/1	0/5	**2/18**
CHL	4/10	0/1	--	**4/11**
CIP	0/12	0/1	/5	**0/18**
COL	--	0/1	0/3	**0/4**
DOR	0/2	--	--	**0/2**
ETP	0/4	0/1	2/4	**2/9**
FEP	0/9	0/1	0/4	**0/14**
FOT	1/8	0/1	0/1	**1/10**
FOX	0/7	0/1	0/3	**0/11**
GEN	0/11	--	0/6	**0/17**
IMI	0/10	0/1	0/3	**0/14**
LEVO	0/6	0/1	0/2	**0/9**
MERO	2/11	0/2	1/4	**3/17**
NAL	0/8	0/1	0/1	**0/10**
PT4	0/10	0/1	0/3	**0/14**
SXT	0/12	0/1	3/5	**3/18**
TET	1/7	0/1	--	**1/8**
TGC	0/2	0/1	0/3	**0/6**
TMP	0/3	--	0/1	**0/4**
TOB	0/6	0/1	0/2	**0/9**

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

Highest proportion of test results outside the expected range was observed in chloramphenicol (4 out of 11) (**Table 10**).

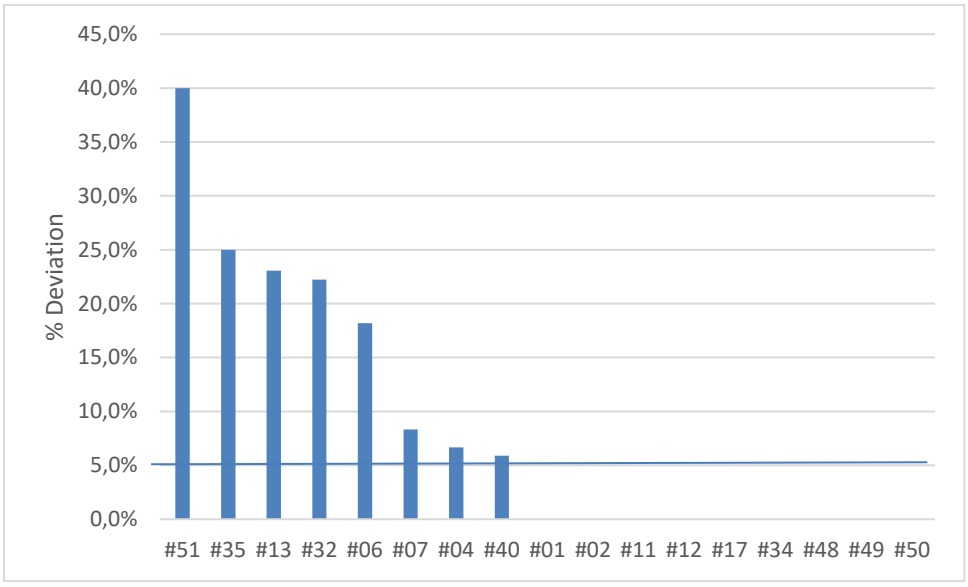


Figure 6. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *E. coli* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 6).** Laboratories #01, #02, #11, #12, #17, #34, #48, #49, and #50 presented no deviation. All other laboratories presented deviations that ranged from 5.9% to 40.0% (**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

18 laboratories from 14 countries submitted results for the *K. pneumoniae* panel.

3.3.1 Bacterial identification

18 participating laboratories submitted results for bacterial identification (**Table 11**). The five target *K. pneumoniae* strains were identified accurately by all 18 laboratories.

Table 11. Bacterial identification of each of the 7 test strains provided within the *K. pneumoniae panel*. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Kp EQASIA 24.1	*Klebsiella pneumoniae*	18/18
Kp EQASIA 24.2	Non- *K. pneumoniae*	16/16
Kp EQASIA 24.3	*Klebsiella pneumoniae*	18/18
Kp EQASIA 24.4	Non- *K. pneumoniae*	17/17
Kp EQASIA 24.5	*Klebsiella pneumoniae*	17/18
Kp EQASIA 24.6	*Klebsiella pneumoniae*	18/18
Kp EQASIA 24.7	*Klebsiella pneumoniae*	18/18

Kp, *K. 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/S) ranged from 72.9% (strain Kp EQASIA 24.6) to 90.5% (strain Kp EQASIA 24.5) (**Table 12**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were tigecycline (61.3%), levofloxacin (36.7%), tetracycline (36.7%), doripenem (33.3%), meropenem (31.8%), colistin (28.6%), nalidixic acid (26.2%), tobramycin (26.2%), azithromycin (23.1%), imipenem (21.7%), chloramphenicol (16.7%), trimethoprim/sulfamethoxazole (15.5%), amikacin (14.3%), cefepime (12.5%), piperacillin/tazobactam (12.0%), and ceftazidime (11.2%), whereas ampicillin, cefotaxime, and trimethoprim sulfamethoxazole revealed no deviation from the expected results (**Figure 7**).

Laboratory-based analysis

All the laboratories had a deviation above 5% in their performance in terms of interpretation of the results (R/S) (**Figure 8**). On average, the deviation was 18.4% (ranging from 6.2% to 63.3%).

Table 12. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 18 HH laboratories for the *K. pneumoniae* panel.

Strain	AST in total	% Correct
Kp EQASIA 24.1	271	89.7
Kp EQASIA 24.3	272	80.5
Kp EQASIA 24.5	252	90.5
Kp EQASIA 24.6	273	72.9
Kp EQASIA 24.7	270	77.4

Kp, *K. pneumoniae*

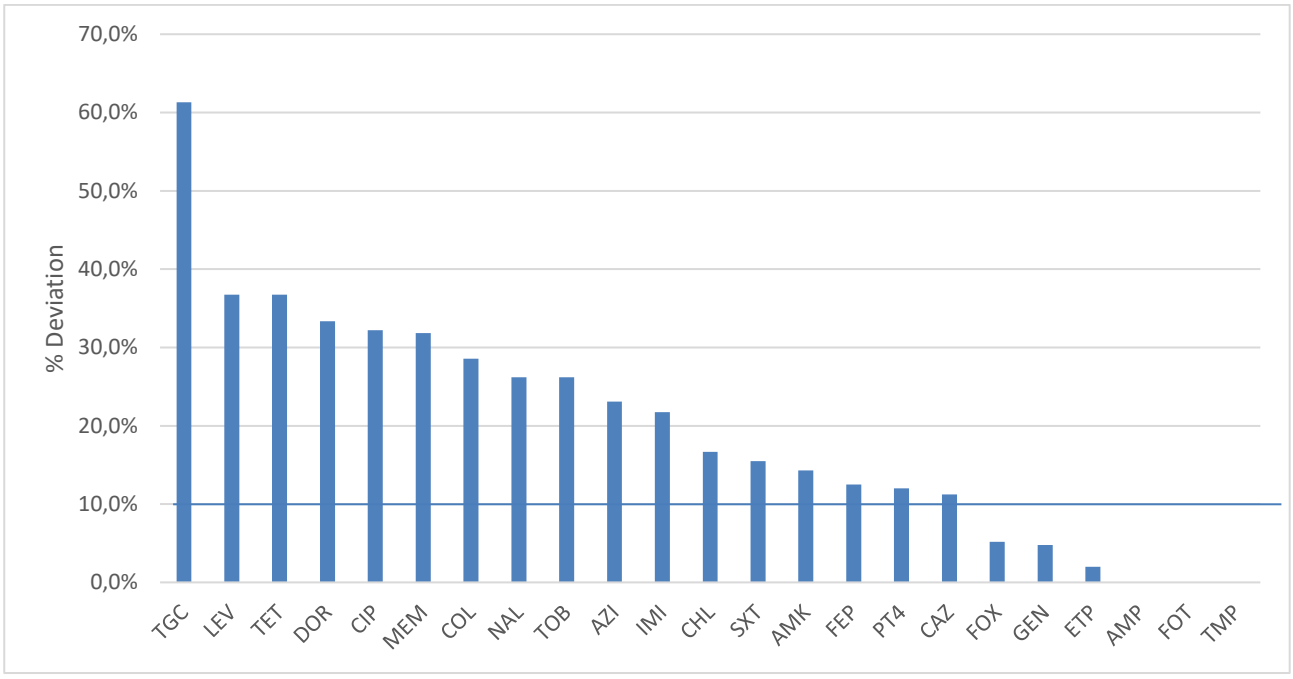


Figure 7. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=18) participating in the 8th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

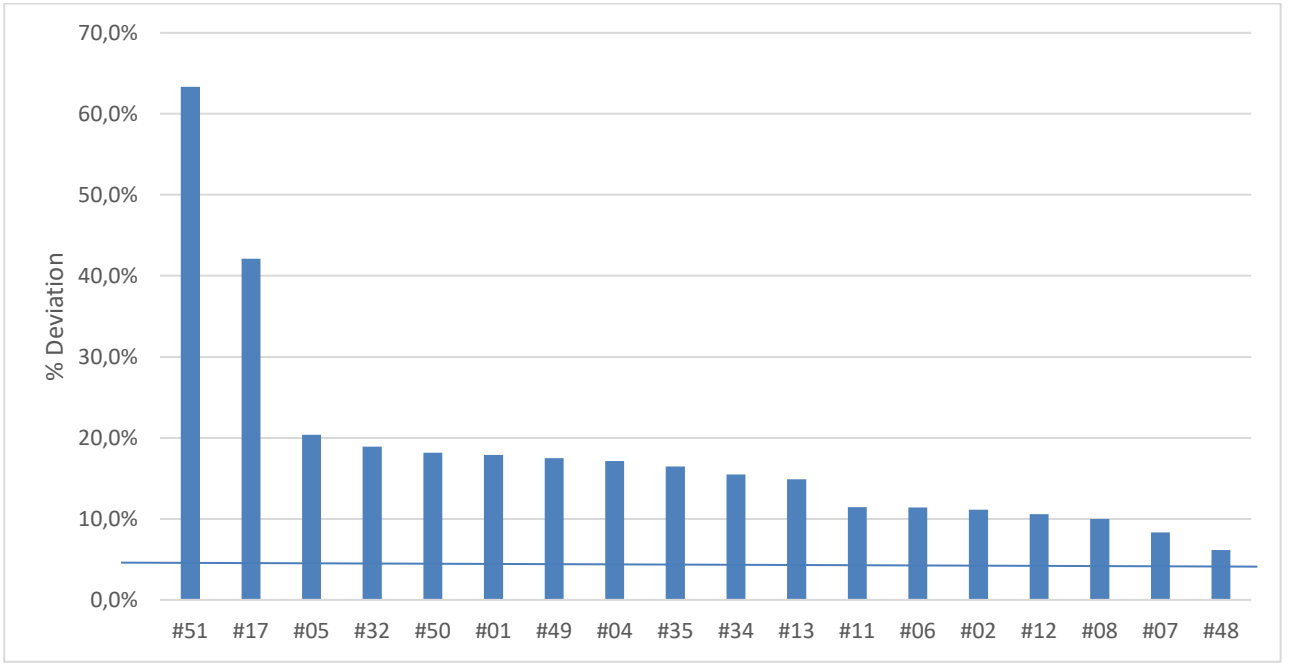


Figure 8. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=18) participating in the 8th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.3.3 β-lactamase producing *K. pneumoniae*

17 out of the 18 participating laboratories tested for ESBL/AmpC/carbapenemase production (**Table 13**). Only one laboratory accurately identified the resistant phenotype of all five *K. pneumoniae* strains. The highest deviation from the expected results was obtained for strain Kp EQASIA 24.7, an ESBL-producer (**Table 13**). Most of the laboratories reported it as a

carbapenemase-producer while the isolate exhibited an ESBL-phenotype combined with porin loss which also leads to decreased susceptibility to some of the carbapenems.

Table 13. 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 17 HH laboratories.

Strain code		Kp EQASIA 24.1	Kp EQASIA 24.3	Kp EQASIA 24.5	Kp EQASIA 24.6	Kp EQASIA 24.7
Expected results		Carbapenemase	ESBL	Carbapenemase	ESBL	ESBL + porin loss
Obtained results (n/N)	ESBL	1/16 (6.3%)	**7/17 (41.2%)**	--	**13/14 (92.9%)**	**2/14 (14.3%)**
	Carbapenemase	**13/16 (81.1%)**	8/17 (47.1%)	**13/13 (100.0%)**	1/14 (7.1%)	7/14 (50.0%)
	ESBL + AmpC	1/16 (6.3%)	2/17 (11.7%)	--	--	2/14 (14.3%)
	AmpC	--	--	--	--	2/14 (14.3%)
	Other	1/16 (6.3%)	--	--	--	**1/14 (7.1%)**

Kp, *K. pneumoniae*
(n/N) number of responses (n) out of the total of reported results (N)

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. 15 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 in this panel and only four performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922. Inhibition zone diameter was determined by disk diffusion, and MIC was determined by either gradient test or broth microdilution (**Table 14**). For testing *E. coli* NCTC 13846, MIC was determined by standard method broth microdilution.

Table 14. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *K. 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**
AMK	0/9	0/1	1/5	**1/15**
AMP	0/9	--	1/6	**1/15**
CAZ	1/9	0/1	1/5	**2/15**
CHL	1/8	0/1	1/1	**2/10**
CIP	1/9	0/1	1/5	**2/15**
COL	--	--	0/4	**0/4**
DOR	0/2	--	--	**0/2**
ETP	0/4	0/1	1/4	**1/9**
FEP	1/6	0/1	0/2	**1/9**
FOT	1/7	0/1	0/1	**1/9**
FOX	0/6	0/1	0/2	**0/9**
GEN	1/9	--	1/6	**2/15**
IMI	0/8	0/1	0/3	**0/12**
LEVO	0/5	0/1	0/2	**0/8**
MERO	0/8	0/2	2/5	**2/15**
NAL	0/7	0/1	0/2	**0/10**
PT4	0/9	0/1	0/3	**0/13**
SXT	0/9	--	4/6	**4/15**
TET	0/7	--	0/1	**0/8**
TGC	0/1	0/1	0/3	**0/5**
TMP	0/2	--	--	**0/2**
TOB	0/5	0/1	0/1	**0/7**

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

Highest proportion of test results outside of the expected range was observed in trimethoprim/sulfamethoxazole (4 out of 15) (**Table 14**).

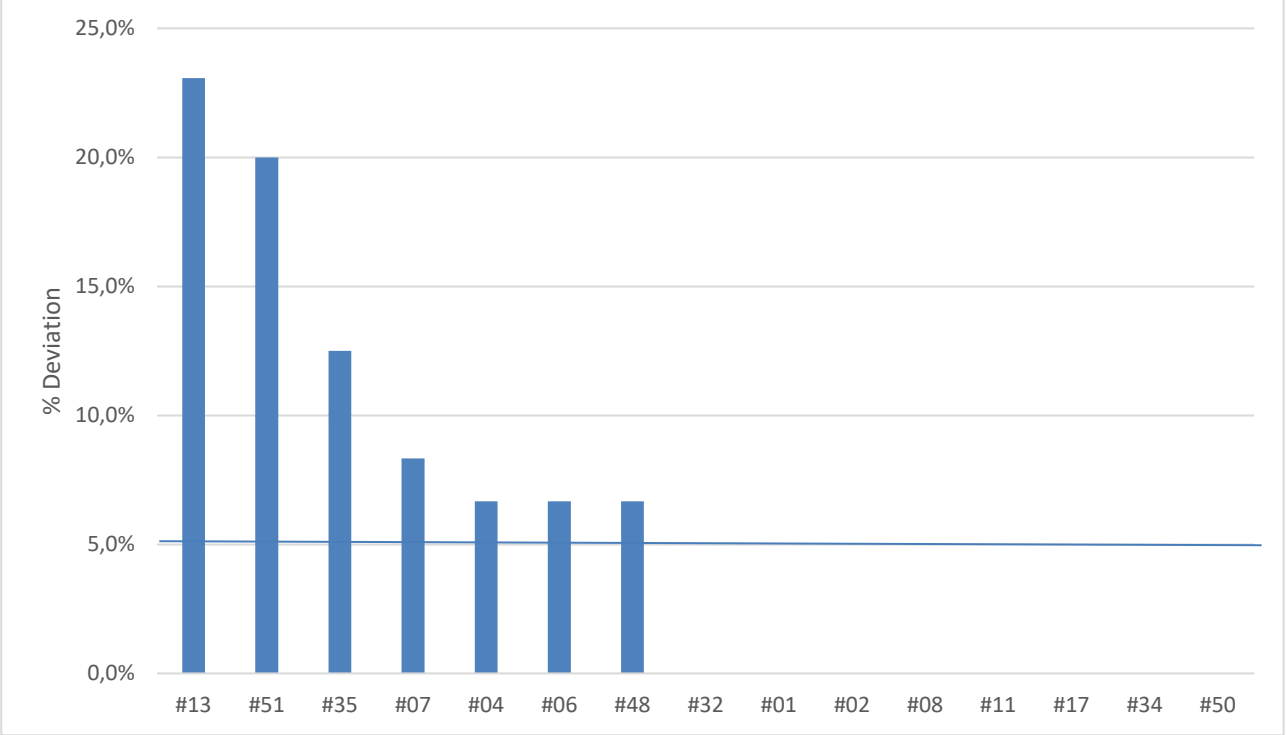


Figure 9. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *K. pneumoniae* panel by the HH laboratories.

