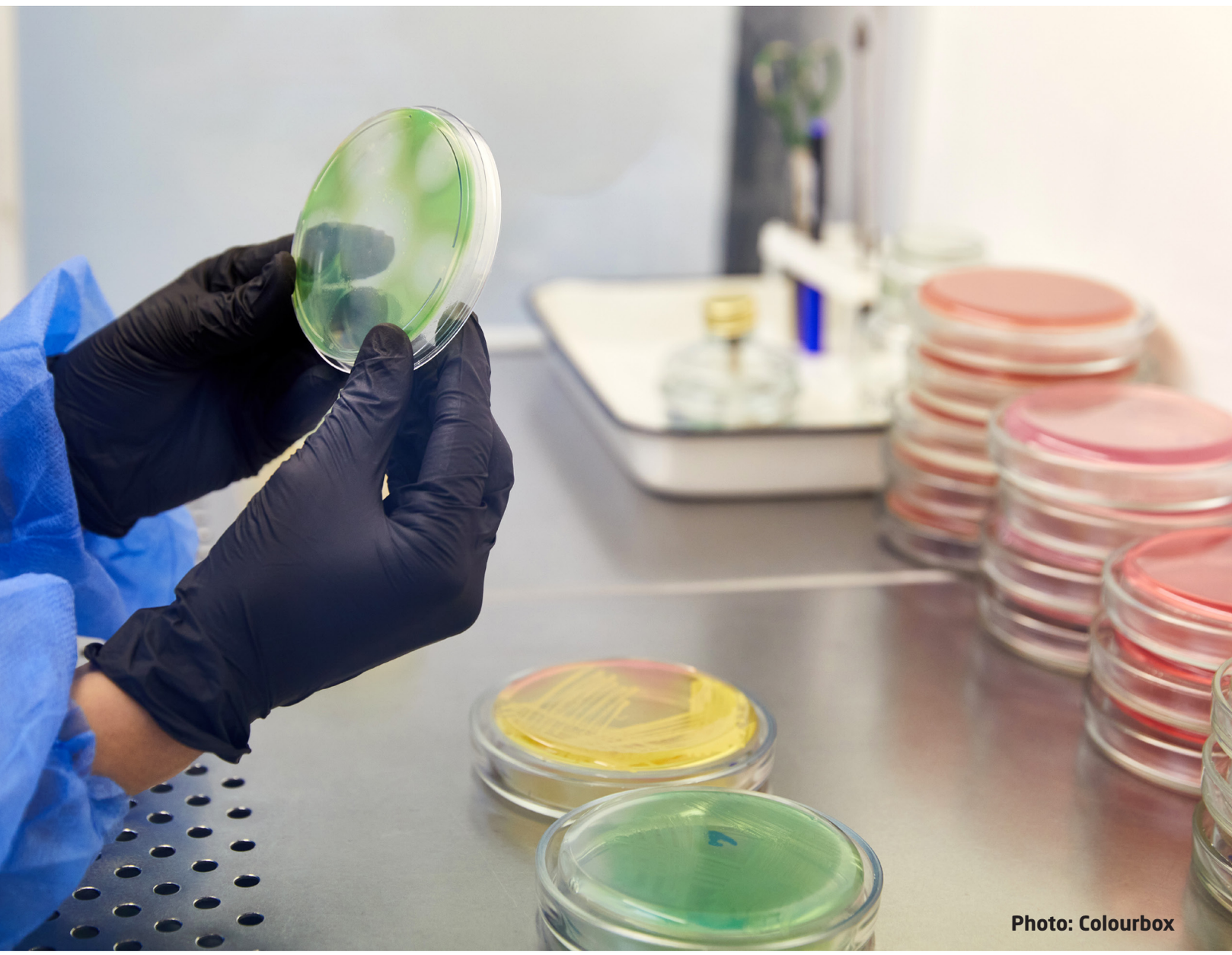
6th EQAsia External Quality Assessment Trial: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* – 2023



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6th EQAsia External Quality Assessment trial:
E. coli, K. pneumoniae, P. aeruginosa, and *S. aureus* – 2023

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Executive Summary

This report summarizes the results of the 6th External Quality Assessment (EQA) trial of EQAsia, the Fleming Fund Regional Grant aiming to strengthen the provision of EQA services across the One Health sector among National Reference Laboratories / Centres of Excellence in South and Southeast Asia. The EQAsia project is entering a second phase (2023 to 2025) in which it will continue to deliver the established EQA programme for both the Human Health (HH sector) and Food and Animal Health (AH sector) laboratories in the region.

The trial was carried out in April – June 2023 and included bacterial identification and antimicrobial susceptibility testing (AST) of four prominent WHO and FAO priority pathogens: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

A total of 23 HH and nine AH laboratories participated in this EQA trial. One HH laboratory 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 were submitted by 29 laboratories for the *E. coli* panel, 28 laboratories for the *K. pneumoniae* panel, 23 – for *P. aeruginosa*, and 28 – 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 in identifying the five strains of the organism in question (target organism) among a total of seven strains. All participating laboratories identified all *P. aeruginosa* isolates correctly. In the other three panels, there were only one or two 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 *E. coli* and *K. pneumoniae* panel was the highest (95.5% and 95.9%, respectively), followed by *P. aeruginosa* – 94.5% and *S. aureus* – 93.9%.

In this EQA trial, laboratories were ranked for the first time based on their average score across the panels in which they participated. The average score varied between 81.8% (rank #31) and 99.3% (rank #1). The total average score among all 31 laboratories that submitted results was 93.6%, while the median was 94.1%.

As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (3 in the *P. aeruginosa* panel and 5 in the *S. aureus* panel) did not submit results from reference strain testing at all. For the *E. coli* EQA round, there were ten laboratories (7 HH and 3 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=19) presented deviations between 5.6% and 63.2%. Since the same quality control strains were used also for the *K. pneumoniae* panel, the submitted results were similar. Nine laboratories (7 HH and 2 AH) showed no deviations, while the results from the other 18 laboratories deviated ranging between 5.6% to 65%. There was much less heterogeneity in the *P. aeruginosa* panel where the deviations were between 9.1% and 42.9%. 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 40%.

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. This rate was however higher for the HH laboratories. 17 HH (59%) and 5 AH (17%) out of 29 laboratories tested and submitted results for *E. coli*, while 19 HH (68%) and 4 AH (14%) out of 28 laboratories tested and submitted results for *K. pneumoniae.*

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. Laboratories need to make sure 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 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 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 WHO GLASS pathogens [1] and FAO priority pathogens [2]. EQAsia is entering a second phase and will continue to deliver the established EQA programme for both the Human Health (HH) sector and Food and Animal Health (AH) sector in the region until 2025.

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

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

A total of six 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*) and *Streptococcus pneumoniae*. In addition, a Matrix EQA trial was offered twice, consisting of a complex food sample spiked with AmpC beta-lactamases (AmpC), extended-spectrum beta-lactamases (ESBLs) or carbapenemase-producing *E. coli* for surveillance purposes. The aim was to align with the scope of WHO Tricycle and suggested by FAO, to assess the veterinary laboratories' ability to detect multidrug-resistant bacteria from food matrices.

For a given organism, candidate strains are assessed and validated by DTU Food and an external partner (The Peter Doherty Institute for Infection and Immunity, Australia). The validation includes both phenotypic determination of minimum inhibitory concentration (MIC) by broth microdilution, and whole-genome sequencing (WGS) to detect antimicrobial resistance (AMR) genes and chromosomal point mutations. The test strains are then selected based on the phenotypic AMR profile to include a heterogeneous panel, allowing for strain variation from almost pan-resistant to fully susceptible isolates.

This report contains results from the sixth EQA trial of the EQAsia project (EQA6) carried out in April – June 2023. 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 further testing was required. For the remaining five test strains of the target organism, AST results were requested.

This sixth EQA trial includes identification and AST of *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*. The aim of this EQA trial was to monitor the quality of AST results produced by the participating laboratories and identify underperforming laboratories in need of assistance to improve their performance in bacterial identification and AST.

The evaluation of the participants' results is based on international guidelines, namely the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Interpretative criteria referring to both disk diffusion and MIC determination are listed in the EQA6 protocol (**Appendix 1**) and allow for the obtained results to be interpreted into categories as resistant, intermediate, or susceptible depending on the method used. Results in agreement with the expected interpretation are scored '4' (correct), while results deviating from the expected interpretation are scored as either '0' (incorrect: very major error), '1' (incorrect: major error) or '3' (incorrect: minor error), as explained in the EQA6 protocol (**Appendix 1**). This standardized interpretation of results is necessary to allow comparison of performance between laboratories. Laboratory performance is considered acceptable if there are < 5 % deviation from the expected results.

Evaluation of a result as "deviating from the expected interpretation" should be carefully analysed in a root cause analysis procedure performed by individual participants (self-evaluation) when the EQA results are disclosed to the respective participating laboratory. The methods applied have limitations in reproducibility, thus, on repeated testing, the same strain/antimicrobial combination can result in different MIC or Inhibition Zone Diameter values differing by one-fold dilution or ± 3 mm, respectively. If the expected MIC / Zone Diameter is close to the threshold for categorising the strain as susceptible, intermediate, or resistant, a one-fold dilution / ± 3 mm difference may result in different interpretations. As this report evaluates the interpretations of MIC / Zone Diameter and not the values, some participants may find their results classified as incorrect (score of 0, 1 or 3) even though the actual MIC / Zone Diameter measured is only one-fold dilution / ± 3 mm apart from the expected MIC / Zone Diameter. In these cases, the participants should be confident about the good quality of their AST performance.

In this report, results from laboratories affiliated with the HH or AH Sectors are presented separately. The laboratories are identified by codes and each code is known only by the corresponding laboratory and the organizers. The full list of laboratory codes is confidential and known only by the EQAsia Consortium.

This report is approved in its final version by a Technical Advisory Group composed by members of the EQAsia Consortium, and by the EQAsia Advisory Board members Ben Howden (The Peter Doherty Institute for Infection and Immunity, Australia), Monica Lahra (WHO Collaborating Centre for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia) and Russel Cole (Pacific Pathology Training Centre, New Zealand).

2. Materials and Methods

2.1 Participants in EQAsia EQA6

A total of 32 laboratories participated in the sixth EQA trial of the EQAsia project: 23 laboratories belonging to the HH Sector and nine 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 could 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 EQA6, 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-c**) 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 free of charge 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 M100-32nd 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 EQA6 protocol (**Appendix 1**) and in **Table 1**. These antimicrobials correspond to several antimicrobial class representatives important 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, 32nd Ed. and VET06, 1st Ed.) [3, 4]. When not available, EUCAST clinical breakpoints (Tables v. 12.0, 2022) [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 EQA6 protocol.

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

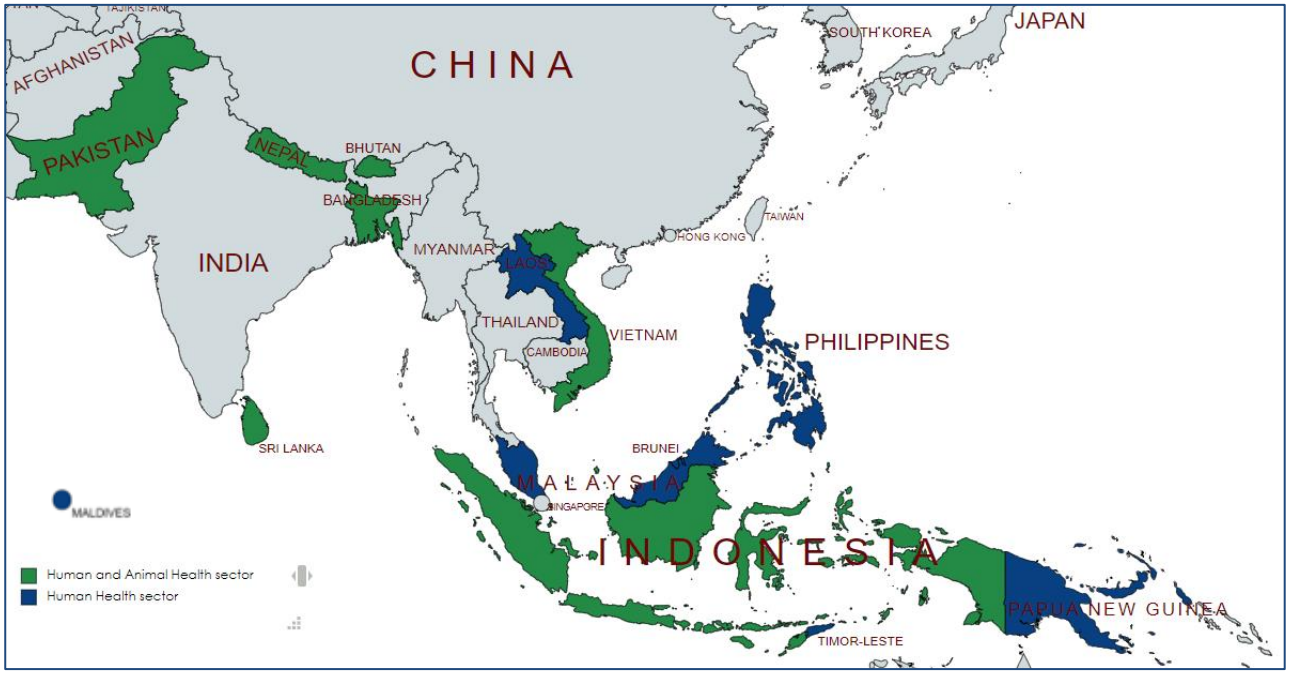


Figure 1: Countries participating in the 6th EQA of the EQAsia project. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA6 2023. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

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

2.4 Distribution

The bacterial strains were dispatched either as lyophilized strains or on swabs in transport medium in March 2023 by CUVET to all participating laboratories. The shipments (UN3373, biological substances category B) were sent according to the International Air Transport Association (IATA) regulations. Participating laboratories received detailed information on how to open, revive and store these lyophilized cultures as part of the EQA6 protocol (**Appendix 1**).