Given the deviations, the laboratories' performance appeared independent of the methodology used for AST of the quality control strains (**Figure 9**). Laboratories #01, #02, #08, #11, #17, #32, #34, and #50 presented no deviation. All other laboratories presented deviations that ranged from 6.7% to 23.1% (**Figure 9**).

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.

3.4 *Acinetobacter spp.* panel

16 laboratories from 13 countries submitted results for the *Acinetobacter spp.* panel.

3.4.1 Bacterial identification

All 16 participating laboratories submitted results for bacterial identification (**Table 15**). The five target *Acinetobacter spp.* strains were identified correctly by 15 laboratories.

Table 15. Bacterial identification of each of the 7 test strains provided within the *Acinetobacter spp.* panel. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ac EQASIA 24.1	*Acinetobacter spp.*	16/16
Ac EQASIA 24.2	*Acinetobacter spp.*	16/16
Ac EQASIA 24.3	Non-*Acinetobacter spp.*	16/16
Ac EQASIA 24.4	*Acinetobacter spp.*	15/15
Ac EQASIA 24.5	*Acinetobacter spp.*	16/16
Ac EQASIA 24.6	Non-*Acinetobacter spp.*	16/16
Ac EQASIA 24.7	*Acinetobacter spp.*	16/16

Ac, *Acinetobacter spp.*

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.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/S) ranged from 70.9% (strain Ac EQASIA 24.1) to 97.3% (strain Ac EQASIA 24.2) (**Table 16**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected results higher than 10% were tigecycline (60.0%), cefotaxime (41.0%), colistin (32.4%), doripenem (25.0%), amikacin (21.8%), piperacillin/tazobactam (21.7%), ceftazidime (21.1%), levofloxacin (20.0%), minocycline (15.8%), cefepime (15.0%), meropenem (14.3%), and doxycycline (13.9%) (**Figure 10**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in only one laboratory, #48 (**Figure 11**). In average, the deviation was 15.7% (ranging from 4.4 to 31.7%).

Table 16. Total number of AST performed and percentage of correct results in agreement with the expected interpretive results (R/S). Results are from 16 HH laboratories for the *Acinetobacter spp.* panel.

Strain	AST in total	% Correct
Ac EQASIA 24.1	182	70.9
Ac EQASIA 24.2	182	97.3
Ac EQASIA 24.4	175	81.7
Ac EQASIA 24.5	184	84.2
Ac EQASIA 24.7	182	86.3

Ac, *Acinetobacter spp.*

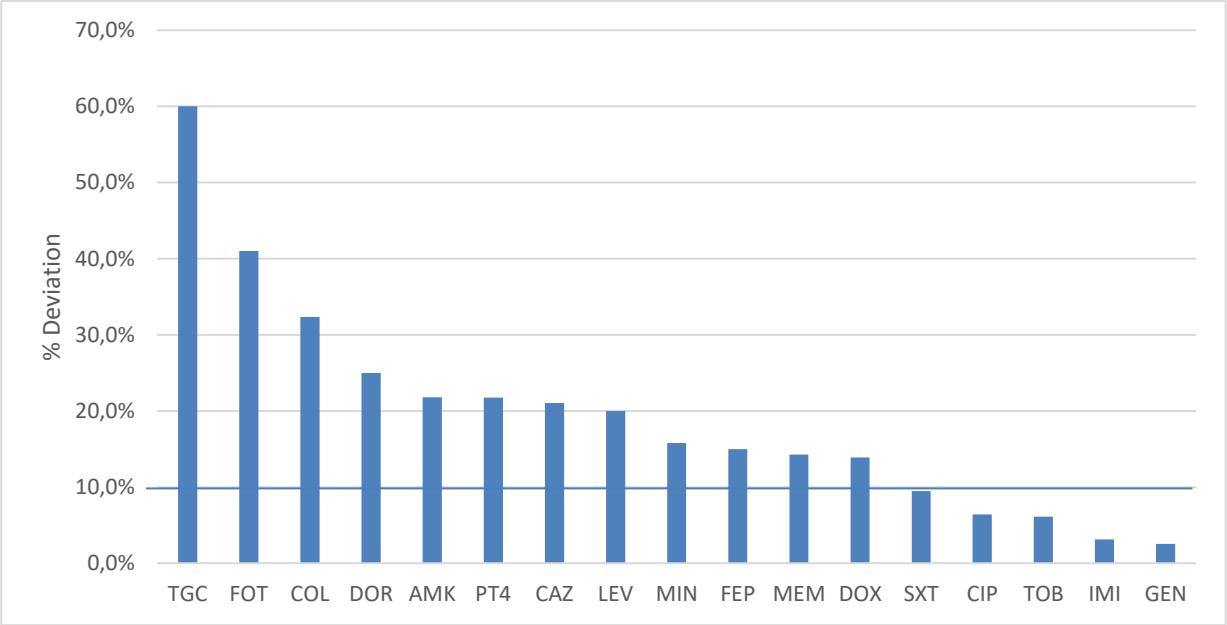


Figure 10. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter spp.* strains by HH laboratories (n=16) participating in the 8th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

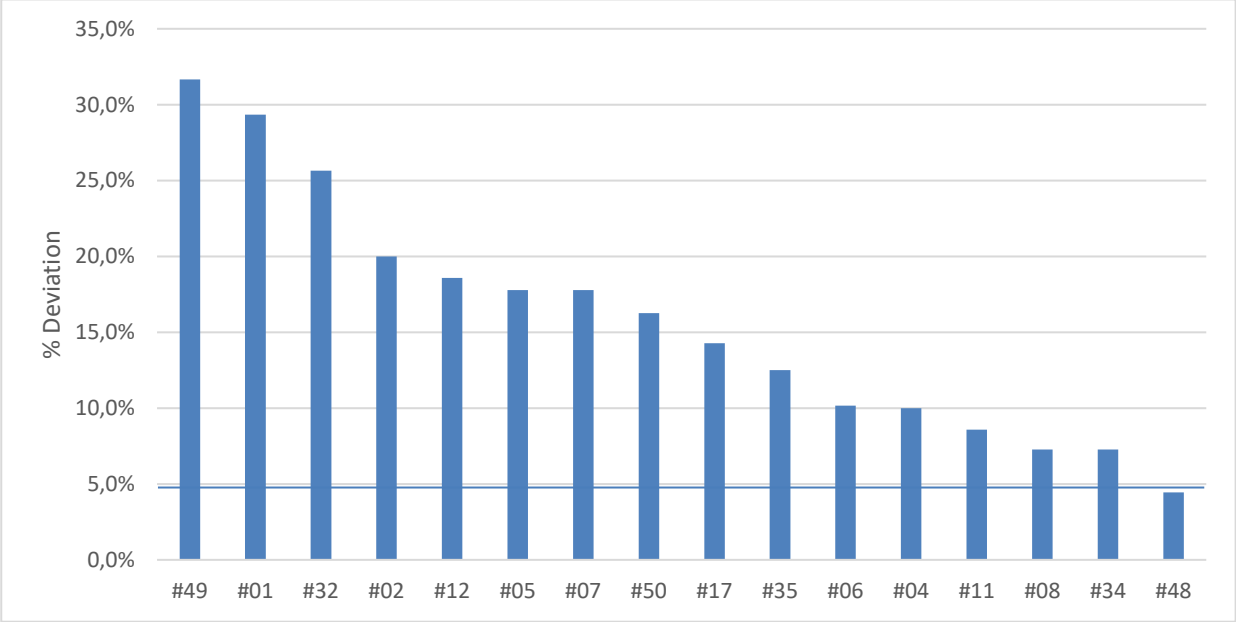


Figure 11. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter spp.* strains by HH laboratories (n=16) participating in the 8th EQA in the EQAsia project. Results are categorized by laboratory ID number.

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* and *Acinetobacter spp.* panels. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the 16 participating laboratories, 12 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**). There was only one deviation each for cefepime and piperacillin/tazobactam (**Table 17**). Disk diffusion was used in both cases of inaccurate results.

Most of the laboratories in this trial had no deviations in the quality control strains results. Ten laboratories (#02, #04, #06, #07, #08, #11, #17, #34, #48, and #50) presented no deviations. The other two laboratories (#01 and #35) had deviations of 10.0% and 16.7%, respectively (**Figure 12**).

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

Antimicrobial	Proportion outside of range			
	Disk diffusion	Gradient*	MIC	**Total**
AMK	0/7	0/1	0/3	**0/11**
FEP	1/4	0/1	0/3	**1/8**
CAZ	0/6	0/1	0/3	**0/10**
CIP	0/7	0/1	0/3	**0/11**
COL	--	--	0/5	**0/5**
FOT	0/2	0/1	--	**0/3**
DOR	0/2	--	--	**0/2**
GEN	0/7	0/1	0/3	**0/11**
IMI	0/5	0/2	0/2	**0/9**
LEVO	0/7	0/1	0/3	**0/11**
MERO	0/5	0/2	0/3	**0/10**
PT4	1/6	0/1	0/3	**1/10**
SXT	--	0/1	0/3	**0/4**
TOB	0/3	0/1	0/1	**0/5**

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

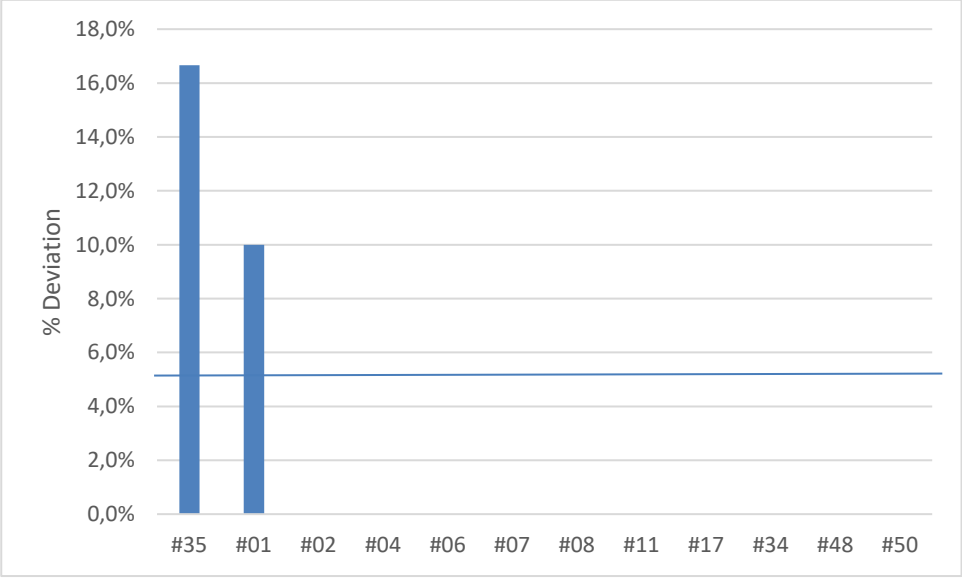


Figure 12. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *Acinetobacter spp.* panel by the HH laboratories.

3.5 *Staphylococcus aureus* panel

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

3.5.1 Bacterial identification

All 19 laboratories that selected the *S. aureus* panel submitted results for bacterial identification. Among these, 17 laboratories accurately identified the five *S. aureus* strains and the two non-*S. aureus* (**Table 18**).

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

Strain	Bacterial ID	No. correct
Sa EQASIA 24.1	Non-*Staphylococcus aureus*	19/19
Sa EQASIA 24.2	*Staphylococcus aureus*	19/19
Sa EQASIA 24.3	*Staphylococcus aureus*	19/19
Sa EQASIA 24.4	*Staphylococcus aureus*	19/19
Sa EQASIA 24.5	*Staphylococcus aureus*	18/19
Sa EQASIA 24.6	Non-*Staphylococcus aureus*	17/19
Sa EQASIA 24.7	*Staphylococcus aureus*	19/19

Sa, *S. 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/S) ranged from 92.5% (strain Sa EQASIA 24.6) to 98.3% (strain Sa EQASIA 24.5) 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 24.2	187	94.1
Sa EQASIA 24.3	189	97.4
Sa EQASIA 24.4	188	93.1
Sa EQASIA 24.5	177	98.3
Sa EQASIA 24.6	187	92.5

Sa, *S. aureus*

Antimicrobial-based analysis

The antimicrobials that resulted in percentage of deviations higher than 10% were clindamycin (20.3%), kanamycin (20.0%), and rifampin (10.2%), whereas chloramphenicol, fusidic acid, linezolid, penicillin, quinupristin/dalfopristin, and trimethoprim/sulfamethoxazole revealed no deviation from the expected results (**Figure 13**).

Laboratory-based analysis

For the *S. aureus* panel, 13 out of the 19 HH laboratories presented a deviation below 5% (laboratories #01, #06, #07, #08, #10, #11, #12, #13, #17, #32, #34, #35, and #48). The average deviation was 4.8% (ranging from 0% to 16.7%) (**Figure 14**).

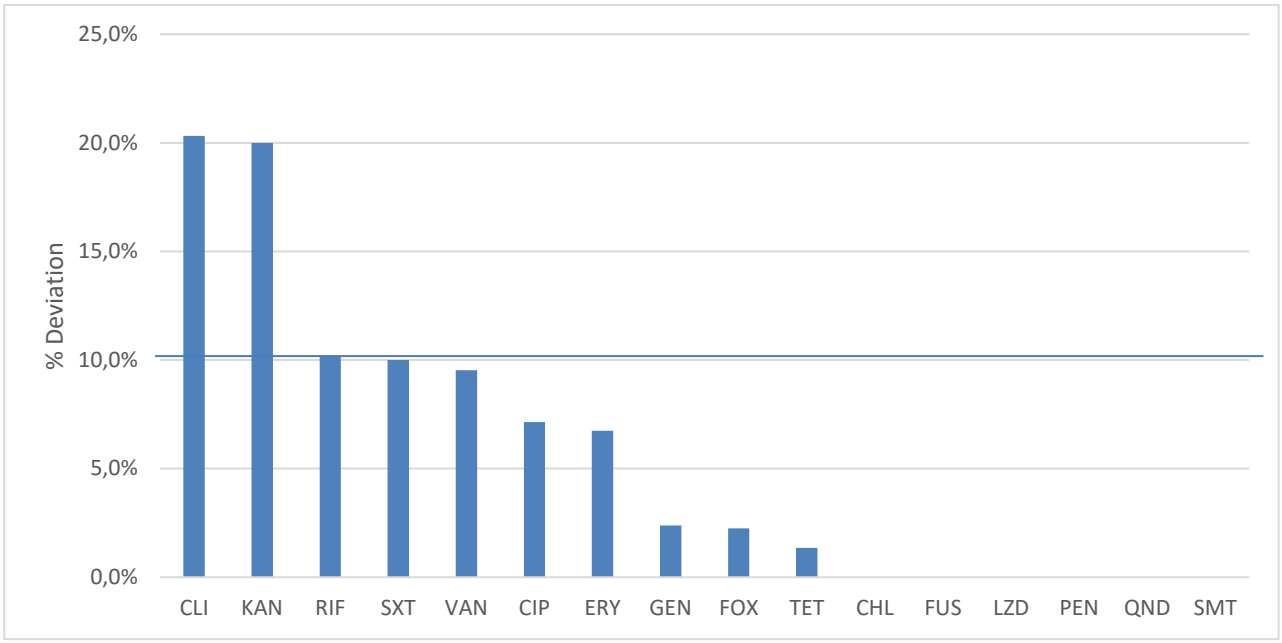


Figure 13. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=19) participating in the 8th EQA of the EQAsia project. Results are categorized by antimicrobial agent.

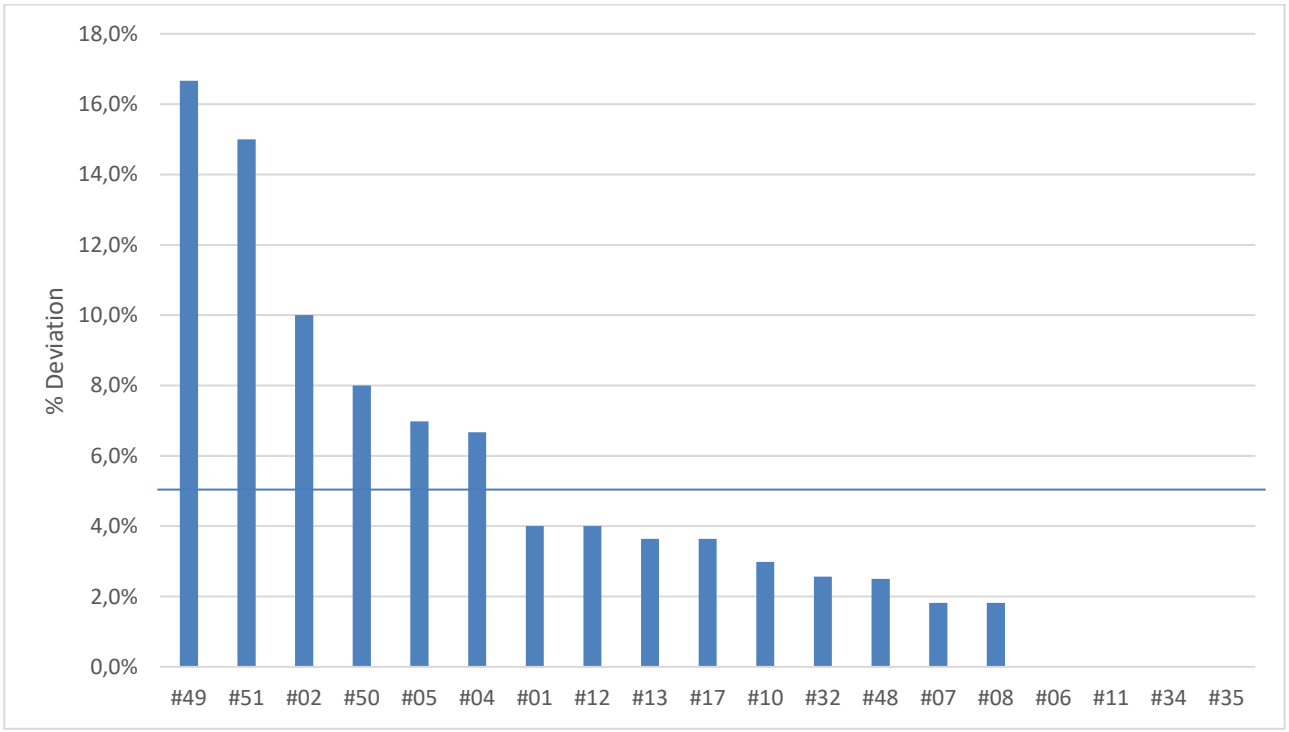


Figure 14. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=19) participating in the 8th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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) 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 19 participating laboratories, 17 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 vancomycin (2 out of 11) (**Table 20**). All deviations occurred when the disk diffusion methodology was applied.

Laboratories #01, #02, #04, #06, #07, #08, #10, #11, #17, #34, #48, and #49 had no deviations. The other five laboratories had deviations ranging from 14.3% to 37.5% (**Figure 15)**. In this panel, all the reported deviations were above the acceptance interval.

Table 20. AST of the reference strains *S. 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**
CHL	0/14	--	0/2	**0/16**
CIP	0/8	0/1	0/6	**0/15**
CLI	0/6	0/1	0/6	**0/13**
ERY	0/10	--	0/7	**0/17**
FOX	1/11	0/1	0/2	**1/14**
FUS	1/4	--	0/2	**1/6**
GEN	0/8	0/1	0/6	**0/15**
KAN	0/2	--	--	**0/2**
LZD	0/5	0/1	0/6	**0/12**
PEN	2/9	0/1	0/5	**2/15**
QND	1/1	--	0/3	**1/4**
RIF	1/4	0/1	0/3	**1/8**
SMX	0/1	--	--	**0/1**
TET	2/9	0/1	0/4	**2/14**
TMP	1/3	--	0/2	**1/5**
VAN	2/2	0/2	0/7	**2/11**

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

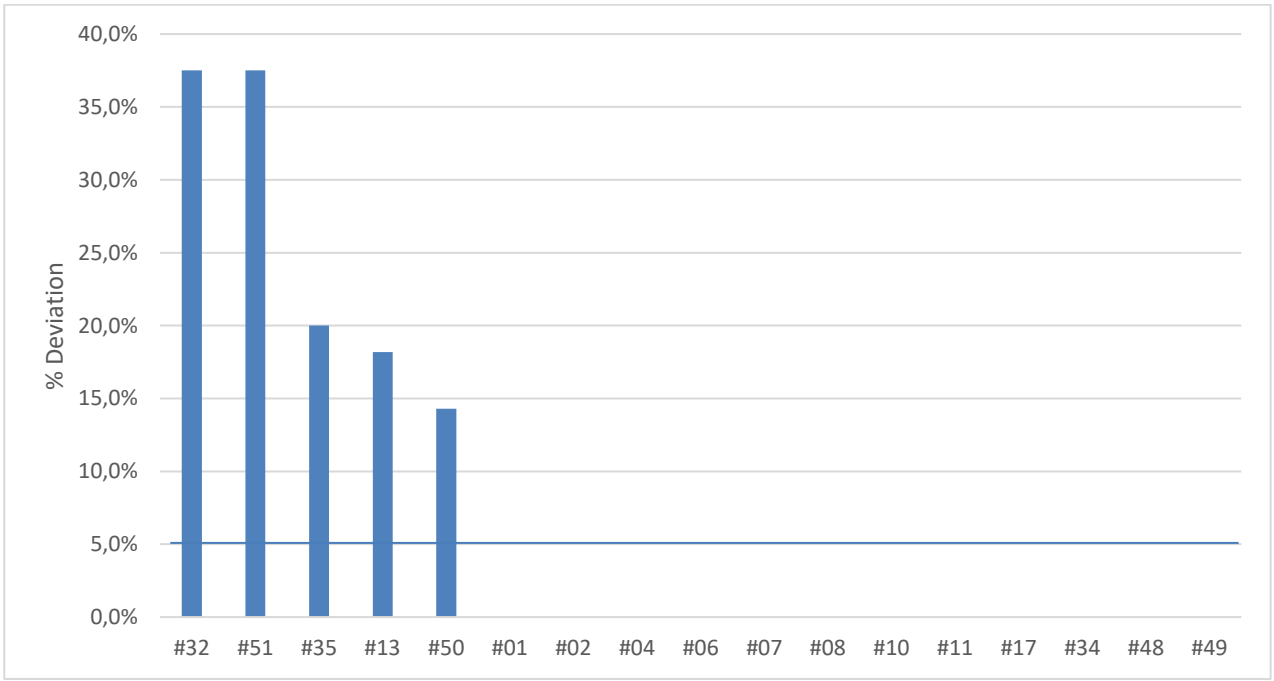


Figure 15. Percentage of deviation in the AST of *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC) in the *S. aureus* panel by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 18 Animal Health laboratories participating in the 8th EQA of the EQAsia programme, 14 laboratories submitted results for the *Escherichia coli* panel, 7 for the *Klebsiella pneumoniae* panel, 5 for the *Acinetobacter spp.*and 14 laboratories submitted results for the *Staphylococcus aureus* panel (**Figure 1**).

Applied AST methodologies for the four trials are presented in **Figure 16**. Disk diffusion as the sole method was the preferred choice for all the trials. Laboratories #38 and #47 were the two participants that used only broth microdilution (automated). Laboratories #18, #42, and #53 used a mixture of disk diffusion and broth microdilution (automated). The remaining laboratories (#37 and #56) applied disk diffusion in combination with broth macrodilution method. Laboratories #33, #46, #58 and #59 did not submit the results.

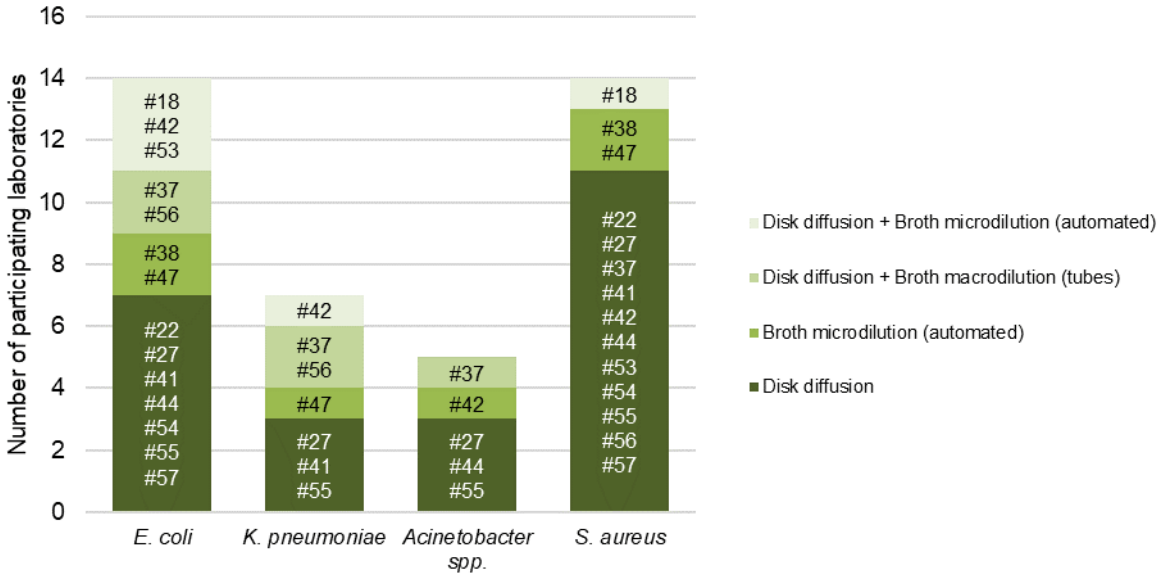


Figure 16. Methodologies applied by the AH laboratories participating for each of the panels.

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

The *E. coli* panel had the highest number of total AST results (n=897) reported by 14 participating laboratories according to the recommended antimicrobials in CLSI (**Table 21**). Some of the most frequently tested antibiotics were ciprofloxacin, ampicillin and cefotaxime. In the *K. pneumoniae* panel, participating laboratories tested and reported most frequently ciprofloxacin, tetracycline, and gentamicin. In the *Acinetobacter* panel, amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem and trimethoprim/sulfamethoxazole were tested by all five participating laboratories, whereas doripenem, levofloxacin, minocycline and tobramycin were tested by only one AH laboratory. For Gram-positive bacteria, erythromycin, gentamicin, ciprofloxacin and tetracycline were tested by most laboratories in the *S. aureus* panel.

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

	E. coli		***K. pneumoniae***		***Acinetobacter* spp.**		***S. aureus***	
Amikacin	57	6.4%	24	5.6%	23	8.9%	-	-
Ampicillin	61	6.8%	28	6.5%	-	-	-	-
Azithromycin	28	3.1%	10	2.3%	-	-	-	-
Cefepime	43	4.8%	10	2.3%	15	5.8%	-	-
Cefotaxime	61	6.8%	26	6.0%	19	7.4%	-	-
Cefoxitin	28	3.1%	9	2.1%	-	-	44	7.6%
Ceftazidime	51	5.7%	28	6.5%	23	8.9%	-	-
Chloramphenicol	51	5.7%	27	6.3%	-	-	43	7.4%
Ciprofloxacin	66	7.4%	32	7.4%	23	8.9%	61	10.6%
Clindamycin	-	-	-	-	-	-	51	8.8
Colistin	23	2.6%	19	4.4%	10	3.9%	-	-
Doripenem	4	0.4%	5	1.2%	5	1.9%	-	-
Doxycycline	-	-	-	-	9	3.5%	-	-
Ertapenem	19	2.1%	10	2.3%	-	-	-	-
Erythromycin	-	-	-	-	-	-	62	10.7%
Fusidic acid	-	-	-	-	-	-	5	0.9%
Gentamicin	57	6.4%	31	7.2%	24	9.3%	62	10.7%
Imipenem	56	6.2%	23	5.3%	23	8.9%	-	-
Kanamycin	-	-	-	-	-	-	22	3.8%
Levofloxacin	25	2.8%	5	1.2%	5	1.9%	-	-
Linezolid	-	-	-	-	-	-	25	4.3%
Meropenem	41	4.6%	24	5.6%	20	7.8%	-	-
Minocycline	-	-	-	-	5	1.9%	-	-
Nalidixic acid	28	3.1%	15	3.5%	-	-	-	-
Penicillin	-	-	-	-	-	-	41	7.1%
Piperacillin/tazobactam	29	3.2%	10	2.3%	10	3.9%	-	-
Quinupristin/dalfopristin	-	-	-	-	-	-	17	2.9%
Rifampin	-	-	-	-	-	-	28	4.8%
Sulfamethoxazole	15	1.7%	5	1.2%	-	-	18	3.1%
Tetracycline	50	5.6%	32	7.4%	-	-	54	9.3%
Tigecycline	29	3.2%	19	4.4%	15	5.8%	-	-
Tobramycin	14	1.6%	10	2.3%	5	1.9%	-	-
Trimethoprim	10	1.1%	5	1.2%	-	-	18	3.1%
Trimethoprim/sulfamethoxazole	51	5.7%	23	5.3%	24	9.3%	-	-
Vancomycin	-	-	-	-	-	-	27	4.7%
Total	**897**		**430**		**258**		**578**	

Missing data or incomplete AST result entries were observed across the four panels (**Tables 22, 23**, **24,** and **25**). Two of the 14 laboratories selecting *E. coli* did not submit complete results (**Table 22**). Regarding the *K. pneumoniae* panel, three out of the seven participating laboratories revealed incomplete results of their own available antimicrobial agents (**Table 23**). One out of five laboratories that submitted AST data for *Acinetobacter* spp. had incomplete results of their own available antimicrobial agents (**Table 24**). Four out of 14 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=14) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Ec EQAsia 24.1	Ec EQAsia 24.3	Ec EQAsia 24.5	Ec EQAsia 24.6	Ec EQAsia 24.7
#22	MERO	-	-	MERO	-
#44	-	-	TET	-	-

Ec, *E. coli*

Table 23. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by AH laboratories (n=7) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Kp EQAsia 24.1	Kp EQAsia 24.3	Kp EQAsia 24.5	Kp EQAsia 24.6	Kp EQAsia 24.7
#27	-	-	GEN	-	-
#37	-	-	FOX	-	-
#42	-	-	-	FOT	-

Kp, *K. pneumoniae*

Table 24. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter* spp. strains reported by AH laboratories (n=5) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Ac EQAsia 24.1	Ac EQAsia 24.2	Ac EQAsia 24.4	Ac EQAsia 24.5	Ac EQAsia 24.7
#55	-	-	AMK, TAZ, IMI	CIP	-

Ac, *Acinetobacter spp.*

Table 25. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by AH laboratories (n=14) participating in the 8th EQA of the EQAsia project.

Lab ID No.	Sa EQAsia 24.2	Sa EQAsia 24.3	Sa EQAsia 24.4	Sa EQAsia 24.5	Sa EQAsia 24.7
#22	-	-	SYN	-	-
#38	-	CIP	-	-	-
#42	-	-	-	-	CLI
#44	FUS	FUS	-	FUS	FUS

Sa, *S. aureus*

4.2 *Escherichia coli* panel

Fourteen laboratories from nine countries submitted results for the *E. coli* panel.

4.2.1 Bacterial identification

14 laboratories submitted results for bacterial identification (**Table 26**). The five target *E. coli* strains were identified correctly by 10 laboratories.

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

Strain	Bacterial ID	No. correct
Ec EQAsia 24.1	*Escherichia coli*	14/14
Ec EQAsia 24.2	Non-*Escherichia coli*	13/14
Ec EQAsia 24.3	*Escherichia coli*	12/13
Ec EQAsia 24.4	Non-*Escherichia coli*	8/13
Ec EQAsia 24.5	*Escherichia coli*	13/14
Ec EQAsia 24.6	*Escherichia coli*	14/14
Ec EQAsia 24.7	*Escherichia coli*	13/14

Ec, *E. coli*

4.2.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 89.1% (strain Ec EQASIA 24.5) to 95.6% (strain Ec EQASIA 24.3) for each strain, with one strain revealing a deviation above 10% (**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 eight AH laboratories for the *E. coli* panel.

Strain	AST in total	% Correct
Ec EQAsia 24.1	760	94.9
Ec EQAsia 24.3	640	95.6
Ec EQAsia 24.5	700	89.1
Ec EQAsia 24.6	760	95.0
Ec EQAsia 24.7	728	94.6

Ec, *E. coli*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were doripenem (25.0%), followed by trimethoprim/sulfamethoxazole (15.7%), and piperacillin/tazobactam (15.5%). In reverse, ciprofloxacin, ertapenem, levofloxacin, meropenem, sulfamethoxazole, tetracycline and trimethoprim revealed no deviation from the expected results (**Figure 17**). Doripenem was tested by only one laboratory. Despite the low number of incorrect results, it caused the high deviation observed.