2.5 Procedure

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

Participating laboratories were advised to perform identification and AST of the test strains according to the methods routinely applied in their laboratory. Participants were encouraged to 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 the obtained results into an informatics module designed within the EQAsia programme and adapted for this trial. The informatics module could be accessed through a secured individual login and password. After release of the results, the participants were invited to login to retrieve an individual database-generated evaluation report.

2.6 Data management

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

3. Results – Human Health Laboratories

3.1 Overall participation

Out of 23 Human Health laboratories participating in the 6th EQA of the EQAsia project, 22 laboratories submitted results. Among these, 21, 22, 19 and 20 laboratories submitted results for *E. coli*, *K. pneumoniae, P. aeruginosa,* and *S. aureus* panels, respectively. The methodologies applied primarily by the laboratories varied and are summarized in **Figure 2**.

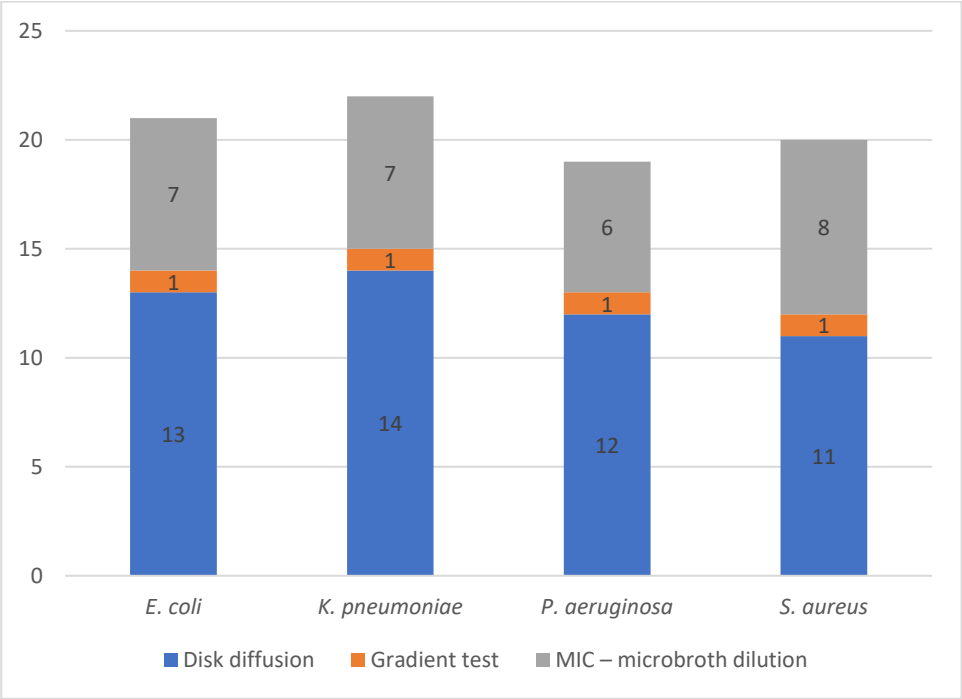


Figure 2. Methodologies primarily used by the laboratories for antimicrobial susceptibility testing in each of the trials.

Participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R'), intermediate ('I') or susceptible ('S') for each drug-bug combination. Only the categorisation was evaluated, whereas the Inhibition Zone Diameters/MIC values were used as supplementary information.

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

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

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

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

	E. coli		***K. pneumoniae***		***P. aeruginosa***		***S. aureus***	
Amikacin	85	*4,6%*	85	*4,5%*	78	*9,2%*	-	-
Ampicillin	90	*4,9%*	89	*4,7%*	-	-	-	-
Azithromycin	65	*3,5%*	66	*3,5%*	-	-	-	-
Aztreonam	-	-	-	-	50	*5,9%*	-	-
Cefepime	80	*4,4%*	86	*4,6%*	70	*8,3%*	-	-
Cefoxitin	-	-	-	-	-	-	84	*8,7%*
Cefotaxime	75	*4,1%*	81	*4,3%*	-	-	-	-
Cefotaxime and clavulanic acid	45	*2,5%*	44	*2,3%*	-	-	-	-
Cefoxitin	75	*4,1%*	76	*4,0%*	-	-	-	-
Ceftazidime	90	*4,9%*	95	*5,0%*	78	*9,2%*	-	-
Ceftazidime and clavulanic acid	43	*2,3%*	45	*2,4%*	-	-	-	-
Chloramphenicol	70	*3,8%*	79	*4,2%*	-	-	64	*6,6%*
Ciprofloxacin	90	*4,9%*	95	*5,0%*	79	*9,3%*	69	*7,2%*
Clindamycin	-	-	-	-	-	-	54	*5,6%*
Colistin	53	*2,9%*	54	*2,9%*	49	*5,8%*	-	-
Doripenem	53	*2,9%*	56	*3,0%*	50	*5,9%*	-	-
Ertapenem	76	*4,1%*	74	*3,9%*	-	-	-	-
Erythromycin	-	-	-	-	-	-	74	*7,7%*
Fusidic acid	-	-	-	-	-	-	44	*4,6%*
Gentamicin	85	*4,6%*	84	*4,5%*	70	*8,3%*	74	*7,7%*
Imipenem	85	*4,6%*	89	*4,7%*	71	*8,4%*	-	-
Kanamycin	-	-	-	-	-	-	43	*4,5%*
Levofloxacin	67	*3,6%*	71	*3,8%*	61	*7,2%*	-	-
Linezolid	-	-	-	-	-	-	59	*6,1%*
Meropenem	85	*4,6%*	90	*4,8%*	71	*8,4%*	-	-
Nalidixic acid	75	*4,1%*	59	*3,1%*	-	-	-	-
Penicillin	-	-	-	-	-	-	74	*7,7%*
Piperacillin and tazobactam	79	*4,3%*	85	*4,5%*	71	*8,4%*	-	-
Quinupristin and dalfopristin	-	-	-	-	-	-	44	*4,6%*
Rifampin	-	-	-	-	-	-	49	*5,1%*
Sulfamethoxazole	50	*2,7%*	51	*2,7%*	-	-	48	*5,0%*
Tetracycline	70	*3,8%*	75	*4,0%*	-	-	78	*8,1%*
Tigecycline	55	*3,0%*	50	*2,7%*	-	-	-	-
Tobramycin	50	*2,7%*	54	*2,9%*	48	*5,7%*	-	-
Trimethoprim	55	*3,0%*	56	*3,0%*	-	-	52	*5,6%*
Trimethoprim and sulfamethoxazole	90	*4,9%*	94	*5,0%*	-	-	-	-
Vancomycin	-	-	-	-	-	-	54	*5,6%*
Total	**1836**		**1883**		**846**		**964**	

Scattering of missing data or incomplete AST results entries were observed in all four types of bacteria. One of the laboratories that received the four panels (#15) did not submit any data.

Eight out of 21 laboratories had partially incomplete results submitted for the *E. coli* panel (**Table 3**). A complete data set was considered when the list of reported antimicrobials was consistent across the five target strains. The highest number of incomplete results in the *E. coli* panel were seen for laboratories #04, #16, and #32.

Eight out of 22 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 #05, #06, #32, and #49.

Five out of 19 laboratories that selected *P. aeruginosa* submitted incomplete results of their own available antimicrobial agents (**Table 5**). The highest number of incomplete results in the *P. aeruginosa* panel was seen for laboratories #04, #16, and #32.

Only 3 out of 20 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 #49.

Table 3. Distribution of incomplete or missing data of antimicrobial agents among *E. coli* strains reported by HH laboratories (n=21) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Ec EQASIA 23.2	Ec EQASIA 23.4	Ec EQASIA 23.5	Ec EQASIA 23.6	Ec EQASIA 23.7
#01	--	--	--	--	--
#02	--	--	ETP	--	--
#04	COL	--	COL	COL	--
#05	--	--	--	--	--
#06	ETP	ETP	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#13	--	--	--	--	--
#14	--	--	--	--	--
#16	--	--	LEV	LEV	PT/4
#17	--	--	--	--	COL, DOR
#32	COL, ETP, LEV	--	COL	COL	--
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	--	--	--
#49	--	DOR	--	--	--
#50	--	--	--	--	--
#51	--	--	--	CAZ	--
#52	--	--	--	--	--

Ec, *E. coli*

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by HH laboratories (n=22) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Kp EQASIA 23.1	Kp EQASIA 23.3	Kp EQASIA 23.5	Kp EQASIA 23.6	Kp EQASIA 23.7
#01	--	--	--	--	--
#02	TET	--	--	--	--
#04	--	--	--	--	--
#05	--	TOB	TOB	TOB	TOB
#06	ETP	ETP	ETP	ETP	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#10	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#13	--	--	--	--	--
#14	--	--	--	--	--
#16	--	SXT	--	LEV	LEV
#17	--	--	ETP, GEN	--	TGC
#32	COL, ETP, IMP	--	CHL, TOB	TOB	CHL
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	--	--	COL
#49	NAL	LEV	LEV, NAL	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--

Kp, *K. pneumoniae*

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *P. aeruginosa* strains reported by HH laboratories (n=19) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Pa EQASIA 23.1	Pa EQASIA 23.3	Pa EQASIA 23.4	Pa EQASIA 23.5	Pa EQASIA 23.7
#01	--	--	--	--	--
#02	CIP	--	--	--	--
#04	COL	--	COL	COL	COL
#06	--	--	--	--	--
#07	--	--	--	--	--
#08	--	--	--	--	--
#11	--	--	--	--	--
#12	--	--	--	--	--
#14	--	--	--	--	--
#16	IMP	--	LEV, MEM, PT/4	MEM	--
#17	--	--	--	--	--
#32	COL	--	AMK, CAZ, COL, IMP, LEV, MEM, PT/4, TOB	COL, IMP, LEV, PT/4	AMK, CAZ, COL, IMP, LEV, MEM, PT/4, TOB
#34	--	--	--	--	--
#35	--	--	--	--	--

#48	--	--	--	COL	COL
#49	--	--	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--

Pa, *P. aeruginosa*

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by HH laboratories (n=20) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Sa EQASIA 23.1	Sa EQASIA 23.2	Sa EQASIA 23.3	Sa EQASIA 23.4	Sa EQASIA 23.6
#01	--	--	--	--	--
#02	--	--	--	--	--
#04	--	--	--	--	--
#05	--	--	--	--	--
#06	--	--	--	--	--
#07	--	TMP	--	--	--
#08	--	--	--	--	TMP
#11	--	--	--	--	--
#12	--	--	--	--	--
#14	--	--	--	--	--
#16	--	--	--	--	--
#17	--	--	--	--	--
#32	--	--	--	--	--
#34	--	--	--	--	--
#35	--	--	--	--	--
#48	--	--	--	--	--
#49	SMT, TET	KAN	--	--	--
#50	--	--	--	--	--
#51	--	--	--	--	--
#52	--	--	--	--	--

Sa, *S. aureus*

3.2 *Escherichia coli* panel

21 laboratories from 14 countries uploaded results for the *E. coli* panel.

3.2.1 Bacterial identification

21 laboratories submitted results for bacterial identification (**Table 7**). Only one laboratory (#15) did not submit results. The five target *E. coli* strains were identified correctly by all 21 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 23.1	*Non- E. coli*	21/21
Ec EQASIA 23.2	*Escherichia coli*	21/21
Ec EQASIA 23.3	Non- *E. coli*	21/21
Ec EQASIA 23.4	*Escherichia coli*	21/21
Ec EQASIA 23.5	*Escherichia coli*	21/21
Ec EQASIA 23.6	*Escherichia coli*	21/21
Ec EQASIA 23.7	*Escherichia coli*	21/21

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 91.8% (strain Ec EQASIA 23.4) to 97.2% (strains Ec EQASIA 23.1, 23.5 and 23.7) (**Table 8**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were tigecycline (42.3%), tobramycin (34.3%), chloramphenicol (20.0%), imipenem (17.6%), azithromycin (16.7%), meropenem (14.0%), cefoxitin (12.9%), doripenem (12.0%), and ceftazidime (10.6%) whereas colistin, levofloxacin, nalidixic acid, sulfamethoxazole and trimethoprim revealed no deviation from the expected results (**Figure 3**).

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 # 34, #16, #06, #11, #32, and #17 (**Figure 4**). In average, the deviation was 8.1% (ranging from 1.5 to 18.1%). As the acceptance level was set to 5% deviation, 15 laboratories (#04, #51, #49, #48, #13, #50, #52, #35, #01, #12, #08, #02, #05, #07, and #14) did not perform within the expected 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 21 HH laboratories for the *E. coli* panel.