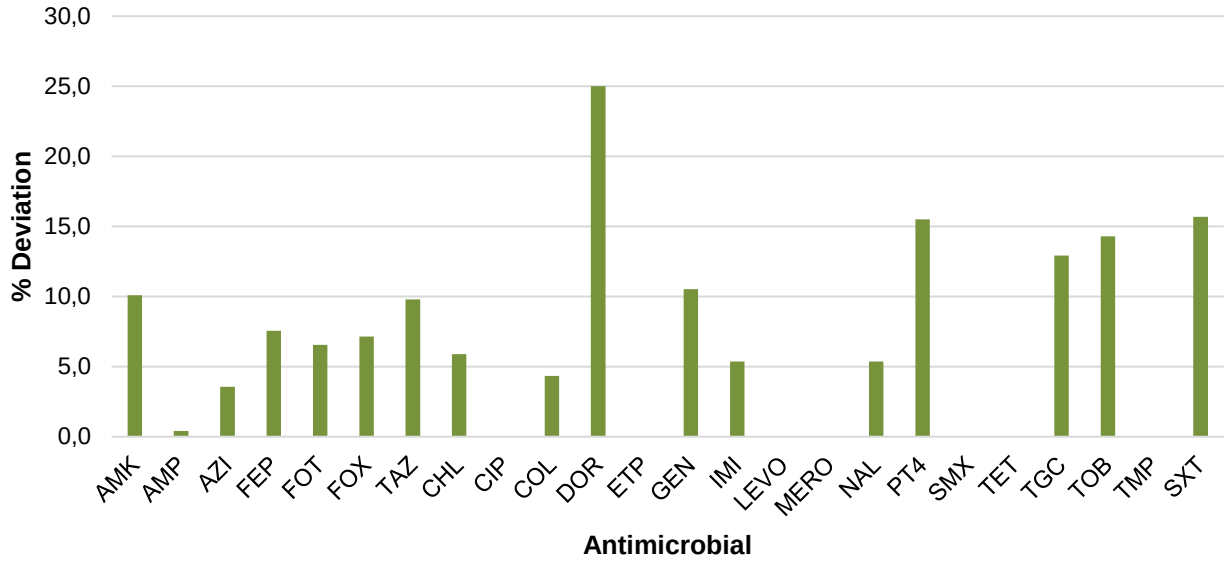


Figure 17. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=14) participating in the 8th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for six out of the 14 participants (**Figure 18**). In average, the deviation was 5.8% (ranging from 2.3 to 13.1%). As the acceptance level was set to 5% deviation, eight laboratories did not perform within the expected range for the panel.

Laboratory #22 deviations were caused by reported inaccuracies in the results for several antimicrobials, such as amikacin and piperacillin and tazobactam.

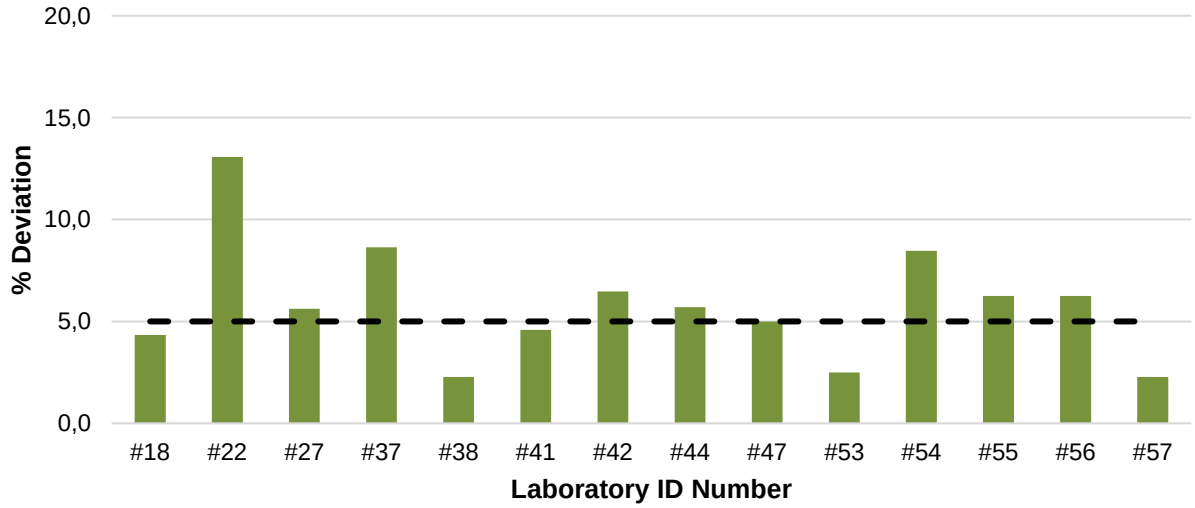


Figure 18. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=14) participating in the 8th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 β-lactamase-producing *E. coli*

Five out of the 14 participating laboratories uploaded results for this component of the *E. coli* trial (laboratories #22, #37, #42, #44, and #47). Discrepancies from the expected results are summarized in **Table 28**.

Of all five laboratories, two laboratories (#22 and #44) correctly identified all the carbapenemase phenotypes. Strain Ec EQAsia 24.7 was correctly identified by all laboratories, followed by strain Ec EQAsia 24.3, which was misclassified by only one laboratory (#44); this laboratory identified this strain as AmpC-producing *E. coli* strains instead of carbapenemase-producers. Strain Ec EQAsia 24.6 was wrongly reported as ESBL+AmpC-producer by two laboratories (#22 and #37).

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 5 AH laboratories.

Strain code		Ec EQASIA 24.1	Ec EQASIA 24.3	Ec EQASIA 24.5	Ec EQASIA 24.6	Ec EQASIA 24.7
Expected results		Carbapenemase	Carbapenemase	ESBLs +AmpC	AmpC	ESBLs
Obtained results (n/N)	ESBLs	1/5 (20.0%)	--	1/4 (25.0%)	--	**4/4 (100.0%)**
	ESBLs + AmpC	--	--	**1/4 (25.0%)**	2/4 (50.0%)	--
	Carbapenemase	**3/5 (60.0%)**	**2/3 (66.7%)**	--	--	--
	AmpC	1/5 (20.0%)	1/3 (33.3%)	1/4 (25.0%)	**2/4 (50.0%)**	--
	Other	--	--	1/4 (25.0%)	--	--

Ec, *E. coli*
(n/N) number of responses (n) out of the total of reported results (N)

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 at no cost (in previous trials) to all participating laboratories to be used as reference strains for the *E. coli* panel.

Among the 14 participating laboratories, 10 laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only two 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**). For testing *E. coli* NCTC 13846, MIC was determined by macrodilution methods.

Ertapenem had the highest proportion of test results falling outside of the expected range (4 out of 5), followed by cefepime (4 out of 6) and ciprofloxacin (6 out of 9) (**Table 29**).

In terms of performance, 10 laboratories presented deviations that ranged from 7.7% to 100.0% (**Figure 19**). Overall, the average deviation for this part of the panel was 39.3%. Laboratory #47 used only MIC method, laboratories #18, #37, #42, and #56 applied disk diffusion, broth microdilution and broth macrodilution, while the other five laboratories used disk diffusion only.

These overall deviations indicate a 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* panel. A proportion of test results outside of the expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk diffusion	MIC	Total
AMK	1/5	0/3	**1/8**
AMP	1/8	0/2	**1/10**
FEP	2/4	2/2	**4/6**
FOT	1/7	1/1	**1/7**
FOX	1/5	-	**1/5**
TAZ	1/4	0/2	**1/6**
CHL	3/7	0/1	**3/8**
CIP	4/6	2/3	**6/9**
COL	-	1/2	**1/2**
DOR	1/1	-	**1/1**
ETP	2/3	2/2	**4/5**
GEN	1/5	0/3	**1/8**
IMI	2/5	0/2	**2/7**
LEVO	1/3	1/1	**2/4**
MERO	1/3	2/3	**3/6**
NAL	0/2	0/1	**0/3**
PT4	0/3	0/2	**0/5**
SMX	0/1	0/1	**0/2**
TET	2/6	0/1	**2/7**
TGC	0/2	2/3	**1/5**
TOB	0/2	-	**0/2**
TMP	0/1	0/1	**0/2**
SXT	2/4	0/2	**2/6**

Disk diffusion – Inhibition zone diameter determination by disk diffusion;
MIC – MIC determination by broth macro- or microdilution, or by agar dilution.

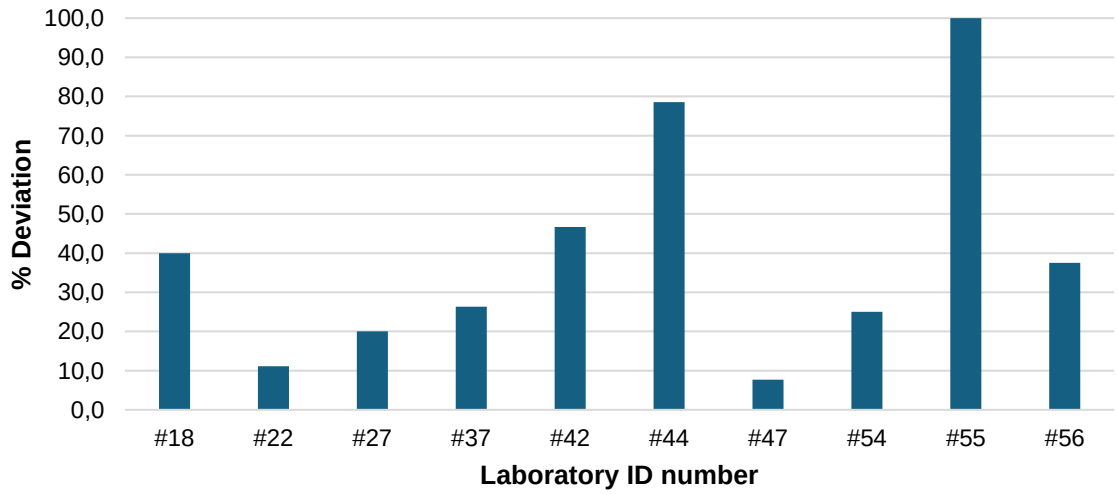


Figure 19. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *E. coli* panel by the AH laboratories.

4.3 *Klebsiella pneumoniae* panel

A total of seven laboratories from five countries submitted results for the *K. pneumoniae* panel.

4.3.1 Bacterial identification

All seven participating laboratories submitted results for bacterial identification (**Table 30**). Four out of seven laboratories accurately identified all seven test strains provided. Strain Kp EQAsia 24.5 was misidentified as non-*K. pneumoniae* by laboratories #55 and #56. Strain Kp EQAsia 24.7 was misidentified as non-*K. pneumoniae* by laboratory #27.

Table 30. Bacterial identification of each of the seven test strains provided related to the *K. pneumoniae* panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Kp EQAsia 24.1	*Klebsiella pneumoniae*	7/7
Kp EQAsia 24.2	Non-*Klebsiella pneumoniae*	6/7
Kp EQAsia 24.3	*Klebsiella pneumoniae*	7/7
Kp EQAsia 24.4	Non-*Klebsiella pneumoniae*	4/7
Kp EQAsia 24.5	*Klebsiella pneumoniae*	5/7
Kp EQAsia 24.6	*Klebsiella pneumoniae*	7/7
Kp EQAsia 24.7	*Klebsiella pneumoniae*	6/7

Kp, *K. 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.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 90.4% (strain Kp EQASIA 24.6) to 97.3% (strain Kp EQASIA 24.5) for each strain (**Table 31**).

Table 31. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 7 AH laboratories for the *K. pneumoniae* panel.

Strain	AST in total	% Correct
Kp EQAsia 24.1	368	95.4
Kp EQAsia 24.3	368	94.8
Kp EQAsia 24.5	292	97.3
Kp EQAsia 24.6	364	90.4
Kp EQAsia 24.7	328	93.3

Kp, *K. pneumoniae*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were doripenem (45.0%) and tigecycline (26.3%), whereas ampicillin, azithromycin, cefotaxime, ertapenem, sulfamethoxazole, tobramycin and trimethoprim revealed no deviation from the expected results (**Figure 20**).

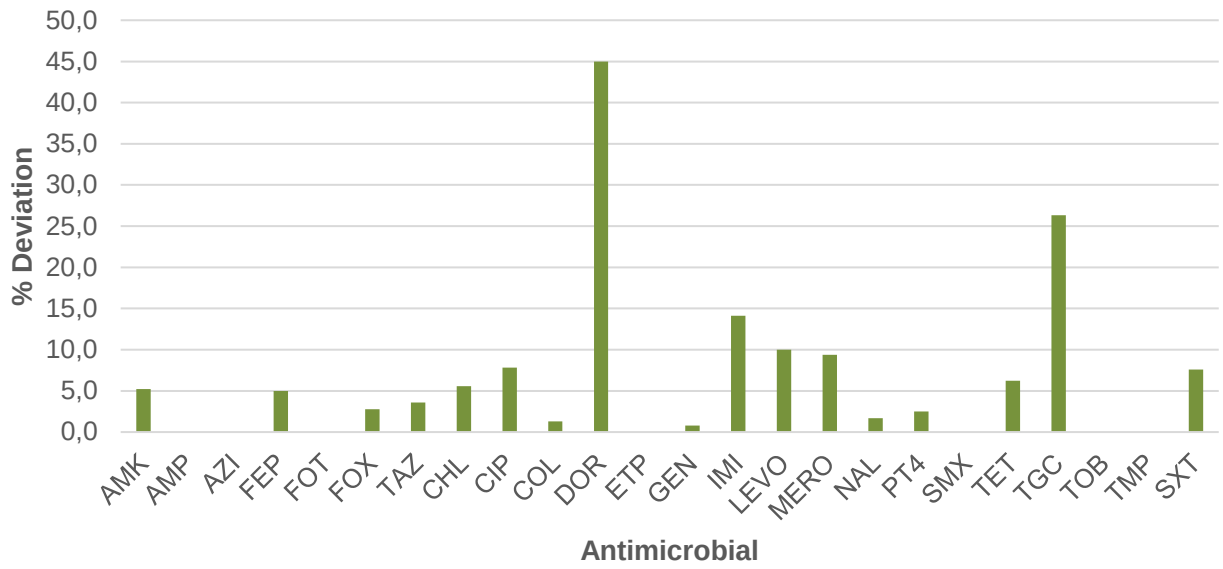


Figure 20. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=7) participating in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed in three participant (#41, #47 and #55) (**Figure 21**). In average, the deviation was 5.9% (ranging from 2.3 to 9.0%). For laboratories #22 and #37, the deviations were only a bit above the acceptance level.

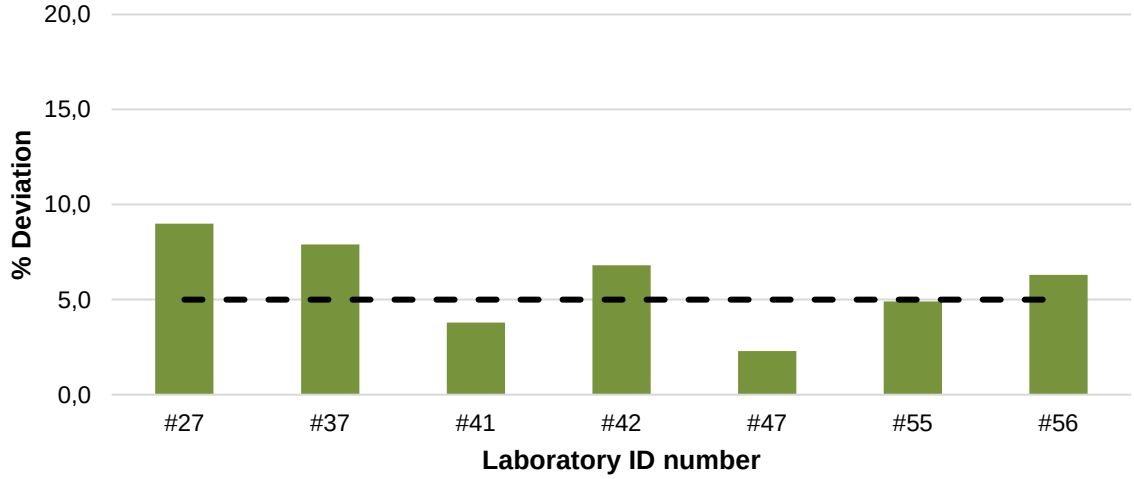


Figure 21. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=7) participating in the 8th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 β-lactamase-producing *K. pneumoniae*

Three out of the seven participating laboratories submitted results for this component of the *K. pneumoniae* panel (laboratories #37, #42 and #47). Discrepancies from the expected results are summarized in **Table 32**.

The laboratories identified the strains that produced ESBL/AmpC/carbapenemase, and then reported the specific phenotype. Strains Kp EQASIA 24.3 and Kp EQASIA 24.7 were expected to be ESBL producers; however, laboratories #37 and #42 wrongly classified strains Kp EQASIA 24.3 and Kp EQASIA 24.7 as carbapenem-producers. Strain Kp EQAsia 24.5 was correctly identified by both laboratories.

Table 32. 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 24.1	Kp EQASIA 24.3	Kp EQASIA 24.5	Kp EQASIA 24.6	Kp EQASIA 24.7
Expected results		Carbapenemase	ESBLs	Carbapenemase	ESBLs	ESBLs
Obtained results (n/N)	ESBLs	1/3 (33.3%)	**1/3 (33.3%)**	**--**	**1/3 (33.3%)**	**1/3 (33.3%)**
	ESBLs + AmpC	--	--	--	1/3 (33.3%)	--
	Carbapenemase	**2/3 (66.7%)**	2/3 (66.7%)	**2/2 (100%)**	1/3 (33.3%)	2/3 (66.7%)
	AmpC	--	**--**	**--**	--	--
	Other	--	--	--	**--**	**--**

Kp, *K. pneumoniae*
(n/N) number of responses (n) out of the total of reported results (N)

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 at no cost (in previous trials) to all participating laboratories to be used as reference strains for the *K. pneumoniae* panel.

Among the seven participating laboratories, five submitted results for the reference strain *E. coli* ATCC 25922 and only two reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli ATCC* 25922: Inhibition zone diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated) and broth macrodilution (**Table 33**).

For testing *E. coli* NCTC 13846, MIC was determined by macrodilution methods.

The highest proportion of test results outside of the expected range was observed for cefepime (1 out of 2), chloramphenicol (2 out of 4) and trimethoprim/sulfamethoxazole (2 out of 3) (**Table 33**).

In terms of performance, five laboratories presented deviations that ranged from 7.7% to 46.7% (**Figure 22**). Overall, the average deviation for this part of the panel was 24.4%. Laboratory #47 used only MIC method, laboratories #37, #42, and #56 applied disk diffusion, broth microdilution and broth macrodilution, while laboratory #27 used disk diffusion only.

Table 33. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *K. pneumoniae* panel. A proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk diffusion	MIC	Total
AMK	0/2	0/2	**0/4**
AMP	0/2	0/2	**0/4**
FEP	0/1	1/1	**1/2**
FOT	0/4	1/1	**1/5**
FOX	0/1	-	**0/1**
TAZ	0/2	0/2	**0/4**
CHL	2/3	0/1	**2/4**
CIP	2/3	1/2	**3/5**
COL	-	1/2	**1/2**
DOR	0/1	-	**0/1**
ETP	1/1	0/1	**1/2**
GEN	1/3	0/2	**1/5**
IMI	1/2	0/1	**1/3**
LEVO	-	1/1	**1/1**
MERO	0/2	1/2	**1/4**
NAL	0/1	0/1	**0/2**
PT4	0/1	0/1	**0/2**
SMX	-	0/1	**0/1**
TET	1/4	0/1	**1/5**
TGC	0/2	1/2	**1/4**
TOB	0/1	-	**0/1**
TMP	-	0/1	**0/1**
SXT	1/2	1/1	**2/3**

Disk diffusion – Inhibition zone diameter determination by disk diffusion
MIC – MIC determination by broth macro- or microdilution, or by agar dilution.

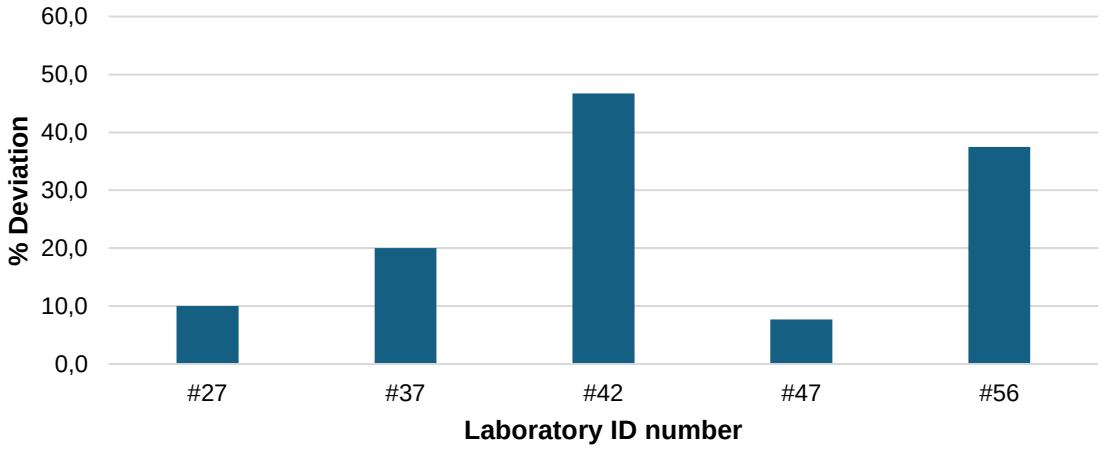


Figure 22. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *K. pneumoniae* trial by the AH laboratories.

4.4 *Acinetobacter spp.* panel

Five laboratories from four countries submitted results for the *Acinetobacter* panel.

4.4.1 Bacterial identification

All five participating laboratories submitted results for bacterial identification (**Table 34**). Four out of five laboratories accurately identified all seven test strains provided.

Table 34 Bacterial identification of each of the seven test strains provided related to the *Acinetobacter* spp. panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Ac EQAsia 24.1	*Acinetobacter* spp.	4/5
Ac EQAsia 24.2	*Acinetobacter* spp.	5/5
Ac EQAsia 24.3	Non-*Acinetobacter* spp.	4/5
Ac EQAsia 24.4	*Acinetobacter* spp.	5/5
Ac EQAsia 24.5	*Acinetobacter* spp.	5/5
Ac EQAsia 24.6	Non-*Acinetobacter* spp.	5/5
Ac EQAsia 24.7	*Acinetobacter* spp.	5/5

Ac, *Acinetobacter spp.*

4.4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 77.2% (strain Ac EQASIA 24.1) to 88.0% (strain Ac EQASIA 24.7) for each strain (**Table 35**). The results from all five strains revealed more than 10% deviation.

Table 35. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 5 AH laboratories for the *Acinetobacter* spp. panel.

Strain	AST in total	% Correct
Ac EQAsia 24.1	184	77.2
Ac EQAsia 24.2	216	81.5
Ac EQAsia 24.4	204	87.3
Ac EQAsia 24.5	212	68.9
Ac EQAsia 24.7	216	88.0

Ac, *Acinetobacter spp.*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were imipenem (30.4%), tigecycline (30.0%) and tobramycin (30.0%) (**Figure 23**).

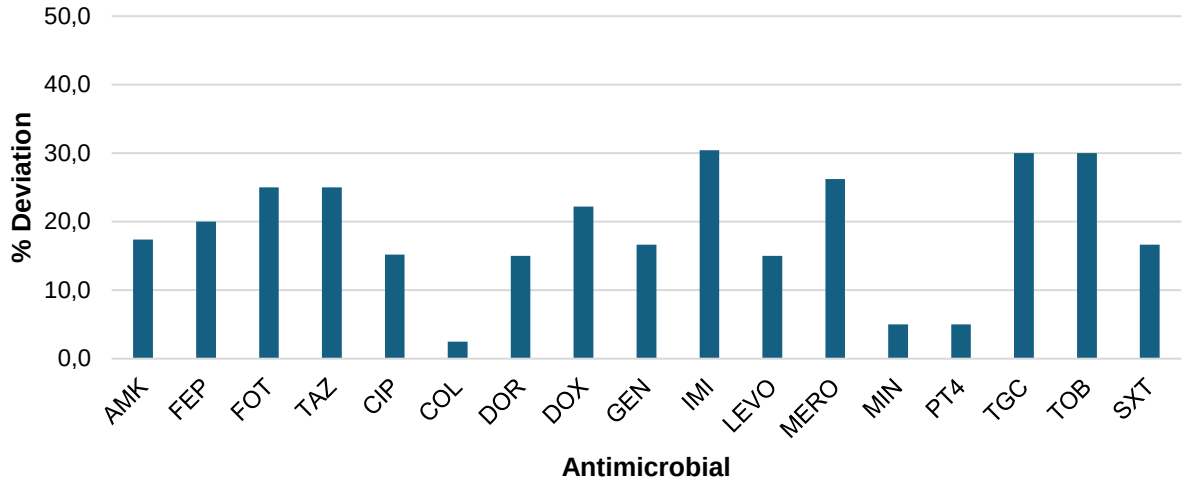


Figure 23. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* spp. strains by AH laboratories (n=5) participating in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

All participating laboratories had a deviation above 5% of laboratory performance in terms of interpretation of the results (R/I/S). The performance of one participant was a bit above the acceptable level (**Figure 24**). In average, the deviation was 19.5% (ranging from 5.4 to 43.5%). Laboratory #44 presented the highest deviation observed for this panel. Half of the submitted results were not in accordance with the expected outcome, resulting in penalties (score of 0, 1 or 3 instead of 4) and in the observed deviation.

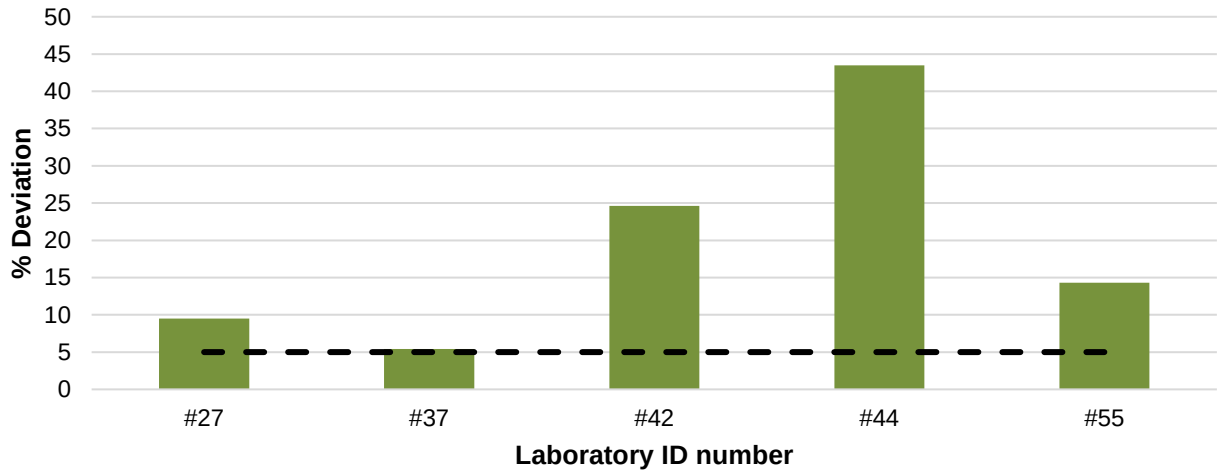


Figure 24. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* spp. strains by AH laboratories (n=5) participating in the 8th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strains *Pseudomonas aeruginosa* ATCC 27853

The quality control strain *P. aeruginosa* ATCC 27853 was sent to all participating laboratories at no cost (in previous trials) to be used as a reference strain for the *Acinetobacter* spp. panel. Among the five participating laboratories, none of laboratory submitted results for the reference strain *P. aeruginosa* ATCC 27853.

4.5 *Staphylococcus aureus* panel

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

4.5.1 Bacterial identification

All 14 participating laboratories submitted results for bacterial identification (**Table 36**). Eight laboratories correctly identified the five *S. aureus* strains and two non-*S. aureus*.

Table 36. Bacterial identification of each of the seven test strains provided related to the *S. aureus* panel. Number of correct results out of the total of AH participating laboratories is presented.

Strain	*Bacterial ID*	No. correct
Sa EQAsia 24.1	Non-*Staphylococcus aureus*	11/13
Sa EQAsia 24.2	*Staphylococcus aureus*	12/13
Sa EQAsia 24.3	*Staphylococcus aureus*	12/14
Sa EQAsia 24.4	*Staphylococcus aureus*	13/14
Sa EQAsia 24.5	*Staphylococcus aureus*	11/13
Sa EQAsia 24.6	Non-*Staphylococcus aureus*	8/14
Sa EQAsia 24.7	*Staphylococcus aureus*	14/14

Sa, *S. 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.

Strain-based analysis

The percentage of results in agreement with the expected interpretative results (R/I/S) ranged from 92.7% (strain Sa EQASIA 24.4) to 95.3% (strain Sa EQASIA 24.3) for each strain (**Table 37**). The results from all five strains revealed deviations below 10%.

Table 37. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 8 AH laboratories for the *S. aureus* panel.

Strain	AST in total	% Correct
Sa EQAsia 24.2	464	95.0
Sa EQAsia 24.3	444	95.3
Sa EQAsia 24.4	492	92.7
Sa EQAsia 24.5	392	94.6
Sa EQAsia 24.7	520	93.3

Sa, *S. aureus*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were kanamycin (21.6%), followed by fusidic acid (20.0%), and quinupristin/dalfopristin (19.1%), whereas gentamycin, linezolid, penicillin, and rifampin revealed no deviation from the expected results (**Figure 25**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for nine out of the 14 participants (**Figure 26**). In average, the deviation was 6.0% (ranging from 0.0 to 24.0%). Laboratory #55 presented the highest deviation. As the acceptance level was set to 5% deviation, five laboratories (#22, #37 #42, #44, and #55) did not perform within the expected range for the *S. aureus* panel.

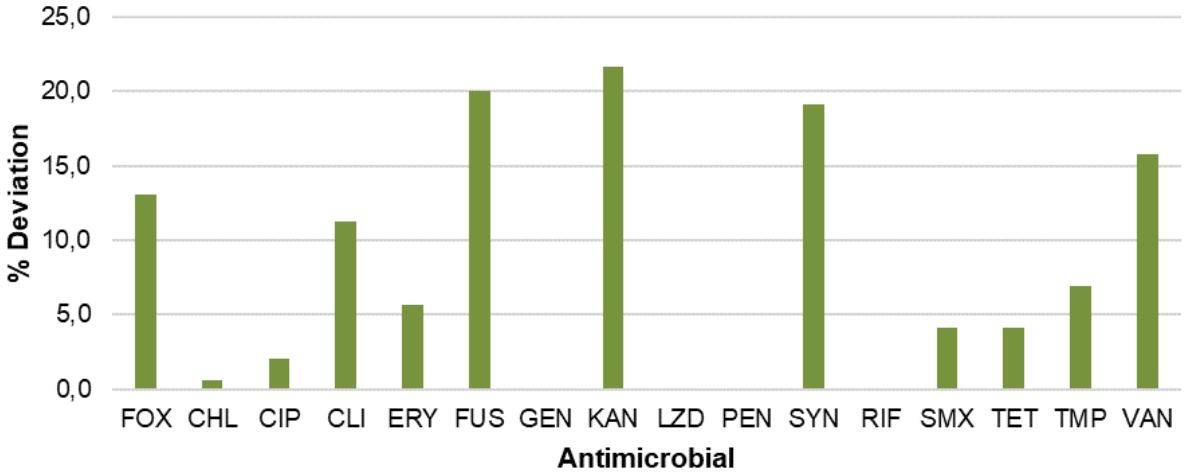


Figure 25. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=14) participating in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

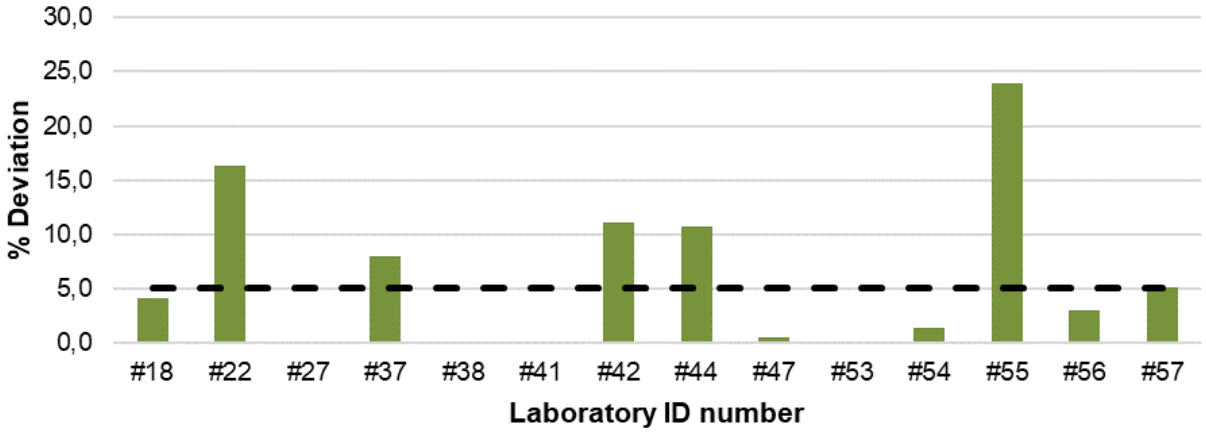


Figure 26. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=14) participating in the 8th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 at no cost (in previous trials) to all participating laboratories to be used as reference strains for the *S. aureus* panel.