Strain	AST in total	% Correct
Ec EQASIA 23.2	335	97.2
Ec EQASIA 23.4	339	91.8
Ec EQASIA 23.5	336	97.2
Ec EQASIA 23.6	336	96.6
Ec EQASIA 23.7	339	97.2

Ec, E. coli

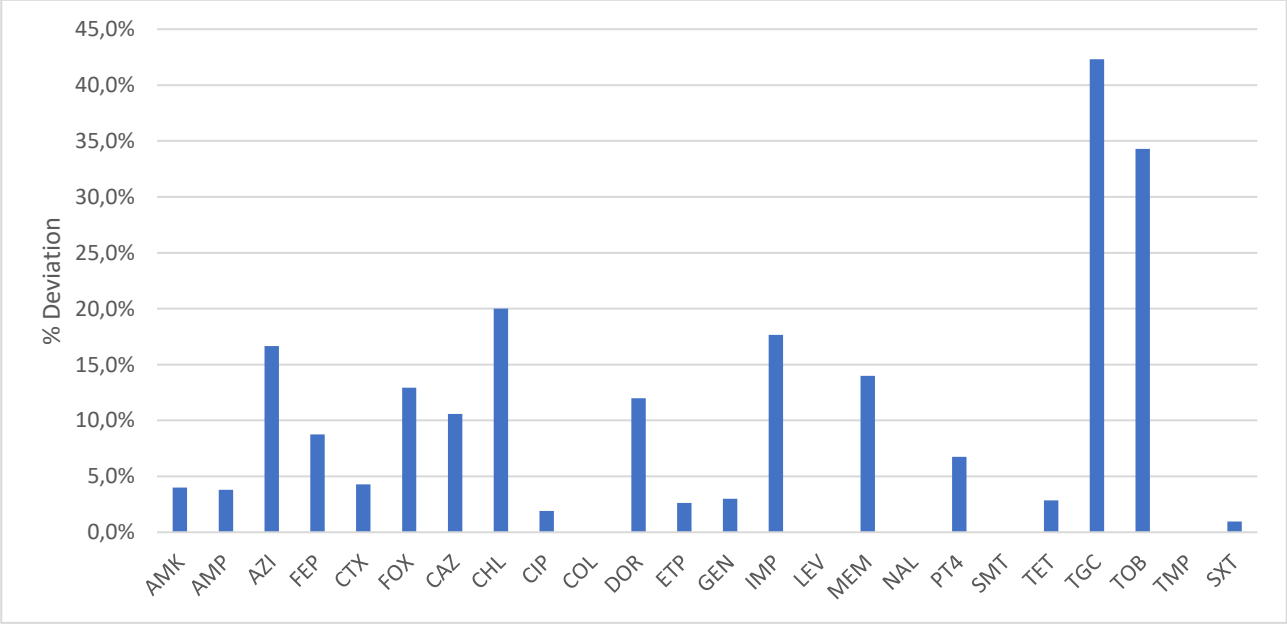


Figure 3. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=21) participating in the 6th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

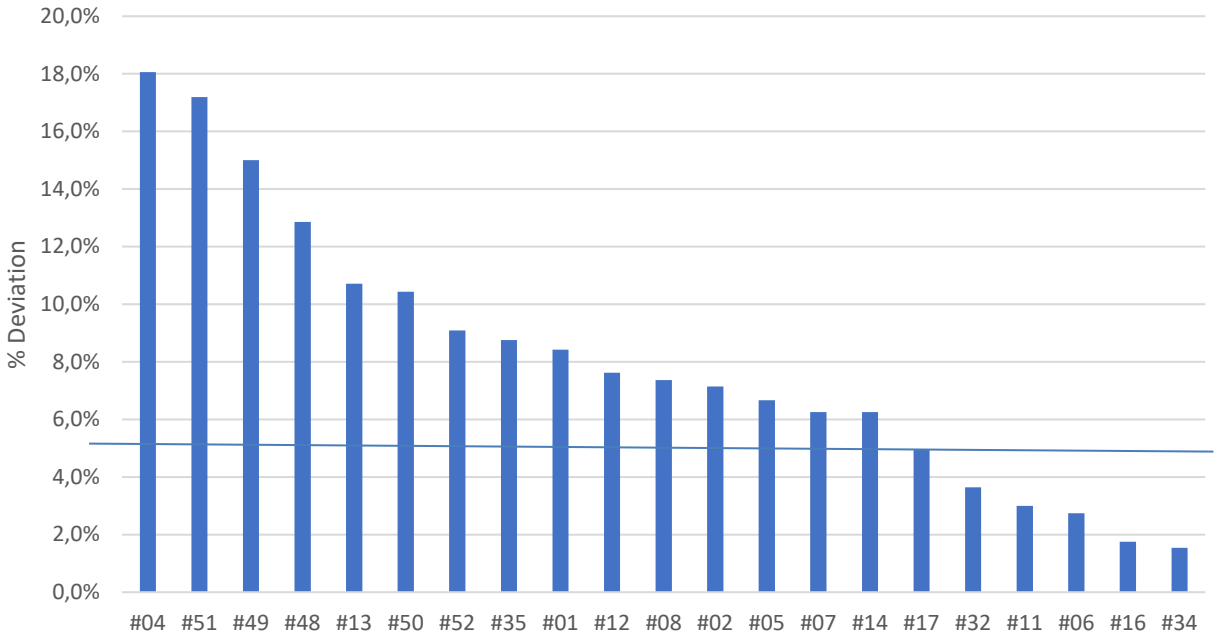


Figure 4. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by HH laboratories (n=21) participating in the 6th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 β-lactamase producing *E. coli*

16, 17 and 15 participating laboratories tested for ESBL/AmpC/carbapenemase-production in strains Ec EQASIA 23.2, Ec EQASIA 23.4 and Ec EQASIA 23.5, respectively. 14 laboratories reported results for strains Ec EQASIA 23.6, and for Ec EQASIA 23.7 (**Table 9**). 11 laboratories correctly identified all phenotypes among the five *E. coli* strains.

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 23.2	Ec EQASIA 23.4	Ec EQASIA 23.5	Ec EQASIA 23.6	Ec EQASIA 23.7
Expected results		Carbapenemase	Carbapenemase	ESBL	-	-
Obtained results (n/N)	ESBL	2/17 (11.7%)	6/17 (35.3%)	--	--	--
	Carbapenemase	**14/17 (82.4%)**	**11/17 (64.7%)**	**14/15 (93.3%)**	--	--
	Other	--	--	--	**1/14 (7.1%)**	**1/14 (7.1%)**
	Susceptible*	1/17 (5.9%)	--	1/15 (6.7%)	**13/14 (92.9%)**	**13/14 (92.9%)**

Ec, *E. coli*
***no AmpC, ESBL and carbapenemase**
(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.

All 21 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only eight performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, agar, or 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 expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diffusion	Gradient	MIC	**Total**
AMK	1/12	--	0/8	**1/20**
AMP	5/14	--	0/7	**5/21**
AZI	0/10	0/1	--	**0/11**
CAZ	3/12	1/1	0/6	**4/19**
CHL	2/15	0/1	--	**2/16**
CIP	3/13	0/1	1/7	**4/21**
COL	--	--	0/8	**0/8**
DOR	0/3	--	0/1	**0/4**
ETP	1/9	--	0/6	**1/15**
FEP	3/10	--	0/6	**3/16**
FOT	2/12	--	0/1	**2/13**
FOX	4/15	0/1	0/1	**4/15**
GEN	1/12	--	1/7	**2/19**
IMI	0/9	0/1	0/5	**0/15**
LEVO	1/7	0/1	2/5	**3/13**
MERO	1/12	--	2/8	**3/20**
NAL	2/8	0/1	0/2	**2/11**
PT4	0/10	--	0/7	**0/17**
SXT	4/14	--	3/6	**7/20**
TET	3/14	--	0/1	**3/15**
TGC	0/2	--	1/3	**1/5**
TMP	1/3	--	0/1	**1/4**
TOB	1/7	--	1/3	**2/10**

Disk Diffusion – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth micro or macrodilution.
*Gradient test is not recommended for colistin testing

Highest proportion of test results outside of the expected range was observed in trimethoprim/sulfamethoxazole (7 out of 20) (**Table 10**).

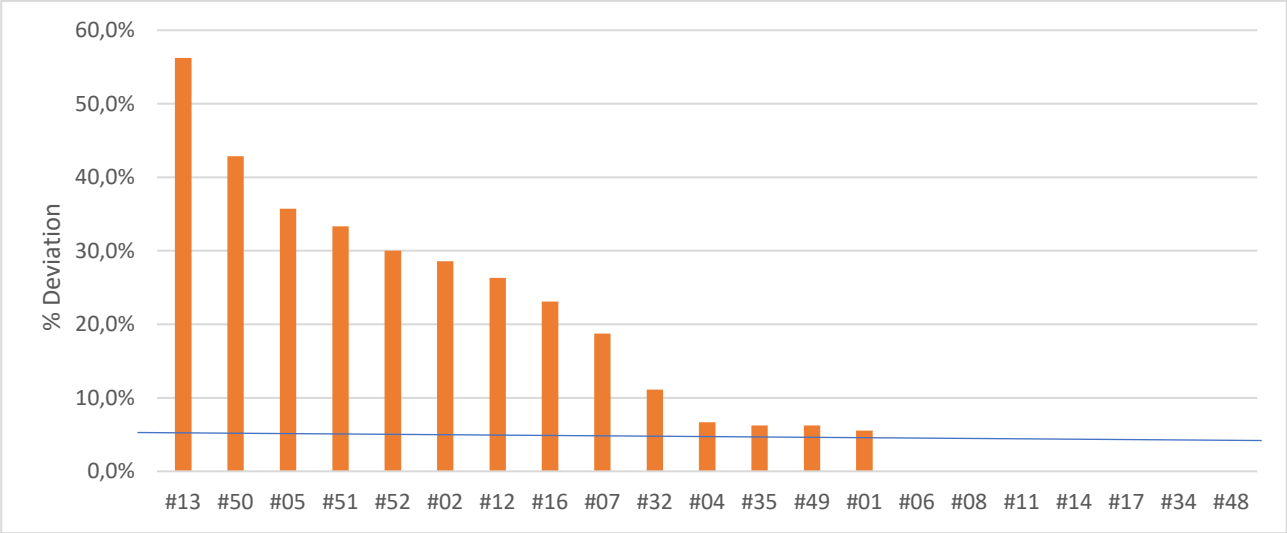


Figure 5. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *E. coli* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 5).** Laboratories #06, #08, #11, #14, #17, #34, and #48 presented no deviation. I.e. laboratory #06 used only MIC method, laboratory #11 applied disk diffusion, gradient MIC, agar and broth microdilution, while the other five laboratories used disk diffusion only. All other laboratories presented deviations that ranged from 5.6% to 56.3% (**Figure 5**).

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

3.3 *Klebsiella pneumoniae* panel

22 laboratories from 14 countries uploaded results for the *K. pneumoniae* panel.

3.3.1 Bacterial identification

22 participating laboratories submitted results for bacterial identification (**Table 11**). Only one laboratory (#15) did not submit results. The five target *K. pneumoniae* strains were identified correctly by all 22 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 23.1	*Klebsiella pneumoniae*	22/22
Kp EQASIA 23.2	Non- *K. pneumoniae*	22/22
Kp EQASIA 23.3	*Klebsiella pneumoniae*	22/22
Kp EQASIA 23.4	Non- *K. pneumoniae*	22/22
Kp EQASIA 23.5	*Klebsiella pneumoniae*	22/22
Kp EQASIA 23.6	*Klebsiella pneumoniae*	22/22
Kp EQASIA 23.7	*Klebsiella pneumoniae*	22/22

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 93.6% (strain Kp EQASIA 23.6) to 98.2% (strain Kp EQASIA 23.3) (**Table 12**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected result higher than 10% were tigecycline (60.0%), tetracycline (29.0%), azithromycin (18.2%), ciprofloxacin (17.3%), meropenem (15.4%) cefepime (13.3%), trimethoprim/sulfamethoxazole (12.8%), nalidixic acid (12.0%), and piperacillin/tazobactam (11.6%) whereas colistin and sulfamethoxazole revealed no deviation from the expected results (**Figure 6**).

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 # 32, #14, #17, #11, #16, and #06 (**Figure 7**). On average, the deviation was 9.3% (ranging from 1.7 to 38.0%). As the acceptance level was set to 5% deviation, 16 laboratories (#51, #52, #05, #50, #01, #02, #08, #12, #49, #04, #48, #34, #10, #07, #35, and #13) did not perform within the expected range for the *K. pneumoniae* panel.

Table 12. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 22 HH laboratories for the *K. pneumoniae* panel.