Among the 14 participating laboratories, 10 submitted results for the reference strains: nine laboratories reported data for *S. aureus* ATCC 25923 reference strain as disk diffusion was the methodology applied (**Table 38,** *).

Laboratories #18 and #47 submitted AST results for *S. aureus* ATCC 29213 reference strain as broth microdilution was the methodology applied (**Table 38,** **).

The highest proportion of test results outside of the expected range was observed for trimethoprim (2 out of 3) and quinupristin/dalfopristin (2 out of 4) (**Table 38**).

Table 38. AST of the reference strain *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* panel. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk diffusion *	MIC **	Total
FOX	3/8	--	**3/8**
CHL	1/6	0/1	**1/7**
CIP	2/8	1/2	**3/10**
CLI	0/6	2/2	**2/8**
ERY	1/8	0/2	**1/10**
FUS	0/1	--	**0/1**
GEN	2/8	1/2	**3/10**
KAN	0/4	--	**0/4**
LZD	1/2	0/2	**1/4**
PEN	0/5	0/2	**0/7**
SYN	2/3	0/1	**2/4**
RIF	0/3	2/2	**2/5**
SMX	1/3	--	**1/3**
TET	2/7	1/2	**3/9**
TMP	2/3	--	**2/3**
VAN	1/3	0/2	**1/5**

Disk diffusion – 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

Examining the laboratories' performance (**Figure 27**) reveals that two laboratories had no deviation from the expected range (#22 and #42). Conversely, laboratory #44 presented a 90.0% deviation, corresponding to incorrect results for 10 out of 11 tested antimicrobials. The remaining four laboratories (#18, #27, #37, #54, #55 and #56) had two, one, three, one, two and one deviations each, respectively.

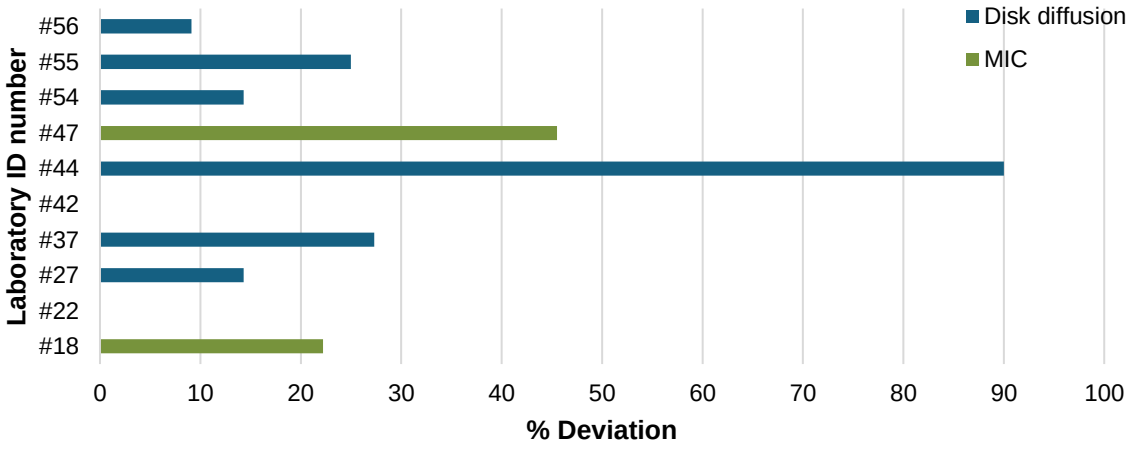


Figure 27. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* panel by the AH laboratories.

5. Results – Overall

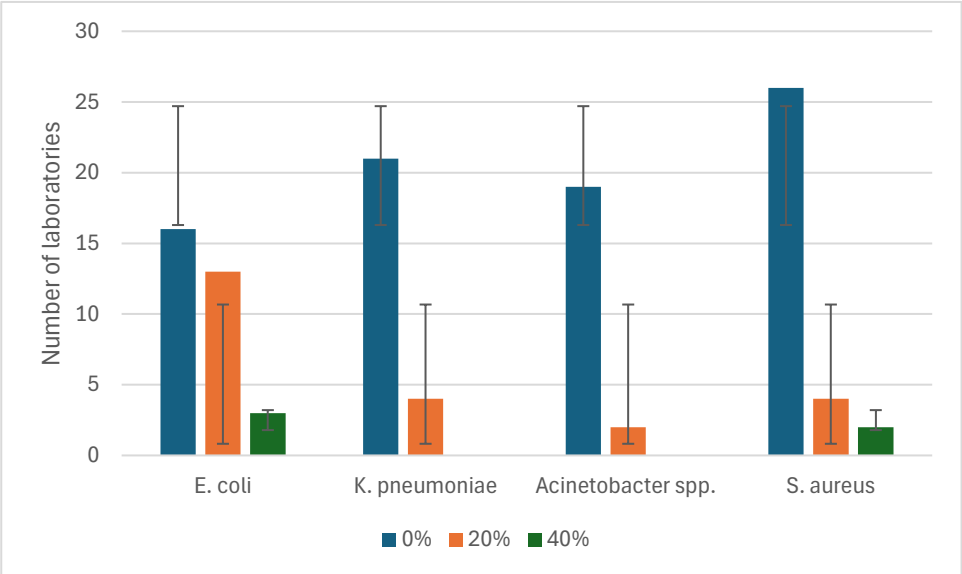
5.1 Bacterial identification

A total of 20 HH and 18 AH laboratories participated in this EQA trial. Four AH laboratories did not submit any results. 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 32 laboratories for the *E. coli* panel, 25 laboratories for the *K. pneumoniae* panel, 21 for *Acinetobacter spp.*, and 33 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 28** 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 28. Percentage of deviation in the bacterial identification of *E. coli*, *K. pneumoniae*, *Acinetobacter spp.* and *S. 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 29-32** 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 ceftazidime and tigecycline (26.0% and 23.8%, respectively) (**Figure 29**). Chloramphenicol, colistin and piperacillin/tazobactam were also some of the antimicrobials with high percentages of deviation (23.2%, 22.0%, and 21.5%, respectively). All other antimicrobials showed deviations below 20%.

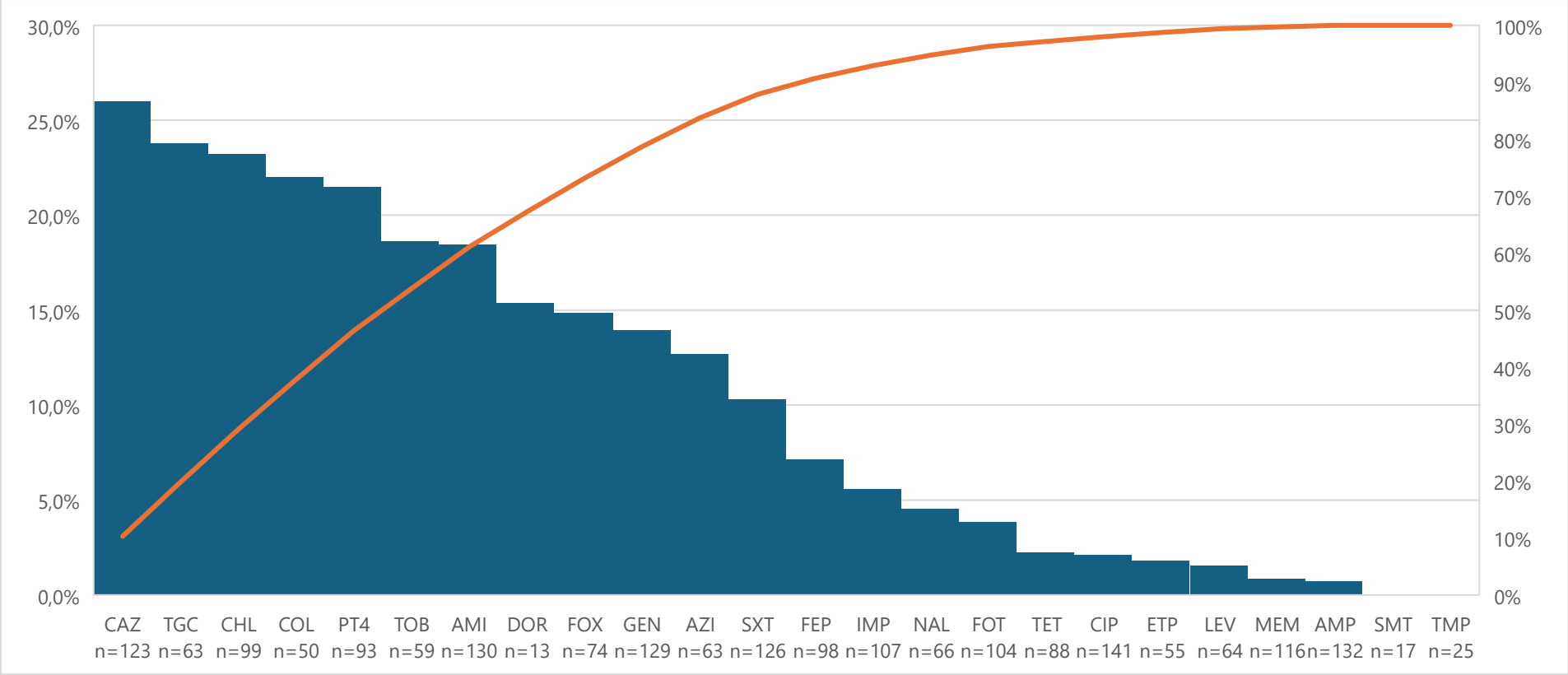


Figure 29. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains of the laboratories that submitted results (n=32) in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests evaluated is indicated below each antimicrobials' abbreviation. The orange line represents the cumulative percentage of deviation.

The results submitted for the *K. pneumoniae* panel showed most deviations for tigecycline (54.0%) mainly because of fewer tests being done (n=50) (**Figure 30**). However, when compared to the *K. pneumoniae* panel provided within EQA6, an improvement of this parameter is observed in EQA8.

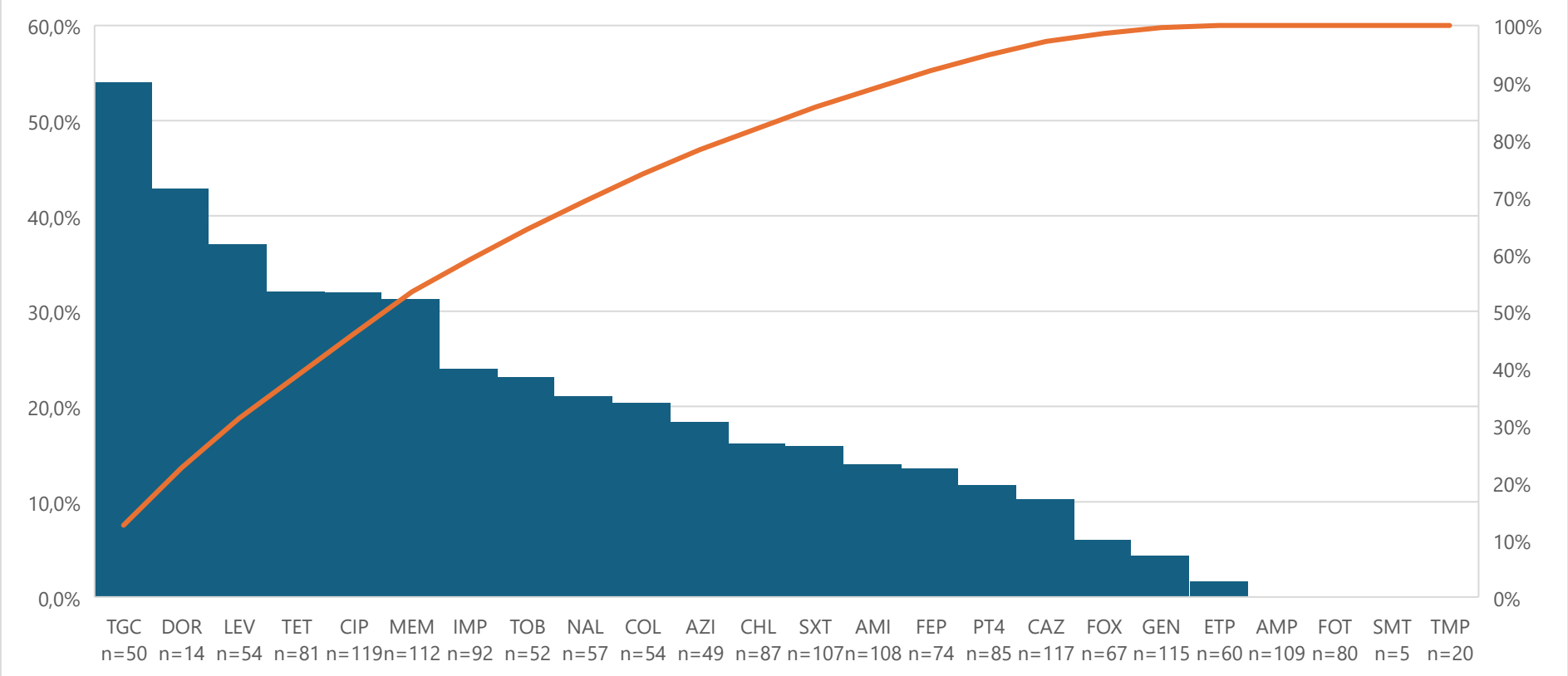


Figure 30. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains of the laboratories that submitted results (n=25) in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the cumulative percentage of deviation.

The results submitted for the *Acinetobacter spp.* panel showed deviations for all reported antimicrobials, mostly for tigecycline (53.3%) and cefotaxime (46.6%) (**Figure 31)**. All other results showed deviations of less than 30%.

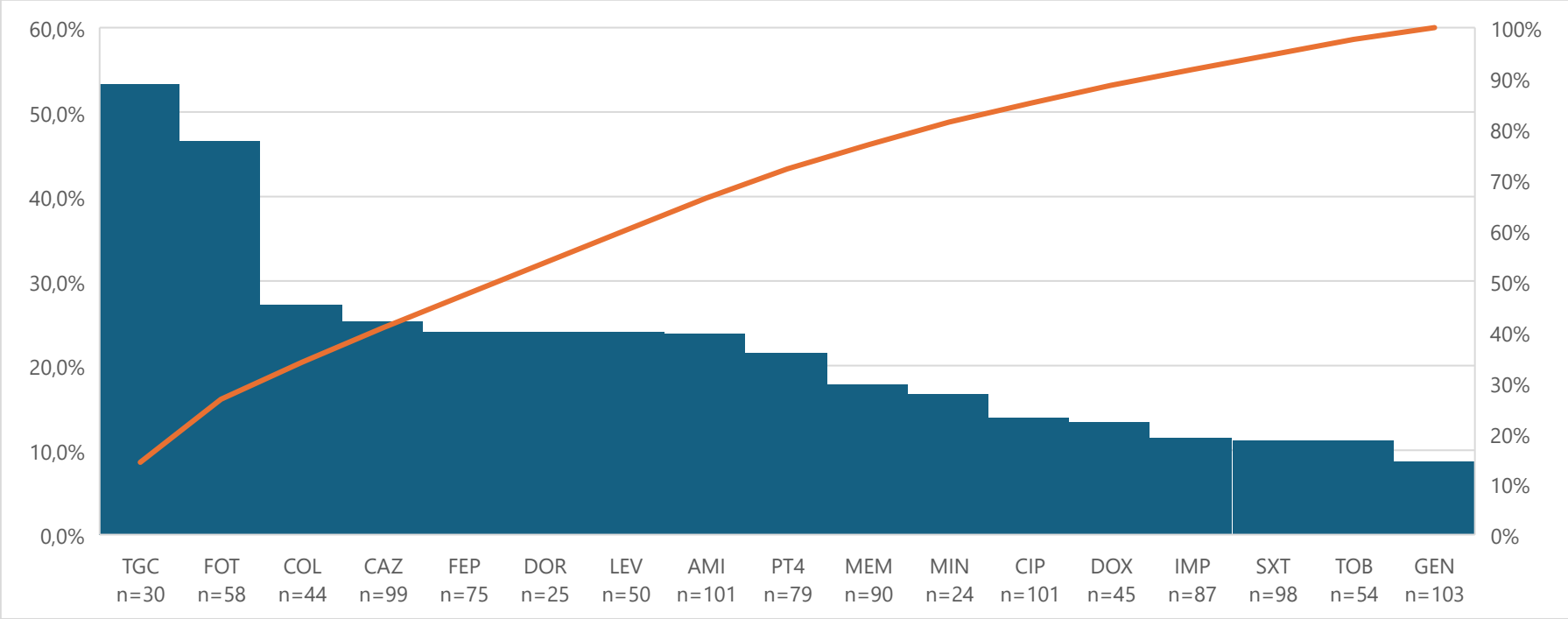


Figure 31. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter spp.* strains of the laboratories that submitted results (n=21) in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the cumulative percentage of deviation.

The results submitted in the *S. aureus* panel showed deviations for all reported antimicrobials, except for linezolid and penicillin. Most of the deviations occurred in the results of kanamycin (25.0%) and clindamycin (20.9%) (**Figure 32)**. All the other tested antibiotics showed deviations of less than 20%.

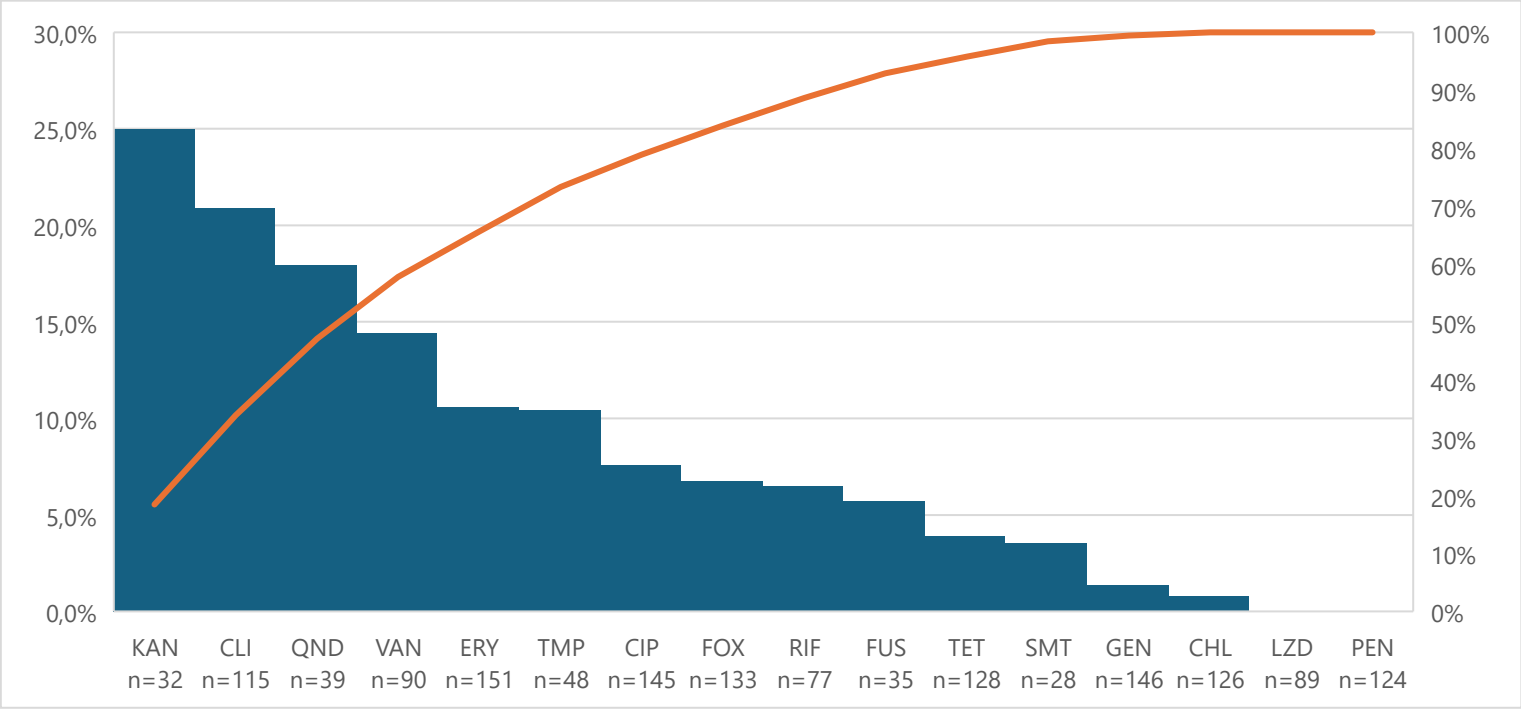


Figure 32. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains of the laboratories that submitted results (n=33) in the 8th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The orange line represents the cumulative percentage of deviation.

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.

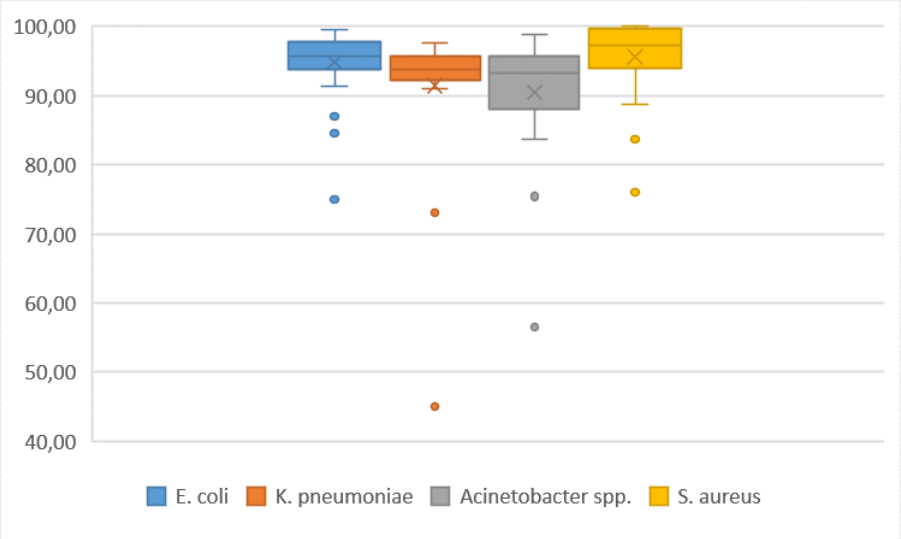


Figure 33. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 8th EQA of the EQAsia project. Most laboratories' performance rate was clustered between 88% and 100%, with a few outliers in each of the four panels.

Out of the four panels included in this trial, the obtained results were the best for the *S. aureus* and *E. coli* panels (average score 95.6% and 94.8%, respectively). The laboratories with minimum score in these two panels had a performance rate of 76.0% and 75.0%, respectively. The performance score of the participating laboratories in the *Acinetobacter spp.* panel were mostly clustered between 88% and 95%, with just two laboratories having a score below 80%. The average score for this panel was 90.5%. The results submitted for the *K. pneumoniae* panel were more heterogenous and generated scores between 45.0% and 97.7%, with an average of 91.4%.

Laboratories were ranked (#1 to #34) based on their average score across the panels in which they participated. The average score varied between 69.6% (rank #34) and 98.9% (rank #1). The total average score among all 34 laboratories that submitted results was 93.5%, while the median was 95.3%.

Overall, a large heterogeneity was observed in this EQA trial which suggests once again that the level of proficiency varies greatly among the participating laboratories. However, the performance rate was not substantially different between the four panels included in this EQA round (**Figure 33**).

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 *Acinetobacter spp.* 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. As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (7 in the *Acinetobacter spp.* panel and 5 in each of the *E. coli, K. pneumoniae* and *S. aureus* panels) did not submit results from reference strain testing at all. For the *E. coli* EQA round, there were 11 laboratories (9 HH and 2 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=16) presented deviations between 5.9% and 78.6%. Since the same quality control strains were used also for the *K. pneumoniae* panel, the submitted results were similar. Nine laboratories (8 HH and 1 AH) showed no deviations, while the results from the other 11 laboratories deviated between 6.7% and 37.5%. There was much less heterogeneity in the *Acinetobacter spp.* panel where the

deviations were between 8.3% and 25.0%. Half of the laboratories (n=7) that reported results did not have any deviations. The results from the quality control testing for *S. aureus* varied substantially between the different laboratories with deviations from the QC ranges between 9.1% and 90.9%.

Compared to the submitted AST results of the target strains, the results from the testing of the quality control strains were more heterogeneous and led to a much lower performance score in this component of the EQA trial. The greatest heterogeneity was observed in the *E. coli* panel (**Figure 34**). The minimum score in this panel was 21.4%, while in the *K. pneumoniae* panel it was 62.5%. The lowest score was seen in the *S. aureus* panel - 9.1%. The testing of the *P. aeruginosa* ATCC 27853 quality control strain was more straightforward and there were less deviations from the expected ranges. Most of the QC results generated a clustered range of scores between 75% and 100%, with a few outliers outside of this range. Overall, the highest average performance was seen in the *Acinetobacter spp.* (93.0%), while the lowest was seen in the *E. coli* panel (85.4%). The *S. aureus* panel had the highest median for the testing of the reference strains (100.0%), while *E. coli* and *K. pneumoniae* panels had the lowest median (93.3%).

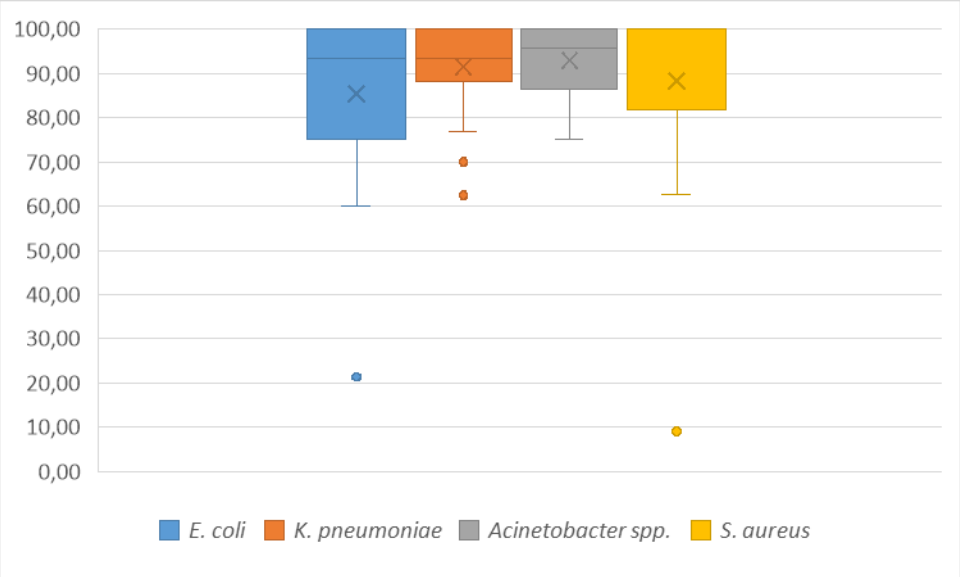


Figure 34. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 6th EQA of the EQAsia project.

6. Discussion

6.1 Human Health Laboratories

All 20 Human Health laboratories participating in the 8th EQA of the EQAsia submitted EQA results for one or more EQA panels. Disk diffusion and broth microdilution as solo methodologies were chosen by most of the participants for testing the recommended antimicrobials in each of the panels. The remaining laboratories opted for disk diffusion along with the other methods, such as gradient test, broth microdilution and/or broth macrodilution.

All laboratories that performed bacterial identification have also submitted AST results. Incomplete AST results' entries were, however, observed in all four panels, meaning that the participating laboratories did not submit complete results of their own available antimicrobial agents. However, that was true to a lesser extent for the *Acinetobacter spp.* and *S. aureus* panels. It would be expected that the isolates of each panel would be tested against the same range of antimicrobials, allowing for a solid assessment of the laboratories' performance and capacity.

Regarding the bacterial identification component of this EQA, the participants showed high proficiency in correctly identifying the *K. pneumoniae, Acinetobacter spp.* and *S. aureus* species among the provided test strains. In the *E. coli* panel, some of the laboratories demonstrated limited capacity to properly identify the target species, as some misidentifications were 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 appropriate training.

The antimicrobial susceptibility testing performance was assessed from different angles to better identify deviations from the expected results. For the Gram-negative bacteria panels, some common antimicrobials presented a high deviation from the expected results, such as tigecycline (29.4% and 61.3% in the *E. coli* and *K. pneumoniae* panels, respectively). This was likely since there are currently no breakpoints for tigecycline in CLSI, as well as to the low number of laboratories testing this antimicrobial. Lastly, colistin was also tested by a handful of labs, which might be due to the need to set up a standard broth microdilution testing. Broth microdilution is a method that requires proper experience for a good performance. For the Gram-positive bacteria panel (*S. aureus*), clindamycin revealed a rather high deviation (20.3%).

Regarding the HH laboratories' AST performance, on average, the deviation was 9.1% in the *E. coli* panel, 18.4% in the *K. pneumoniae* panel, 15.7% in the *Acinetobacter spp.* panel and only 4.8% in the *S. aureus* panel.

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. 17 participating laboratories submitted results for *E. coli* and *K. pneumoniae*, each, and, in most of the cases, were able to differentiate the carbapenemase-producers from ESBL/AmpC-producers. Only one laboratory correctly identified the resistant phenotype of all five *K. pneumoniae* strains. The main mistake observed was the incorrect classification of the carbapenemase phenotypes, even though the strains were reported as resistant to at least one of the carbapenems. The observations suggest a need for further clarification and capacity building.

Among all laboratories, there were four laboratories that did not submit antimicrobial susceptibility testing results for all or some of the quality control strains: laboratory #05 did not submit results for any of the reference strains, while the other three laboratories submitted results for some of the reference strains - #12 (only for the *E. coli* panel), #32 (for *E. coli, K.*

pneumoniae and *S. aureus*), and #49 (only for *E. coli* and *S. aureus*). According to the CLSI recommendations, quality of laboratory performance is determined by the quality control management, indicating accuracy and precision of data produced by an individual laboratory. Therefore, the correct AST results of test strains without quality control may not imply a reliable laboratory AST performance.

6.2 Animal Health Laboratories

For the Animal Health sector, 13 laboratories participated in the 8th 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 or broth macrodilution.

The participants were asked to firstly perform bacterial identification and then proceed with AST of the target strains. Incomplete AST results' entries were observed in all panels. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance.

Regarding the bacterial identification component of this EQA, the participants showed proficiency in correctly identifying the *E. coli, K. pneumoniae, Acinetobacter* and *S. aureus* species among the provided test strains. Some of the laboratories demonstrated limited capacity to properly identify the target species, as some misidentifications were 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 appropriate training.

The antimicrobial susceptibility testing performance revealed that *Klebsiella pneumoniae* test strains exhibited the lowest deviation from the expected results. In contrast, the *Acinetobacter* strains showed deviations as high as 19.4%. Regarding the antimicrobials, the ones with the highest deviation from the expected results varied from panel to panel. In the *K. pneumoniae* panel, doripenem and tigecycline presented the highest deviations with 45% and 26.3%, respectively, whereas the imipenem, tigecycline and tobramycin were problematic in the *Acinetobacter* panel (30.4%, 30.0%, and 30.0%, respectively). Doripenem presented the highest deviation in the *E. coli* panel (25.0%), which can be explained by the fact that these antimicrobials were tested by very few laboratories. For the Gram-positive bacteria *S. aureus* panel, the highest deviations from the expected results were observed for kanamycin (21.6%).

Regarding laboratories' performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. A deviation below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for six out of the 14 participants in the *E. coli* panel and for three out of the seven participants in the *K. pneumoniae* panel. In the *S. aureus* panel, eight out of fourteen participants exhibited a deviation below 5%. However, all five participants in the *Acinetobacter* panel showed a deviation above 5%.

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. Five and three participating laboratories submitted results for *E. coli* and *K. pneumoniae*, respectively. Two laboratories (#22 and #44) correctly identified all the carbapenemase phenotypes among the five *E. coli* strains. However, none of the 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 regarding capacity building.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains

relevant for each of the panels. Four laboratories (#38, #41, #53, and #57) did not submit results for 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 presents the results of the EQAsia 8th EQA trial, which was carried out in April – June 2024 and included bacterial identification and antimicrobial susceptibility testing (AST) of four prominent WHO and FAO priority pathogens: *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.* and *Staphylococcus aureus*.

The ultimate goal of EQAsia is to enable EQA participation for both Human and Food and Animal Health laboratories to assist in assessing quality of bacterial identification and antimicrobial susceptibility testing of pathogens for clinical and surveillance purposes. As in previous EQAsia EQAs, any result deviation level below 5% was tackled on an individual laboratory level and underperformance was addressed by providing additional support, feedback and technical guidance through follow ups and capacity building.

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

In terms of bacterial identification, the pathogens included in this trial were more readily identified by participating laboratories, compared to other pathogens included in previous EQAsia trials (i.e. *S. pneumoniae*, *Campylobacter spp.,* enterococci and *N. gonorrhoeae*).