Strain	AST in total	% Correct
Kp EQASIA 23.1	336	96.1
Kp EQASIA 23.3	339	98.2
Kp EQASIA 23.5	335	95.3
Kp EQASIA 23.6	338	93.6
Kp EQASIA 23.7	341	97.6

Kp, *K. pneumoniae*

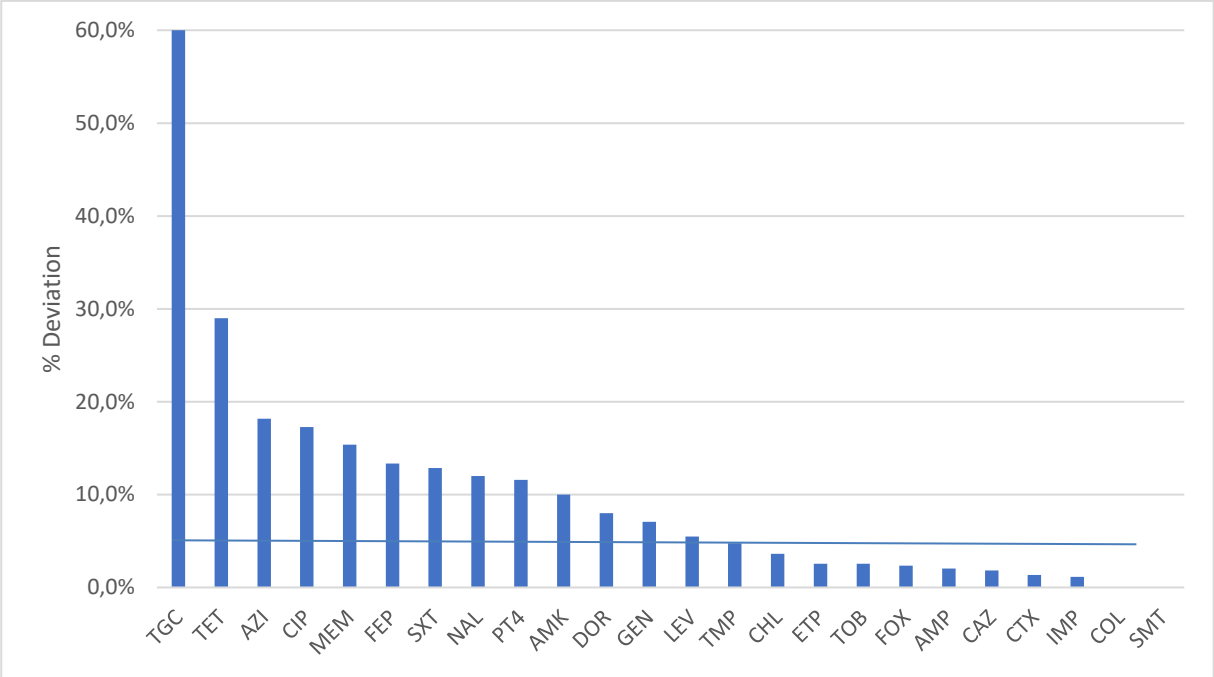


Figure 6. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=22) participating in the 6th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

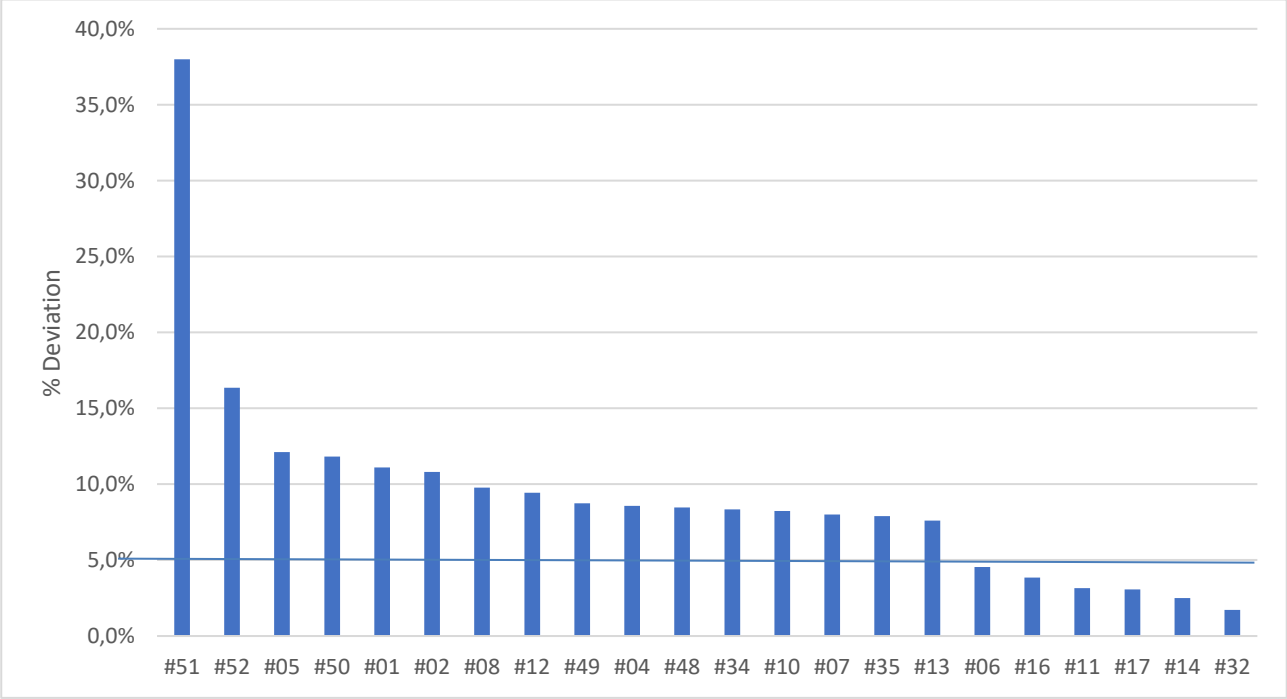


Figure 7. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=22) participating in the 6th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.3.3 β-lactamase producing *K. pneumoniae*

19 out of the 22 participating laboratories tested for ESBL/AmpC/carbapenemase-production in strains Kp EQASIA 23.1, Kp EQASIA 23.3 and Kp EQASIA 23.5, respectively. 18 and 15 laboratories reported results for strains Kp EQASIA 23.6, and Kp EQASIA 23.7, respectively

(**Table 13**). Two laboratories correctly identified all phenotypes among the five *K. pneumoniae* strains. The highest deviation from the expected results was obtained for strain Kp EQASIA 23.5 (**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 19 HH laboratories.

Strain code		Kp EQASIA 23.1	Kp EQASIA 23.3	Kp EQASIA 23.5	Kp EQASIA 23.6	Kp EQASIA 23.7
Expected results		Carbapenemase	Carbapenemase	ESBL + porin loss	ESBL	--
Obtained results (n/N)	ESBL	2/19 (10.5%)	2/19 (10.5%)	**2/19 (10.5%)**	**15/18 (83.3%)**	--
	Carbapenemase	**14/19 (73.7%)**	**15/19 (79.0%)**	11/19 (58.0%)	**--**	**--**
	ESBL + AmpC	**--**	**--**	**2/19 (10.5%)**	**--**	**--**
	AmpC	**--**	**--**	**--**	1/18 (5.6%)	**--**
	Other	--	--	2/19 (10.5%)	--	--
	Susceptible*	3/19 (15.8%)	2/19 (10.5%)	2/19 (10.5%)	2/18 (11.1%)	**15/15 (100%)**

Kp, *K. pneumoniae*
*no AmpC, ESBL and carbapenemase
(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 free of charge to all participating laboratories as part of previous EQAsia EQA trials to be used as reference strains for *K. pneumoniae*. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials. All 22 participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only eight 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, broth macro or microdilution (**Table 14**). For testing *E. coli* NCTC 13846, MIC was determined by standard method either broth macro- or 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 Diff.	Gradient	MIC	**Total**
AMK	1/13	--	0/8	**1/21**
AMP	2/13	--	0/8	**2/21**
AZI	0/9	0/1	0/1	**0/11**
CAZ	3/13	0/1	1/6	**4/20**
CHL	2/17	0/1	--	**2/18**
CIP	2/14	0/1	0/7	**2/22**
COL	--	--	0/8	**0/8**
DOR	0/3	--	0/1	**0/4**
ETP	1/8	0/1	0/6	**1/15**
FEP	3/11	--	0/7	**3/18**
FOT	3/13	--	0/1	**3/14**
FOX	4/16	0/1	0/1	**4/18**
GEN	1/12	--	0/7	**1/19**
IMI	0/9	0/2	1/5	**1/16**
LEVO	2/8	0/1	0/5	**2/14**
MERO	1/12	0/1	0/8	**1/21**
NAL	1/7	--	0/2	**1/9**

PT4	0/11	--	0/7	**0/18**
SXT	5/15	--	3/6	**8/21**
TET	2/15	--	0/1	**2/16**
TGC	0/1	--	1/2	**1/3**
TMP	1/3	--	0/1	**1/4**
TOB	1/7	--	1/2	**2/9**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth micro- or macrodilution.

*Gradient test is not recommended for colistin testing

Highest proportion of test results outside of the expected range was observed in trimethoprim/sulfamethoxazole (8 out of 21) (**Table 14**).

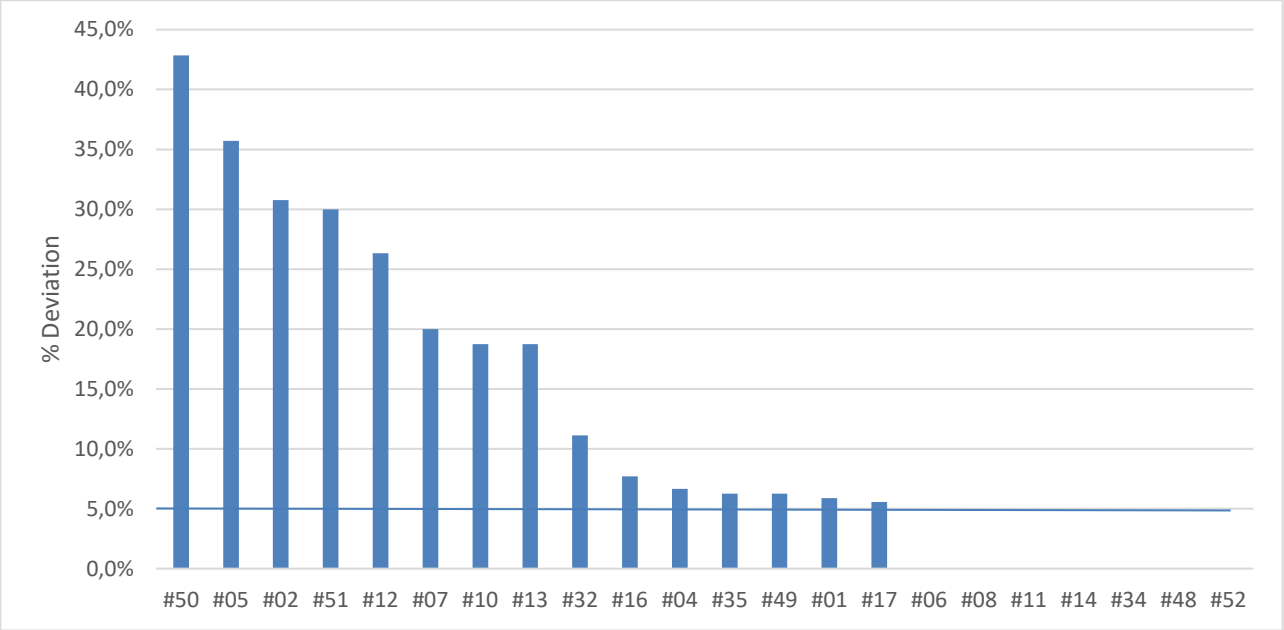


Figure 8. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *K. pneumoniae* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 8**). Laboratories #06, #08, #11, #14, #34, #48, and #52 presented no deviation. I.e. laboratory #06 used only MIC method, laboratory #11 applied disk diffusion, gradient MIC, agar and broth microdilution, while the other 5 laboratories used disk diffusion only.

All other laboratories presented deviations that ranged from 5.6% to 42.9% (**Figure 8**).

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

3.4 *Pseudomonas aeruginosa* panel

19 laboratories from 13 countries uploaded results for the *P. aeruginosa* panel.

3.4.1 Bacterial identification

All 19 participating laboratories submitted results for bacterial identification (**Table 15**). The five target *P. aeruginosa* strains were identified correctly by all 19 laboratories.

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

Strain	Bacterial ID	No. correct
Pa EQASIA 23.1	*Pseudomonas aeruginosa*	19/19
Pa EQASIA 23.2	Non-*P. aeruginosa*	19/19
Pa EQASIA 23.3	*Pseudomonas aeruginosa*	19/19
Pa EQASIA 23.4	*Pseudomonas aeruginosa*	19/19
Pa EQASIA 23.5	*Pseudomonas aeruginosa*	19/19
Pa EQASIA 23.6	Non-*P. aeruginosa*	19/19
Pa EQASIA 23.7	*Pseudomonas aeruginosa*	19/19

Pa, *P. aeruginosa*

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 93.7% (strain Pa EQASIA 23.4) to 99.9% (strain Pa EQASIA 23.7) (**Table 16**).

Antimicrobial-based analysis

Antimicrobials with deviations from the expected results higher than 10% were meropenem (32.6%), doripenem (31.0%), and aztreonam (20.5%) (**Figure 9**).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed in 4 laboratories (#07, #16, #49, and #34) (**Figure 10**). In average, the deviation was 11.0% (ranging from 2.2 to 28.0%).

Table 16. Total number of AST performed and percentage of correct results in agreement with the expected interpretive results (R/S). Results are from 19 HH laboratories for the *P. aeruginosa* panel.