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

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

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

Overall, the results from this EQAsia EQA flag once more the necessity to focus on continuous training and capacity building that underlines the importance of quality control testing in laboratories from both HH and AH sector,

especially for laboratory that have not engaged in many EQA programs. Laboratories need to ensure they have a good quality management system set in place that allows for constant improvement in their routine practice. Providing and maintaining a standardized level of credible diagnostic services is critical for laboratories to generate accurate and reliable results for clinical testing and surveillance data.

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*. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

[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 13.0, 2023. 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://antimicrobialresistance.dk/eqasia.aspx

9. Appendices

Appendix 1: EQA8 Protocol

EQAsia EQA8 trial

Protocol

Identification and antimicrobial susceptibility testing (AST) of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.* and *Staphylococcus aureus* test strains

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 EQA8 trial** includes four EQA panels each composed of seven test strains – *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.*, 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). These reference strains are original CERTIFIED cultures provided free of charge and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. Therefore, please take proper care of these strains.

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 *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.*, and *Staphylococcus aureus*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

EQA8 OUTLINE

Shipping and receipt of strains

Your laboratory is one of the 38 human health and animal health laboratories from South and Southeast Asia participating in EQA8. In April 2024, 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) reference strains have been provided in previous EQA rounds.

- ***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) reference strains have been provided in previous EQA rounds.

- ***Acinetobacter spp.* panel** - seven test strains of which five are *Acinetobacter spp.* and two are non-target species. The reference strain to be used for this panel is *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 and it has been provided in a previous EQA round.

- ***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) reference strains have been provided in previous EQA rounds.

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

N.B.!!! All the isolates are shipped lyophilized. The *E. coli* and *S. aureus* isolates are sent in ampoules. The *K. pneumoniae* and *Acinetobacter spp.* isolates are sent in vials.

Reviving and storage of 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)

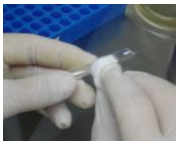
- To open and reconstitute the **ampoules**:

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.

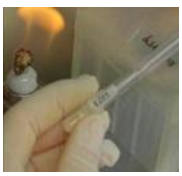
   Note: The ampoule can be cut in the middle or below the cotton wool plug.

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.

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.

Identification of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.,* 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.

Antimicrobial susceptibility testing of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.,* and *Staphylococcus aureus* test strains, and of the reference strains

The strains identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.* and *Staphylococcus 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, 33rd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 13.0, 2023) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.* 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	≤ 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*	≤ 0.5	-	≥ 1	≥ 18	-	≤ 17
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, 33rd Ed.
*Reference values are based on Enterobacterales clinical breakpoints from www.eucast.org (Tables v. 13.0, 2023)

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	≤ 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*	≤ 2	-	≥ 4	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, 33rd Ed.
*Reference values are based on *K. pneumoniae* epidemiological cut off values from https://mic.eucast.org/ on January 2023.

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 *Acinetobacter spp.*

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
Cefepime, FEP	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Cefotaxime, FOT	≤ 8	16-32	≥ 64	≥ 23	15-22	≤ 14
Ceftazidime, TAZ	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 2	4	≥ 8	≥18	15-17	≤ 14
Doxycycline, DOX	≤ 4	8	≥ 16	≥13	10-12	≤ 9
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Imipenem, IMI	≤ 2	4	≥ 8	≥ 22	19-21	≤ 18
Levofloxacin, LEVO	≤ 2	4	≥ 8	≥ 17	14-16	≤ 13
Meropenem, MERO	≤ 2	4	≥ 8	≥ 18	15-17	≤ 14
Minocycline, MIN	≤ 4	8	≥ 16	≥ 16	13-15	≤ 12
Piperacillin/tazobactam, PT4	≤ 16/4	32/4-64/4	≥ 128/4	≥ 21	18-20	≤ 17
Tigecycline, TGC*	≤ 0.5	-	≥ 1	NA	NA	NA
Tobramycin, TOB	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Trimethoprim/sulfamethoxazole, SXT	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10

Reference values are based on *Acinetobacter spp.* breakpoints from CLSI M100, 33rd Ed.

*Reference values are based on *Acinetobacter spp.* epidemiological cut off values from https://mic.eucast.org/ on January 2023.

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*	≤ 1	-	≥ 2	≥ 24	-	≤ 23
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Kanamycin, KAN*	≤ 8	-	≥ 16	≥ 18	-	≤ 17
Linezolid, LZD	≤ 4	-	≥ 8	≥ 21	-	≤ 20
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, 33rd Ed.
*Reference values are based on *Staphylococcus aureus* clinical breakpoints from www.eucast.org (Tables v. 13.0, 2023).

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 June 7th, 2024.

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

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

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

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*

	Amikacin (AMK)		Ampicillin (AMP)		Azithromycin (AZI)		Cefepime (FEP)		Cefotaxime (FOT)		Cefotaxime+clav (F/C)	
Ec EQAsia 24.1	≤ 4	S	> 32	R	64	R	32	R	> 64	R	> 64/4	
Ec EQAsia 24.3	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	
Ec EQAsia 24.5	≤ 4	S	> 32	R	> 64	R	16	R	> 64	R	≤ 0.06/4	
Ec EQAsia 24.6	≤ 4	S	> 32	R	8	S	0.25	S	8	R	8/4	
Ec EQAsia 24.7	≤ 4	S	> 32	R	8	S	16	R	64	R	≤ 0.06/4	

R, Resistant; I, Intermediate; S, Susceptible

	Cefoxitin (FOX)		Ceftazidime (TAZ)		Ceftazidime+clav (T/C)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)	
Ec EQAsia 24.1	> 64	R	> 128	R	> 128/4		≤ 8	S	> 8	R	≤ 0.25	I
Ec EQAsia 24.3	> 64	R	> 128	R	> 128/4		16	I	> 8	R	≤ 0.25	I
Ec EQAsia 24.5	16	I	8	I	0.25/4		> 64	R	> 8	R	4	R
Ec EQAsia 24.6	64	R	16	R	8/4		≤ 8	S	0.03	S	8	R
Ec EQAsia 24.7	4	S	4	S	0.25/4		≤ 8	S	≤ 0.015	S	≤ 0.25	I

R, Resistant; I, Intermediate; S, Susceptible

	Doripenem (DOR)		Ertapenem (ETP)		Gentamicin (GEN)		Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Nalidixic acid (NAL)	
Ec EQAsia 24.1	> 2	R	> 4	R	≤ 1	S	8	R	> 8	R	> 8	R	> 64	R
Ec EQAsia 24.3	> 2	R	> 4	R	> 16	R	16	R	> 8	R	> 16	R	> 64	R
Ec EQAsia 24.5	≤ 0.12	S	≤ 0.25	S	> 16	R	0.25	S	> 8	R	≤ 0.03	S	> 64	R
Ec EQAsia 24.6	≤ 0.12	S	0.03	S	≤ 0.5	S	≤ 0.12	S	≤ 1	S	≤ 0.03	S	≤ 4	S
Ec EQAsia 24.7	≤ 0.12	S	≤ 0.25	S	≤ 0.5	S	≤ 0.12	S	≤ 1	S	≤ 0.03	S	≤ 4	S

R, Resistant; I, Intermediate; S, Susceptible

	Pip/Tazo (PT/4)		Sulfamethoxazole (SMX)		Tetracycline (TET)		Tigecycline (TGC)		Tobramycin (TOB)		Trimethoprim (TMP)		Trime/Sulfa (SXT)	
Ec EQAsia 24.1	> 64	R	> 512	R	> 32	R	≤ 0.25	S	≤ 1	S	> 16	R	> 4/76	R
Ec EQAsia 24.3	> 64	R	> 512	R	> 32	R	≤ 0.25	S	> 8	R	> 16	R	> 4/76	R
Ec EQAsia 24.5	≤ 8	S	> 512	R	> 32	R	≤ 0.25	S	4	I	> 16	R	> 4/76	R
Ec EQAsia 24.6	≤ 8	S	≤ 8	S	> 32	R	≤ 0.25	S	≤ 1	S	0.5	S	≤ 0.5/9.5	S
Ec EQAsia 24.7	≤ 8	S	≤ 8	S	≤ 2	S	≤ 0.25	S	≤ 1	S	≤ 0.25	S	≤ 0.5/9.5	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Amikacin (AMK)		Ampicillin (AMP)		Azithromycin (AZI)		Cefepime (FEP)		Cefotaxime (FOT)		Cefotaxime+clav (F/C)	
Kp EQAsia 24.1	≤ 4	S	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	
Kp EQAsia 24.3	≤ 4	S	> 32	R	16	S	> 32	R	> 64	R	0.5/4	
Kp EQAsia 24.5	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	
Kp EQAsia 24.6	≤ 4	S	> 32	R	16	S	≤ 2	S	16	R	≤ 0.06/4	
Kp EQAsia 24.7	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	4/4	

R, Resistant; I, Intermediate; S, Susceptible

	Cefoxitin (FOX)		Ceftazidime (TAZ)		Ceftazidime+clav (T/C)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)	
Kp EQAsia 24.1	> 64	R	128	R	> 128/4		64	R	> 8	R	≤ 0.25	I
Kp EQAsia 24.3	32	R	128	R	1/4		≤ 8	S	1	R	≤ 0.25	I
Kp EQAsia 24.5	> 64	R	> 128	R	> 128/4		> 64	R	> 8	R	≤ 0.25	I
Kp EQAsia 24.6	4	S	≤ 1	S	≤ 0.12/4		≤ 8	S	0.5	I	≤ 0.25	I
Kp EQAsia 24.7	> 64	R	> 128	R	4/4		> 64	R	> 8	R	≤ 0.25	I

R, Resistant; I, Intermediate; S, Susceptible

	Doripenem (DOR)		Ertapenem (ETP)		Gentamicin (GEN)		Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Nalidixic acid (NAL)	
Kp EQAsia 24.1	> 2	R	> 4	R	> 16	R	8	R	> 8	R	> 16	R	> 64	R
Kp EQAsia 24.3	1	S	4	R	≤ 0.5	S	2	I	≤ 1	S	1	S	8	S
Kp EQAsia 24.5	> 2	R	> 4	R	> 16	R	> 16	R	> 8	R	> 16	R	> 64	R
Kp EQAsia 24.6	≤ 0.12	S	0.03	S	> 16	R	0.5	S	≤ 1	S	0.06	S	8	S
Kp EQAsia 24.7	1	S	> 4	R	> 16	R	≤ 1	S	> 8	R	2	I	> 64	R

R, Resistant; I, Intermediate; S, Susceptible

	Pip/Tazo (PT/4)		Sulfamethoxazole (SMX)		Tetracycline (TET)		Tigecycline (TGC)		Tobramycin (TOB)		Trimethoprim (TMP)		Trime/Sulfa (SXT)	
Kp EQAsia 24.1	> 64	R	> 512	R	> 32	R	2	S	> 8	R	> 16	R	> 4/76	R
Kp EQAsia 24.3	> 64	R	> 512	R	≤ 4	S	≤ 0.25	S	≤ 1	S	> 16	R	> 4/76	R
Kp EQAsia 24.5	> 64	R	> 512	R	8	I	1	S	> 8	R	> 16	R	> 4/76	R
Kp EQAsia 24.6	≤ 8	S	> 512	R	> 32	R	0.5	S	4	I	> 16	R	> 4/76	R
Kp EQAsia 24.7	> 64	R	> 512	R	8	I	≤ 1	S	> 8	R	2	S	1/19	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Identification	Amikacin (AMK)		Cefepime (FEP)		Cefotaxime (FOT)		Ceftazidime (CAZ)		Ciprofloxacin (CIP)		Colistin (COL)	
Ac EQAsia 24.1	*Acinetobacter pittii*	32	I	16	I	8	S	8	S	1	S	1	I
Ac EQAsia 24.2	*Acinetobacter baumannii*	> 32	R	> 16	R	> 32	R	> 16	R	> 2	R	0.5	I
Ac EQAsia 24.4	*Acinetobacter baumannii*	≤ 4	S	≤ 2	S	8	S	4	S	> 2	R	≤ 0.25	I
Ac EQAsia 24.5	*Acinetobacter baumannii*	> 32	R	> 16	R	> 32	R	> 16	R	> 2	R	≤ 0.25	I
Ac EQAsia 24.7	*Acinetobacter pittii*	≤ 4	S	4	S	16	I	8	S	≤ 0.25	S	1	I

R, Resistant; I, Intermediate; S, Susceptible

	Doripenem (DOR)		Doxycycline (DOX)		Gentamicin (GEN)		Imipenem (IMI)		Levofloxacin (LVX)		Meropenem (MERO)		Minocycline (MIN)	
Ac EQAsia 24.1	> 4	R	1	S	> 8	R	> 8	R	≤ 1	S	> 8	R	≤ 1	S
Ac EQAsia 24.2	> 4	R	> 16	R	> 8	R	> 8	R	> 8	R	> 8	R	> 16	R
Ac EQAsia 24.4	0.25	S	1	S	≤ 1	S	≤ 1	S	8	R	≤ 1	S	≤ 1	S
Ac EQAsia 24.5	> 4	R	32	R	> 8	R	> 8	R	8	R	> 8	R	≤ 2	S
Ac EQAsia 24.7	> 4	R	1	S	2	S	> 8	R	≤ 1	S	> 8	R	≤ 2	S

R, Resistant; I, Intermediate; S, Susceptible

	Piperacillin/tazo (PT/4)		Tigecycline (TGC)		Tobramycin (TOB)		Trime/Sulfa (SXT)	
Ac EQAsia 24.1	> 64	R	≤ 0.5	S	> 8	R	> 4/76	R
Ac EQAsia 24.2	> 64	R	4	R	> 8	R	> 4/76	R
Ac EQAsia 24.4	8	S	≤ 0.25	S	≤ 1	S	≤ 0.5/9.5	S
Ac EQAsia 24.5	> 64	R	≤ 0.25	S	> 8	R	> 4/76	R
Ac EQAsia 24.7	32	I	≤ 0.25	S	≤ 1	S	≤ 0.5/9.5	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Cefoxitin (FOX)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Clindamycin (CLI)		Erythromycin (ERY)		Fusidic acid (FUS)	
Sa EQAsia 24.2	> 16	R	8	S	≤ 0.25	S	0.25	S	> 8	R	≤ 0.25	S
Sa EQAsia 24.3	4	S	8	S	≤ 0.25	S	≤ 0.12	S	0.5	S	≤ 0.25	S
Sa EQAsia 24.4	32	R	8	S	0.5	S	≤ 0.12	S	0.5	S	0.5	S
Sa EQAsia 24.5	16	R	8	S	0.25	S	≤ 0.12	S	0.5	S	> 4	R
Sa EQAsia 24.7	4	S	8	S	≤ 0.25	S	≤ 0.12	S	> 8	R	≤ 0.25	S

R, Resistant; I, Intermediate; S, Susceptible

	Gentamicin (GEN)		Kanamycin (KAN)		Linezolid (LZD)		Penicillin (PEN)		Quinupristin/Dalfo (SYN)	
Sa EQAsia 24.2	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 24.3	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 24.4	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 24.5	≤ 0.5	S	> 32	R	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 24.7	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S

R, Resistant; I, Intermediate; S, Susceptible

	Rifampin (RIF)		Sulfamethoxazole (SMX)		Tetracycline (TET)		Trimethoprim (TMP)		Vancomycin (VAN)	
Sa EQAsia 24.2	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQAsia 24.3	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQAsia 24.4	≤ 0.015	S	≤ 64	S	32	R	> 16	R	≤ 1	S
Sa EQAsia 24.5	≤ 0.015	S	≤ 64	S	16	R	1	S	≤ 1	S
Sa EQAsia 24.7	≤ 0.015	S	≤ 64	S	≤ 0.5	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	1-4	24-30
Sulfamethoxazole, SMX	8-32	15-23
Tetracycline, TET	0.5-2	18-25
Tigecycline, TGC	0.03-0.25	20-27
Tobramycin, TOB	0.25-1	18-26
Trimethoprim, TMP	0.5-2	21-28
Trimethoprim and sulfamethoxazole, SXT	≤ 0.5	23-29

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

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

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

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

P. aeruginosa ATCC 27853		
Antimicrobial	**MIC (mg/L)**	**Inhibition Zone Diameter (mm)**
Amikacin, AMK	1-4	20-26
Cefepime, FEP	0.5-4	25-31
Cefotaxime, FOT	8-32	18-22
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
Doxycycline, DOX	--	--
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
Minocycline, MIN	--	--
Piperacillin and tazobactam, P/T4	1-8	25-33
Tigecycline, TGC	--	9-13
Tobramycin, TOB	0.25-1	20-26
Trimethoprim and sulfamethoxazole, SXT	8-32	--

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

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