Strain	AST in total	% Correct
Pa EQASIA 23.1	181	97.7
Pa EQASIA 23.3	186	97.0
Pa EQASIA 23.4	174	93.7
Pa EQASIA 23.5	180	93.8
Pa EQASIA 23.7	177	99.9

Pa, *P. aeruginosa*

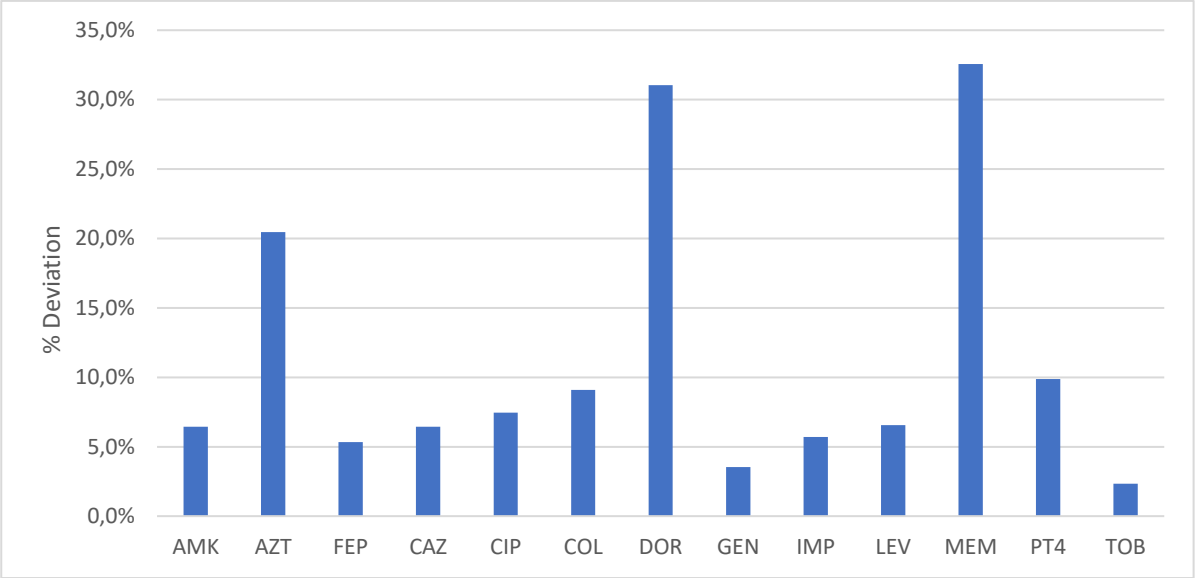


Figure 9. Percentage of deviation in the AST interpretation (R/S) among *P. aeruginosa* strains by HH laboratories (n=19) participating in the 6th EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

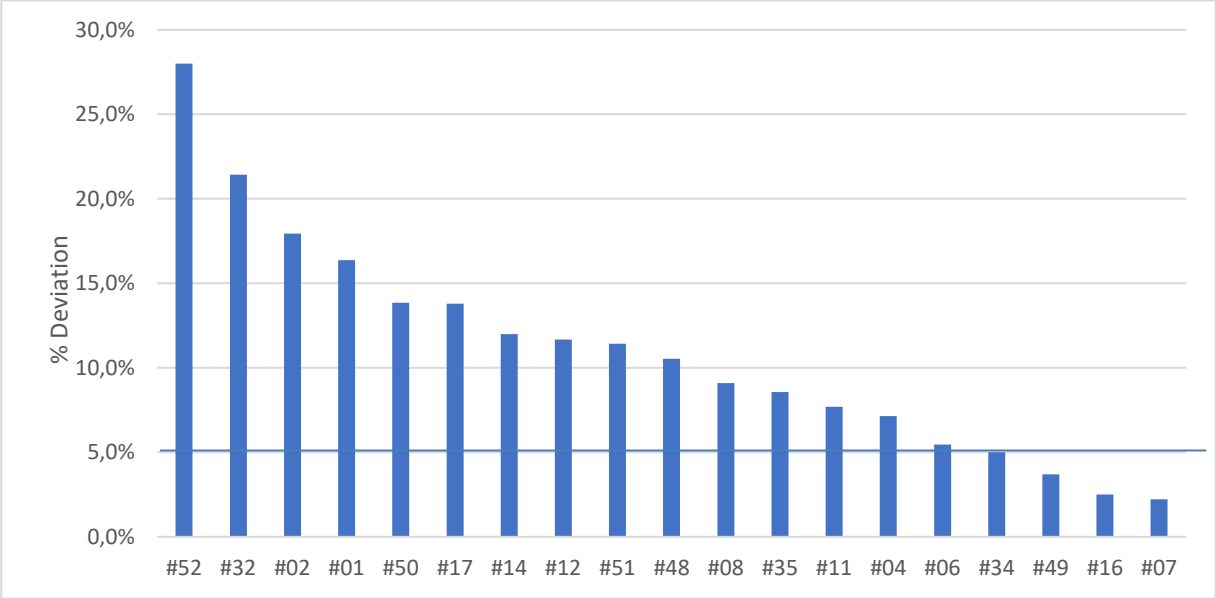


Figure 10. Percentage of deviation in the AST interpretation (R/S) among *P. aeruginosa* strains by HH laboratories (n=19) participating in the 6th EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.4.3 Quality control strains *P. aeruginosa* ATCC 27853

The quality control strains *P. aeruginosa* ATCC 27853 were sent free of charge to all participating laboratories within previous EQAsia EQA trials to be used as reference strains also for subsequent *P. aeruginosa* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the 19 participating laboratories, 18 submitted results for the reference strain *P. aeruginosa* ATCC 27853 and only ten performed colistin testing and reported results. 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, broth macro- or microdilution (**Table 17**).

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

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	Total
AMK	0/12	--	0/6	**0/18**
AZT	1/5	--	0/4	**1/9**
FEP	1/9	--	0/5	**1/14**
CAZ	3/12	0/1	0/5	**3/18**
CIP	0/11	0/1	0/5	**0/17**
COL	--	--	0/10	**0/10**
DOR	0/4	--	0/2	**0/6**
GEN	2/10	--	0/6	**2/16**
IMI	0/8	0/1	1/4	**1/13**
LEVO	0/7	0/1	0/3	**0/11**
MERO	1/11	--	0/6	**1/17**
PT4	1/10	--	0/6	**1/16**
TOB	0/7	0/1	0/2	**0/10**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth micro- or macrodilution.
*Gradient test is not recommended for colistin testing

The highest proportion of test results outside of the expected range was observed for ceftazidime (3 out of 18) (**Table 17**). Moreover, most of the inaccurate results seemed to be caused by disk diffusion.

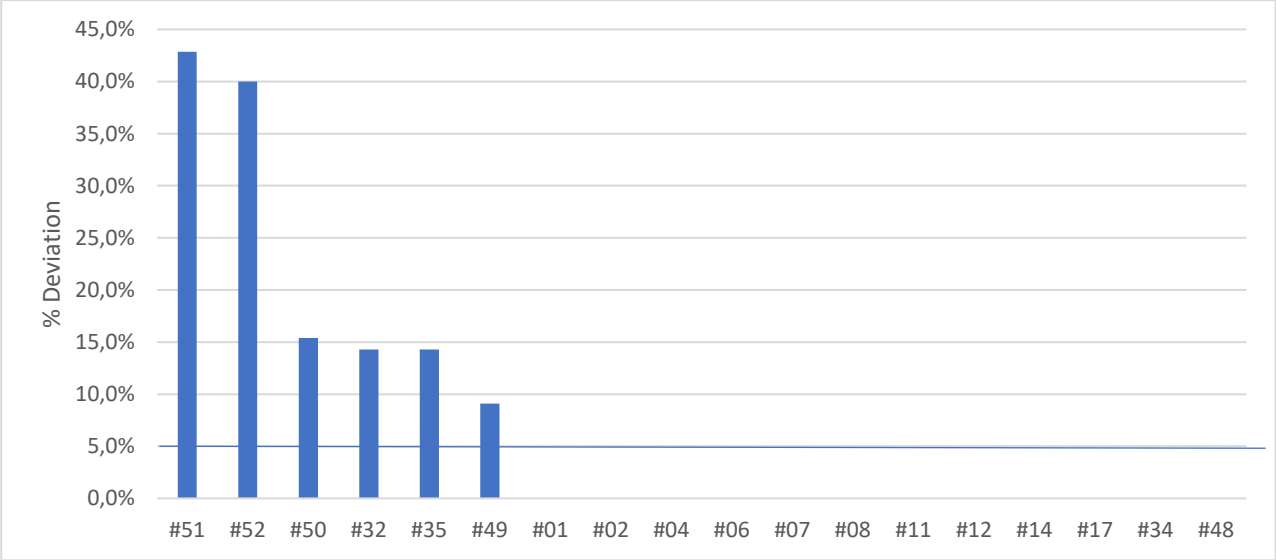


Figure 11. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* panel by the HH laboratories.

Considering the deviations, the laboratories' performance seemed to be independent of the methodology applied for AST of the quality control strains (**Figure 11**). Most of the laboratories in this trial had no deviations in the quality control strains results. Six laboratories (#51, #52, #50, #32, #35 and #49) presented deviations that ranged from 9.1% to 42.9% (**Figure 11**). As mentioned above, most of the deviations were seen when disk diffusion methodology was applied. For those inaccurate results, the Inhibition Zone Diameters reported were usually below the expected range.

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

3.5 *Staphylococcus aureus* panel

20 laboratories from 13 countries uploaded results for the *S. aureus* panel.

3.5.1 Bacterial identification

All 20 laboratories that selected the *S. aureus* panel submitted results for bacterial identification. Among these, 18 laboratories correctly identified the five *S. aureus* strains and the two non-*S. aureus* (**Table 18**). Two non-*S. aureus* strains (strain Sa EQASIA 23.5 and Sa EQASIA 23.7) were misidentified as *S. aureus* by laboratory #06.

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 23.1	*Staphylococcus aureus*	20/20
Sa EQASIA 23.2	*Staphylococcus aureus*	20/20
Sa EQASIA 23.3	*Staphylococcus aureus*	18/20
Sa EQASIA 23.4	*Staphylococcus aureus*	20/20
Sa EQASIA 23.5	Non-*Staphylococcus aureus*	19/20
Sa EQASIA 23.6	*Staphylococcus aureus*	19/20
Sa EQASIA 23.7	Non-*Staphylococcus aureus*	19/20

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 91.8% (strain Sa EQASIA 23.3) to 97.3% (strain Sa EQASIA 23.1) for each strain (**Table 19**).

Table 19. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/S). Results are from 20 HH laboratories for the *S. aureus* panel.

Strain	AST in total	% Correct
Sa EQASIA 23.1	188	97.3
Sa EQASIA 23.2	186	94.8
Sa EQASIA 23.3	165	91.8
Sa EQASIA 23.4	186	95.7
Sa EQASIA 23.6	177	94.9

Sa, *S. aureus*

Antimicrobial-based analysis

The antimicrobials that resulted in percentage of deviations higher than 5% were sulfamethoxazole (66.7%), clindamycin (28.1%), ciprofloxacin (18.3%), erythromycin (12.6%), trimethoprim (11.1%), quinupristin/dalfopristin (10.5%), and cefoxitin (6.2%) (**Figure 12**).

Laboratory-based analysis

For the *S. aureus* panel, nine out of the 20 HH laboratories presented a deviation below 5% (laboratories #52, #32, #16, #07, 08, #01, #35, #17, and #11). The average deviation was 8.0% (ranging from 0% to 25.9%) (**Figure 13**).

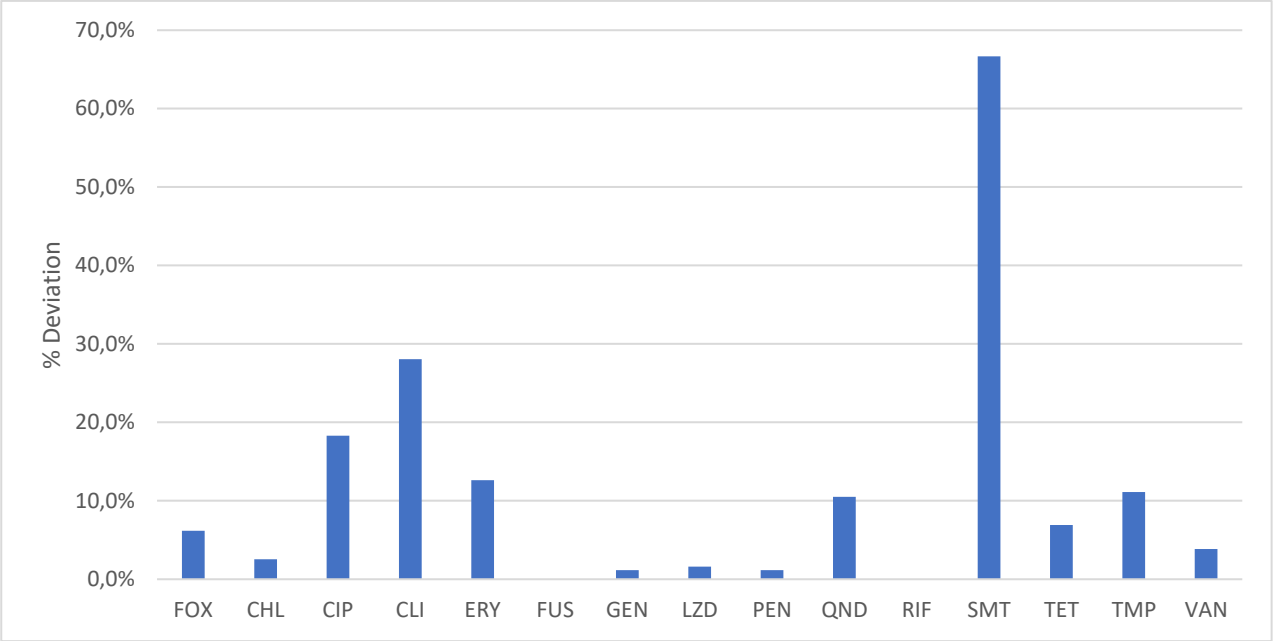


Figure 12. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=20) participating in the 6th EQA of the EQAsia project. Results are categorized by antimicrobial agent.

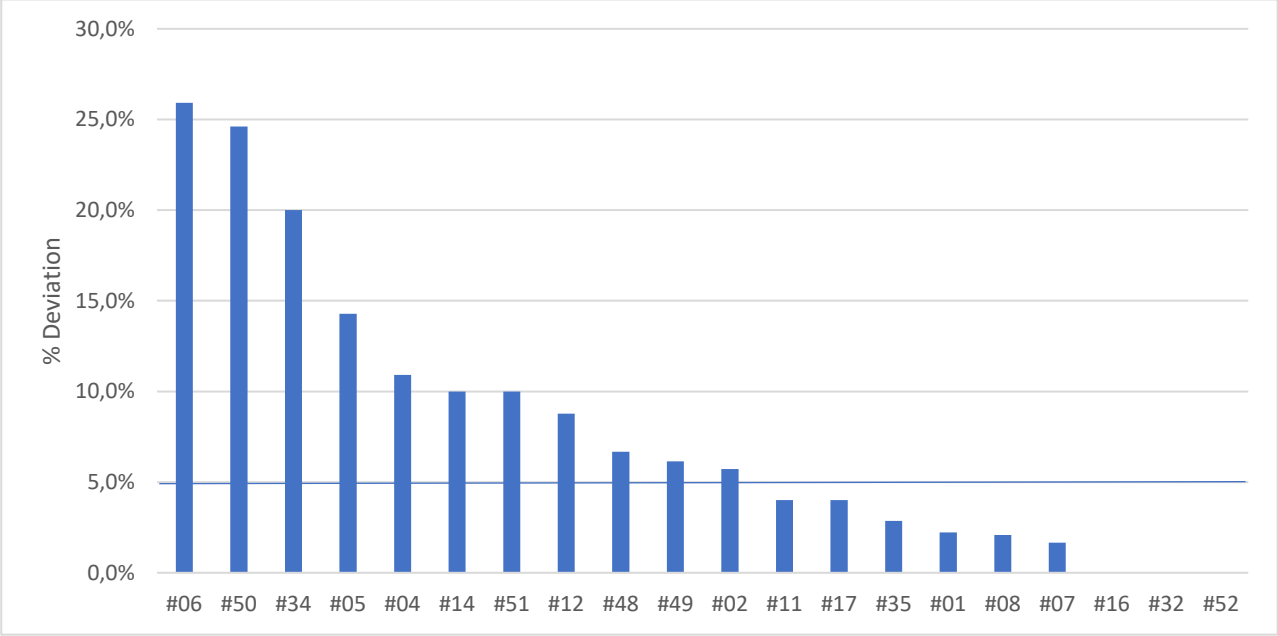


Figure 13. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=20) participating in the 6th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 evaluated separately for each of the trials.

Among the 20 participating laboratories, 16 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) (**Table 20**).

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 expected range are presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	**Total**
CHL	1/10	0/2	0/3	**1/15**
CIP	1/7	0/2	0/8	**1/17**
CLI	1/3	0/2	0/10	**1/15**
ERY	1/7	0/2	0/10	**1/19**
FOX	2/13	0/2	0/5	**2/20**
FUS	0/1	--	0/2	**0/3**
GEN	1/7	--	0/11	**1/18**
LZD	0/4	0/2	0/8	**0/14**
PEN	3/6	0/2	0/10	**3/18**
QND	--	--	0/5	**0/5**
RIF	0/1	--	0/7	**0/5**
SMX	0/1	--	--	**0/1**
TET	2/9	--	0/8	**2/17**
TMP	1/3	--	0/2	**1/5**
VAN	1/2	0/3	0/8	**1/13**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; Gradient – MIC determination by Gradient test; MIC – MIC determination by broth macro- and microdilution
* Gradient test is not recommended for colistin testing

The highest proportion of test results outside of the expected range were observed for penicillin (3 out of 18) (**Table 20**). All deviations occurred when the disk diffusion methodology was applied.

Laboratories #04, #06, #08, #11, #14, #16, #34, #48, and #49 had no deviations. The other seven laboratories had deviations ranging from 9.1% to 40.0% (**Figure 14)**. In this trial, the reported deviations were both above and below the acceptance interval.

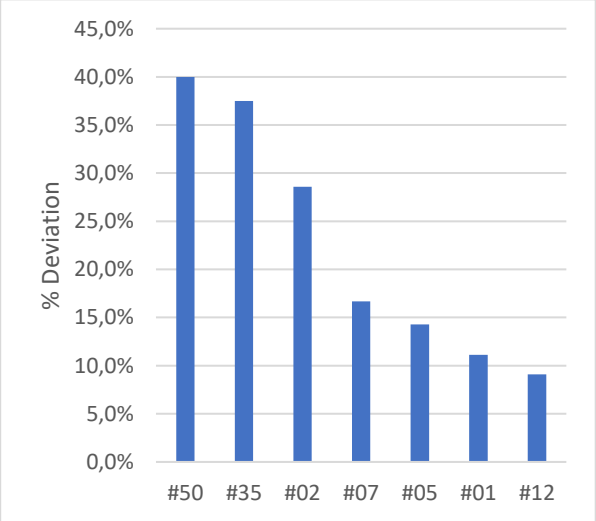


Figure 14. Percentage of deviation in the AST of *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC) in the *S. aureus* panel by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the nine Animal Health laboratories participating in the 6th EQA of the EQAsia project, eight laboratories submitted results for the *E. coli* panel, six for the *K. pneumoniae* panel, four for *P. aeruginosa* panel and eight laboratories submitted results for the *S. aureus* panel (**Figure 1**).

Regarding the methodologies applied by the laboratories, most of the participants opted for disk diffusion alone, followed by broth microdilution (automated) or a mixture of the two methodologies. The remaining laboratories applied disk diffusion in combination with other methodologies, such as broth microdilution (conventional) (**Figure 15**). Laboratory #47 did not report AST results for *K. pneumoniae* and *P. aeruginosa.*

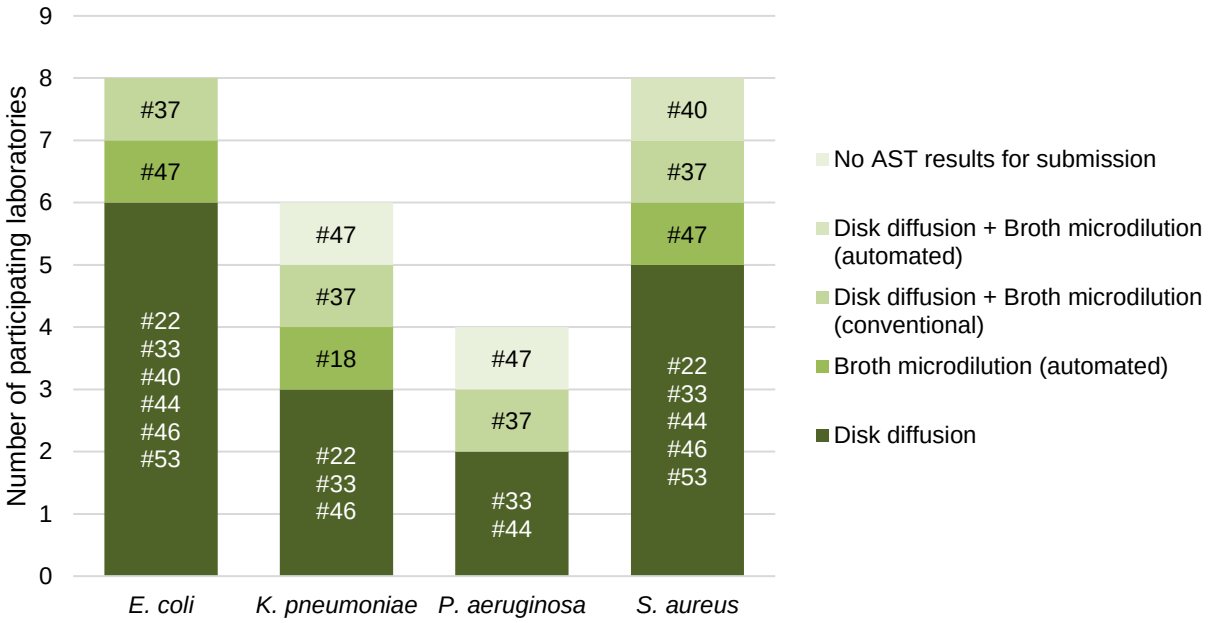


Figure 15. Methodologies applied by the AH laboratories participating for each of the panels.

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

Among the antimicrobial agents included in the *E. coli* panel, ampicillin, chloramphenicol, ciprofloxacin, gentamicin, meropenem, nalidixic acid and tetracycline were tested by all eight participating laboratories; in contrast, doripenem was tested by only one laboratory (**Table 21**). For the *K. pneumoniae* panel, the most tested antimicrobials were cefepime, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, meropenem, nalidixic acid and tetracycline (tested by all five laboratories), whereas doripenem was tested by only one AH laboratory. For the *P. aeruginosa* panel, ceftazidime, ciprofloxacin, gentamicin, levofloxacin and meropenem were tested by all three participating laboratories; in contrast, aztreonam, colistin, doripenem, imipenem and tobramycin were tested by only one laboratory. Lastly, in the *S. aureus* panel, chloramphenicol, erythromycin, gentamicin, and tetracycline were tested by all eight participating laboratories, while fusidic acid was tested by only one AH laboratory.

Table 21. Antimicrobial agents tested by the AH laboratories for each panel. For a given panel (Ec, Kp, Pa, Sa), the number of participating laboratories that tested each antimicrobial is shown (n), as well as the percentage (%) of laboratories out of the total number of participating laboratories (N) for the trial (% of n/N). The antimicrobials not included in a given panel are represented as --.

Antimicrobial	Laboratories in total: n (% of n/N)			
	Ec	Kp	Pa	Sa
AMK	5 (62.5%)	4 (80.0%)	2 (66.7%)	--
AMP	8 (100.0%)	4 (80.0%)	--	--
AZI	5 (62.5%)	3 (60.0%)	--	--
AZT	--	--	1 (33.3%)	--
FEP	7 (87.5%)	5 (100.0%)	2 (66.7%)	--
FOT	7 (87.5%)	4 (80.0%)	--	--
FOX	5 (62.5%)	5 (100.0%)	--	5 (62.5%)
TAZ	6 (75.0%)	4 (80.0%)	3 (100.0%)	--
CHL	8 (100.0%)	5 (100.0%)		8 (100.0%)
CIP	8 (100.0%)	5 (100.0%)	3 (100.0%)	7 (87.5%)
CLI	--	--	--	7 (87.5%)
COL	3 (37.5%)	2 (40.0%)	1 (33.3%)	--
DOR	1 (12.5%)	1 (20.0%)	1 (33.3%)	--
ERY	--	--	--	8 (100.0%)
ETP	6 (75.0%)	4 (80.0%)	--	--
FUS	--	--	--	2 (25.0%)
GEN	8 (100.0%)	5 (100.0%)	3 (100.0%)	8 (100.0%)
IMI	5 (62.5%)	4 (80.0%)	1 (33.3%)	--
KAN	--	--	--	3 (37.5%)
LEVO	3 (37.5%)	3 (60.0%)	3 (100.0%)	--
LZD	--	--	--	6 (75.0%)
MERO	8 (100.0%)	5 (100.0%)	3 (100.0%)	--
NAL	8 (100.0%)	5 (100.0%)	--	--
PEN	--	--	--	6 (75.0%)
PT4	4 (50.0%)	4 (80.0%)	2 (66.7%)	--
RIF	--	--	--	4 (50.0%)
SMX	3 (37.5%)	2 (40.0%)	--	3 (37.5%)
SYN	--	--	--	5 (62.5%)
TET	8 (100.0%)	5 (100.0%)	--	8 (100.0%)
TGC	4 (50.0%)	3 (60.0%)	--	--
TOB	2 (25.0%)	2 (40.0%)	1 (33.3%)	--
TMP	5 (62.5%)	3 (60.0%)	--	5 (62.5%)
SXT	6 (75.0%)	4 (80.0%)	--	--
VAN	--	--	--	4 (50.0%)
Total (N)	**8**	**5**	**3**	**8**

Ec, *E. coli*; Kp, *K. pneumoniae;* Pa, *P. aeruginosa;* Sa, *S. aureus*
(n) number of laboratories that reported results for the antimicrobial; (N) total number of participating laboratories for the trial

Scattering of missing data or incomplete AST results entries were observed in the *E. coli, K. pneumoniae* and *P. aeruginosa* panels (**Tables 22, 23** and **24**). Two of the eight laboratories selecting *E. coli* did not submit complete results. A closer look to laboratory #44 missing data suggests that this laboratory may have wrongly selected colistin instead of ciprofloxacin for strain Ec EQASIA 23.6 when submitting results (**Table 22**). Laboratory #46 missed reporting results for meropenem for strain Ec EQAsia 23.5 and strains Ec EQAsia 23.6, respectively (**Table 22**). Regarding the *K. pneumoniae* panel, one out of the five participating laboratories revealed incomplete results of their own available antimicrobial agents (**Table 23**). Similarly, laboratory #44 may have wrongly selected levofloxacin instead of meropenem for strain Pa

EQASIA 23.3 when submitting results (**Table 24**). 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=8) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Ec EQAsia 23.2	Ec EQAsia 23.4	Ec EQAsia 23.5	Ec EQAsia 23.6	Ec EQAsia 23.7
#44	COL	COL	COL	CIP	COL
#46	-	-	MERO	MERO	-

Ec, *E. coli*

Table 23. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by AH laboratories (n=5) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Kp EQAsia 23.1	Kp EQAsia 23.3	Kp EQAsia 22.5	Kp EQAsia 22.6	Kp EQAsia 22.7
#46	AMK, TAZ, GEN, TET, TGC	-	LEVO	LEVO, SXT	LEVO

Kp, *K. pneumoniae*

Table 24. Distribution of incomplete or missing data of antimicrobial agents among *P. aeruginosa* strains reported by AH laboratories (n=3) participating in the 6th EQA of the EQAsia project.

Lab ID No.	Pa EQAsia 23.1	Pa EQAsia 23.3	Pa EQAsia 22.4	Pa EQAsia 22.5	Pa EQAsia 22.7
#44	LEVO	MERO	LEVO	LEVO	LEVO

Pa, *P. aeruginosa*

4.2 *Escherichia coli* panel

Eight laboratories from six countries uploaded results for the *E. coli* panel.

4.2.1 Bacterial identification

All eight participating laboratories correctly identified the five *E. coli* strains and the two non-*E. coli.* Laboratory #46 did not submit data for Ec EQAsia 23.2 strains (**Table 25**).

Table 25. Bacterial identification of each of the seven test strains provided within 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 23.1	Non-*Escherichia coli*	8/8
Ec EQAsia 23.2	*Escherichia coli*	7/7
Ec EQAsia 23.3	Non-*Escherichia coli*	8/8
Ec EQAsia 23.4	*Escherichia coli*	8/8
Ec EQAsia 23.5	*Escherichia coli*	8/8
Ec EQAsia 23.6	*Escherichia coli*	8/8
Ec EQAsia 23.7	*Escherichia coli*	8/8

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 87.7% (strain Ec EQASIA 23.7) to 99.3% (strain Ec EQASIA 23.2) for each strain, with two strains revealing a deviation above 10% (**Table 26**). Strain Ec EQASIA 23.7 owes its high deviation to laboratory #37, which reported a resistant strain as susceptible to most of the tested antimicrobials.

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were colistin (20.5%), followed by tigecycline (19.7%), piperacillin/tazobactam (15.3%) and doripenem (15.0%). In reverse, sulfamethoxazole, trimethoprim, and trimethoprim/sulfamethoxazole revealed no deviation from the expected results (**Figure 16**). Colistin was tested by only three laboratories. Despite the low number of incorrect results, it caused the high deviation observed. Most of laboratories misinterpreted the results by reporting susceptible instead of intermediate.

Table 26. 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 23.2	448	99.3
Ec EQAsia 23.4	528	91.3
Ec EQAsia 23.5	524	96.6
Ec EQAsia 23.6	524	95.6
Ec EQAsia 23.7	528	87.7

Ec, *E. coli*

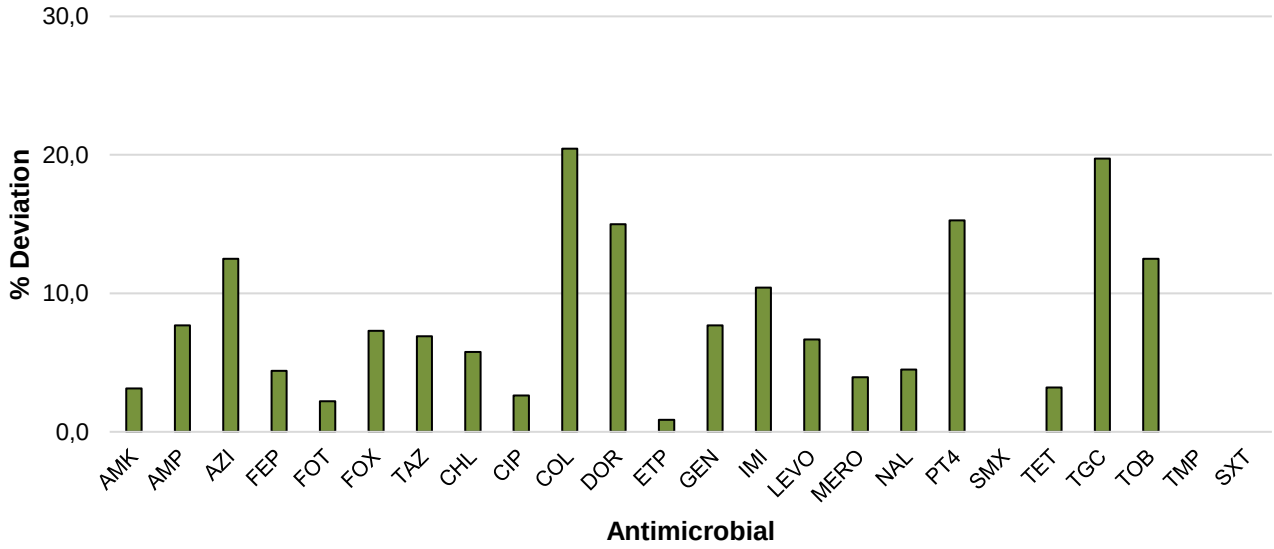


Figure 16. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=8) participating in the 6th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for four out of the eight participants (**Figure 17**). In average, the deviation was 5.5% (ranging from 1.7 to 11.5%). As the acceptance level was set to 5% deviation, four laboratories did not perform within the expected range for the trial.

The highest deviation was observed for laboratory #46 and can be explained by the incorrect results reported for azithromycin, cefepime, piperacillin and tazobactam, as well as

tigecycline. Laboratory #37 underperformance seems to be caused by the obtained results for strain Ec EQAsia 23.7, which reported a resistant strain as susceptible to most of the tested antimicrobials.

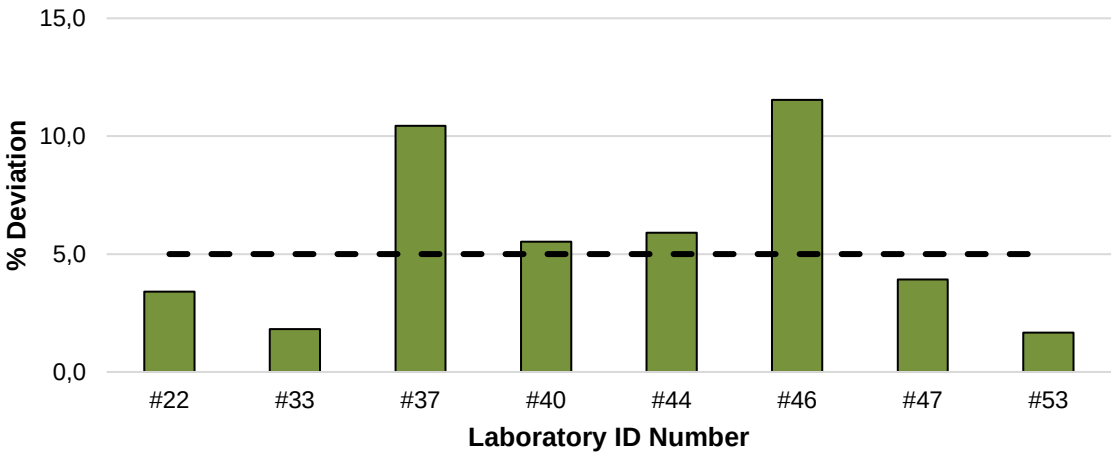


Figure 17. Percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by AH laboratories (n=8) participating in the 6th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 β-lactamase-producing *E. coli*

Five out of the eight participating laboratories uploaded results for this component of the *E. coli* panel (laboratories #22, #33, #37, #40, and #46). Laboratory #46 did not test strain Ec EQAsia 23.2. Discrepancies from the expected results are summarized in **Table 27**.

Of all five laboratories, three laboratories (#22, #33 and #37) correctly identified all the carbapenemase phenotypes. Strain Ec EQAsia 23.5 was correctly identified by all laboratories, followed by strain Ec EQAsia 23.2, which was wrongly classified by only one laboratory (#40); this laboratory classified this strain and Ec EQAsia 23.4 as ESBL+AmpC-producing *E. coli* strains instead of carbapenemase-producers. Strain Ec EQAsia 23.6 was wrongly reported as other phenotype by two laboratories (#37 and #40). Lastly, strain Ec EQAsia 23.7 was wrongly identified as ESBL+AmpC-producer and other phenotype by laboratories #46 and #37, respectively.

Table 27. 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 23.2	Ec EQASIA 23.4	Ec EQASIA 23.5	Ec EQASIA 23.6	Ec EQASIA 23.7
Expected results		Carbapenemase	Carbapenemase	ESBLs	Susceptible	Susceptible
Obtained results (n/N)	ESBLs	--	--	**5/5 (100.0%)**	--	--
	ESBLs + AmpC	1/4 (25.0%)	2/5 (40.0%)	--	--	1/5 (20.0%)
	Carbapenemase	**3/4 (75.0%)**	**3/5 (60.0%)**	**--**	**--**	**--**
	AmpC	--	**--**	**--**	--	--
	Other	--	--	--	2/5 (40.0%)	1/5 (20.0%)
	Susceptible*	--	--	--	**3/5 (60.0%)**	**3/5 (60.0%)**

Ec, *E. coli*

*no AmpC, ESBL and carbapenemase. (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 free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *E. coli* panel.

All eight participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only one reported result for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli ATCC* 25922. Inhibition zone diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated and conventional) (**Table 28**). For testing *E. coli* NCTC 13846, MIC was determined by microdilution methods.

The highest proportion of test results outside of the expected range was observed for cefepime (3 out of 7) and trimethoprim (2 out of 5) (**Table 28**).

Regarding the laboratories' performance (**Figure 18**), laboratories #22, #33 and #47 presented no deviation. While laboratories #22 and #33 applied disk diffusion, laboratory #47 used broth microdilution. The remaining five laboratories presented deviations that ranged from 8.3% to 63.2% (**Figure 18**).

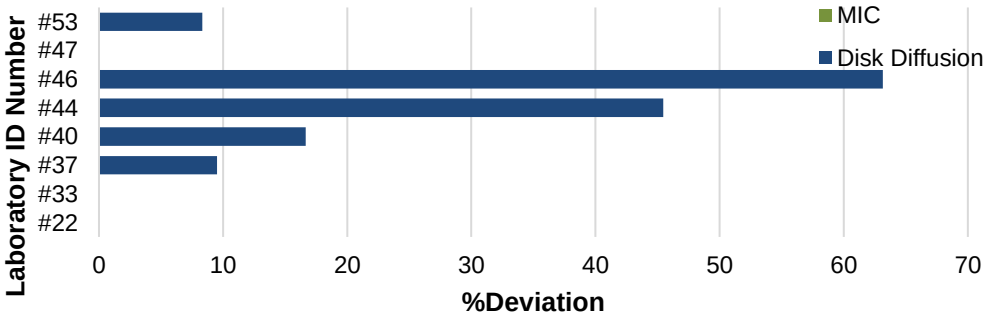


Figure 18. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *E. coli* panel by the AH laboratories.

Table 28. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *E. coli* panel. Proportion of test results outside of expected range is presented by methodology used.

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

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

4.3 *Klebsiella pneumoniae* panel

A total of six laboratories from four countries uploaded results for the *K. pneumoniae* panel.

4.3.1 Bacterial identification

All six participating laboratories submitted results for bacterial identification (**Table 29**). Four out of six laboratories correctly identified all seven test strains provided. Strain Kp EQAsia 23.3 was misidentified as non- *K. pneumoniae* by laboratory #18, whereas the non- *K. pneumoniae* strain Kp EQAsia 23.4 was reported as *K. pneumoniae* by laboratories #18 and #37. Strain Kp EQAsia 23.5 was misidentified as non- *K. pneumoniae* by laboratory #37.

Table 29. Bacterial identification of each of the seven test strains provided within 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 23.1	*Klebsiella pneumoniae*	6/6
Kp EQAsia 23.2	Non-*Klebsiella pneumoniae*	6/6
Kp EQAsia 23.3	*Klebsiella pneumoniae*	5/6
Kp EQAsia 23.4	Non-*Klebsiella pneumoniae*	4/6
Kp EQAsia 23.5	*Klebsiella pneumoniae*	5/6
Kp EQAsia 23.6	*Klebsiella pneumoniae*	6/6
Kp EQAsia 23.7	*Klebsiella pneumoniae*	6/6

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 84.6% (strain Kp EQASIA 23.6) to 97.2% (strain Kp EQASIA 23.7) for each strain, with only one strain revealing a deviation above 10% (**Table 30**). The highest deviation seen for strain Kp EQASIA 23.6 can be explained by the testing results for gentamicin, where almost all laboratories found the strain to be susceptible to the drug when it was expected to be resistant.

Table 30. 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 *K. pneumoniae* panel.

Strain	AST in total	% Correct
Kp EQAsia 23.1	344	96.5
Kp EQAsia 23.3	304	96.1
Kp EQAsia 23.5	268	93.3
Kp EQAsia 23.6	356	84.6
Kp EQAsia 23.7	360	97.2

Kp, *K. pneumoniae*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected results were tigecycline (56.3%) and gentamicin (29.5%), whereas ampicillin, azithromycin, cefotaxime, cefoxitin, ceftazidime, chloramphenicol, doripenem, ertapenem, sulfamethoxazole, tobramycin and trimethoprim revealed no deviation from the expected results (**Figure 19**).

The deviation observed for tigecycline was mostly due to laboratory #46, which reported four strains as resistant to the drug when they were expected to be susceptible, as well as by few incorrect results reported by laboratories #18 and #37.

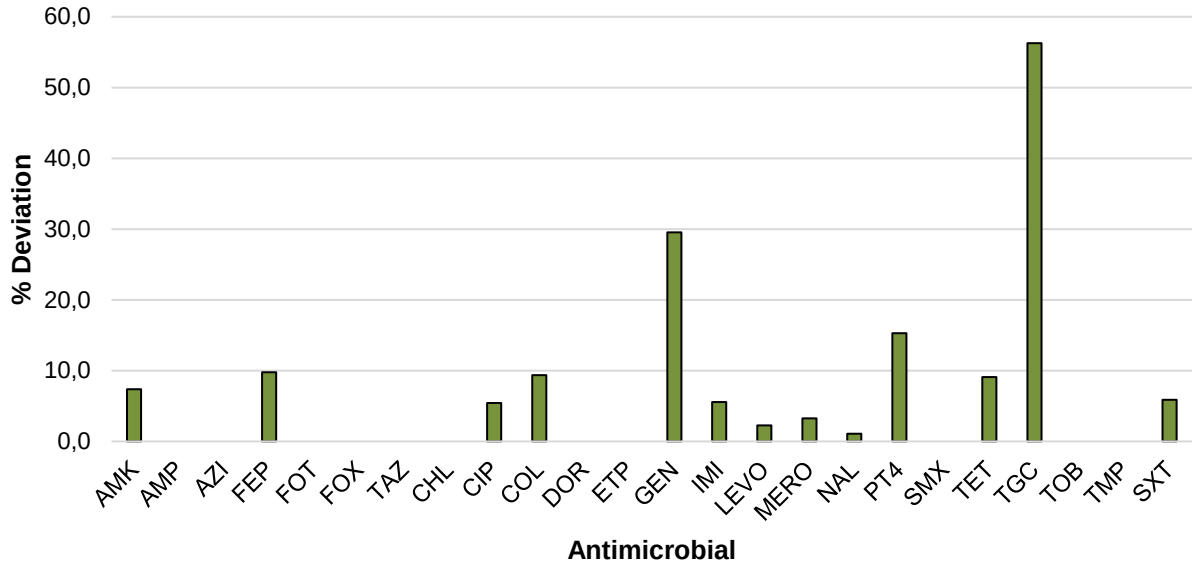


Figure 19. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=5) participating in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for only one participant (**Figure 20**). In average, the deviation was 6.7% (ranging from 3.2 to 11.7%). For laboratories #22 and #37, the deviations were slightly above the acceptance level.

Laboratory #18 deviations were due to reported incorrect results for several antimicrobials, such as tigecycline, amikacin, gentamicin, tetracycline, and imipenem.

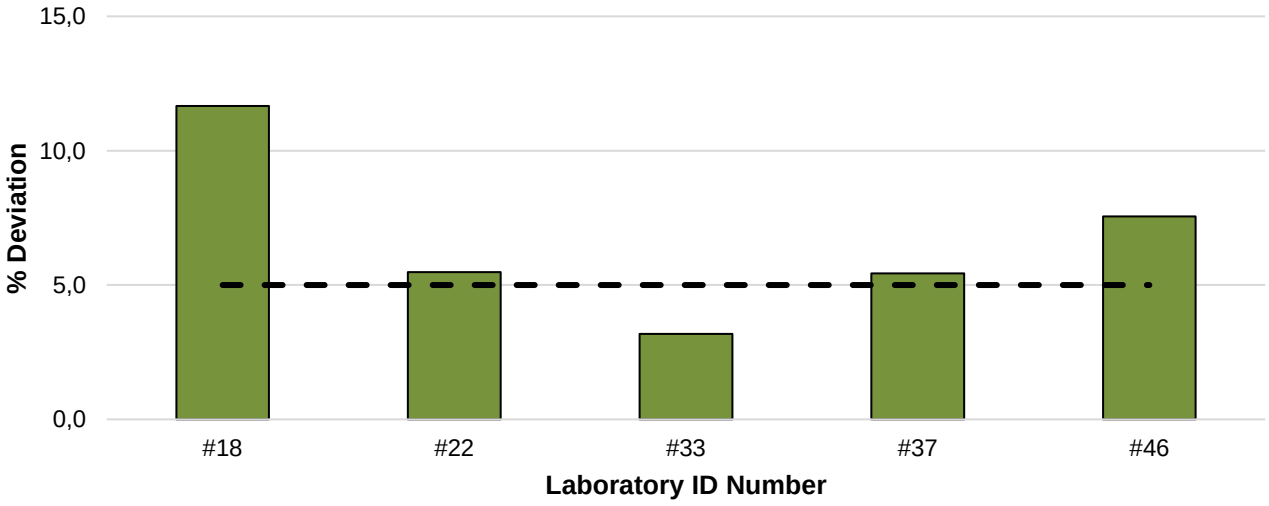


Figure 20. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=5) participating in the 6th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 β-lactamase-producing *K. pneumoniae*

Four out of the five participating laboratories uploaded results for this component of the *K. pneumoniae* panel (laboratories #22, #33, #37 and #46). Discrepancies from the expected results are summarized in **Table 31**.

Firstly, laboratories identified the strains that produced ESBL/AmpC/carbapenemase, and then reported the specific phenotype. Strains Kp EQASIA 23.1, Kp EQASIA 23.3 and Kp EQASIA 23.5 were expected to be carbapenemase-producers; however, laboratory #46 had incorrectly classified the strains Kp EQASIA 23.1 and Kp EQASIA 23.5 as ESBL+AmpC-producers. Laboratory #22 had also incorrectly classified the strain Kp EQASIA 23.5 as ESBL+AmpC-producer. Strain Kp EQAsia 23.3 was correctly identified by all laboratories. Strain Kp EQAsia 23.6 was reported as other phenotype by two laboratories (#22 and #37). Lastly, strain Kp EQAsia 23.7 was incorrectly classified as other phenotype by laboratory #37.

Table 31. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *K. pneumoniae* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 4 AH laboratories.

Strain code		Kp EQASIA 23.1	Kp EQASIA 23.3	Kp EQASIA 23.5	Kp EQASIA 23.6	Kp EQASIA 23.7
Expected results		Carbapenemase	Carbapenemase	Carbapenemase	ESBLs	Susceptible
Obtained results (n/N)	ESBLs	--	--	**--**	**2/4 (50.0%)**	--
	ESBLs + AmpC	1/4 (25.0%)	--	2/3 (66.7%)	--	--
	Carbapenemase	**3/4 (75.0%)**	**4/4 (100.0%)**	**1/3 (33.3%)**	**--**	**--**
	AmpC	--	**--**	**--**	--	--
	Other	--	--	--	2/4 (50.0%)	1/4 (25.0%)
	Susceptible*	--	--	--	**--**	**3/4 (75.0%)**

Kp, *K. pneumoniae*

*no AmpC, ESBL and carbapenemase. (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 free of charge (in previous trials) to all participating laboratories to be used as reference strains for the *K. pneumoniae* panel.

All five participating laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only one reported result for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli ATCC* 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by broth microdilution (automated and conventional) (**Table 32**). For testing *E. coli* NCTC 13846, MIC was determined by microdilution methods.

The highest proportion of test results outside of the expected range was observed for trimethoprim (2 out of 3), tigecycline (2 out of 4) and trimethoprim/sulfamethoxazole (2 out of 4) (**Table 32**).

Regarding the laboratories' performance (Figure 7), laboratories #22 and #33 presented no deviation. The methodology they applied was disk diffusion. The remaining three laboratories presented deviations that ranged from 9.1% to

65.0% (**Figure 21**). Laboratory #46 presented 13 deviations, where inhibition zone diameters reported were below the expected range. Laboratory #18 presented five deviations when applying broth microdilution.

Table 32. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (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	MIC	Total
AMK	0/3	0/1	**0/4**
AMP	1/4	--	**1/4**
FEP	1/4	1/1	**2/5**
FOT	0/4	--	**0/4**
FOX	1/4	--	**1/4**
TAZ	0/4	--	**0/4**
CHL	1/4	--	**1/4**
CIP	1/4	1/1	**2/5**
COL	--	0/1	**0/1**
DOR	0/1	--	**0/1**
ETP	0/3	1/1	**1/4**
GEN	0/4	0/1	**0/5**
IMI	0/3	0/1	**0/4**
LEVO	1/3	--	**1/3**
MERO	0/4	1/1	**1/5**
NAL	2/4	0/1	**2/5**
PT4	1/2	0/1	**1/3**
SMX	1/2	--	**1/2**
TET	1/4	--	**1/4**
TGC	1/3	1/1	**2/4**
TOB	0/2	--	**0/2**
TMP	2/3	--	**2/3**
SXT	1/3	1/1	**2/4**

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

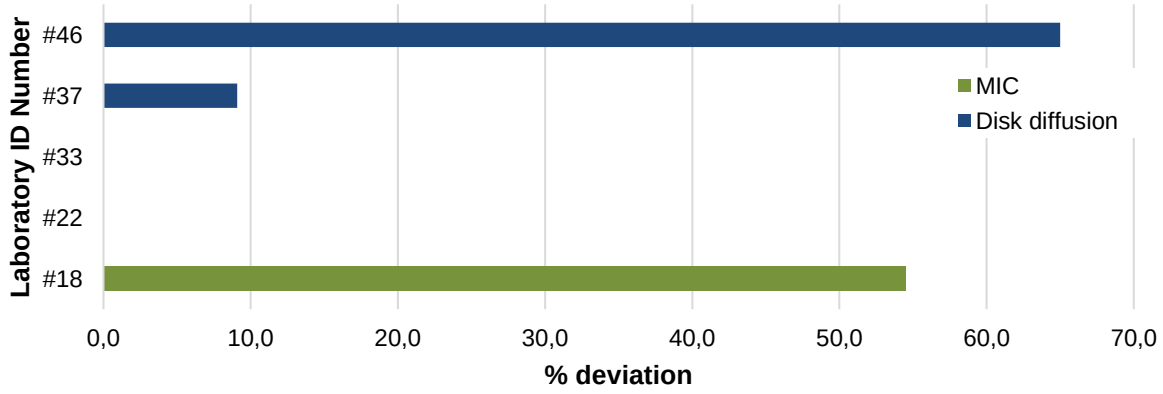


Figure 21. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *K. pneumoniae* panel by the AH laboratories.

4.4 *Pseudomonas aeruginosa* panel

Four laboratories from three countries uploaded results for the *P. aeruginosa* panel.

4.4.1 Bacterial identification

All four participating laboratories submitted results for bacterial identification and correctly identified the tested *P. aeruginosa* and non- *P. aeruginosa* (**Table 33**).

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

Strain	Bacterial ID	No. correct
Pa EQAsia 23.1	*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.2	Non-*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.3	*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.4	*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.5	*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.6	Non-*Pseudomonas aeruginosa*	4/4
Pa EQAsia 23.7	*Pseudomonas aeruginosa*	4/4

Pa, *P. aeruginosa*

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 64.6% (strain Pa EQASIA 23.7) to 96.0% (strain Pa EQASIA 23.1 and 23.5) for each strain (**Table 34**). The results from two strains revealed more than 10% deviation (Pa EQASIA 23.3 and Pa EQASIA 23.7) and only two strains had a deviation below to 5% (**Table 34**). Strain Pa EQASIA 23.3 owes its high deviation to laboratory #37, which reported susceptible strain to most of the tested antimicrobials, even though that outcome was not expected. For the strain Pa EQASIA 23.7, laboratory #37 had reported a susceptible strain as resistant to several antimicrobials.

Table 34. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 3 AH laboratories for the *P. aeruginosa* panel.

Strain	AST in total	% Correct
Pa EQAsia 23.1	100	96.0
Pa EQAsia 23.3	100	55.0
Pa EQAsia 23.4	100	92.0
Pa EQAsia 23.5	100	96.0
Pa EQAsia 23.7	96	64.6

Pa, *P. aeruginosa*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were doripenem (35.0%), imipenem (35%), and tobramycin (35%) (**Figure 22**). Doripenem, imipenem and tobramycin were tested by laboratory #37 only, the laboratory reported incorrect results for two of the five strains.

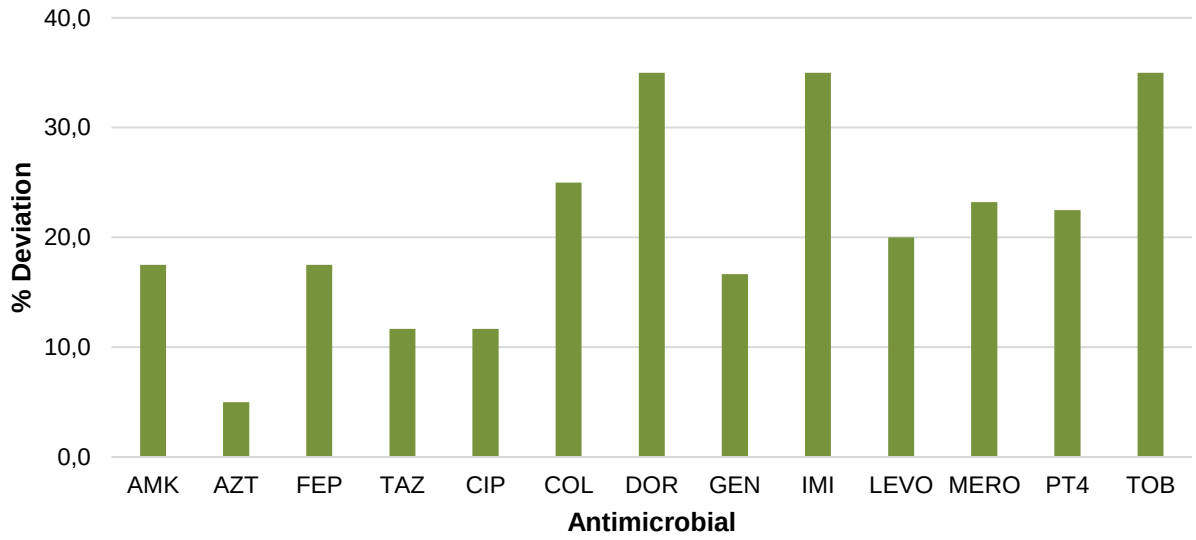


Figure 22. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=3) participating in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for two out of the three participants (**Figure 23**). In average, the deviation was 12.9% (ranging from 2.0% to 34.6%). Laboratory #37 presented the highest deviation, which can be explained by the already mentioned incorrect results reported for strain Pa EQASIA 23.3 and Pa EQASIA 23.7.

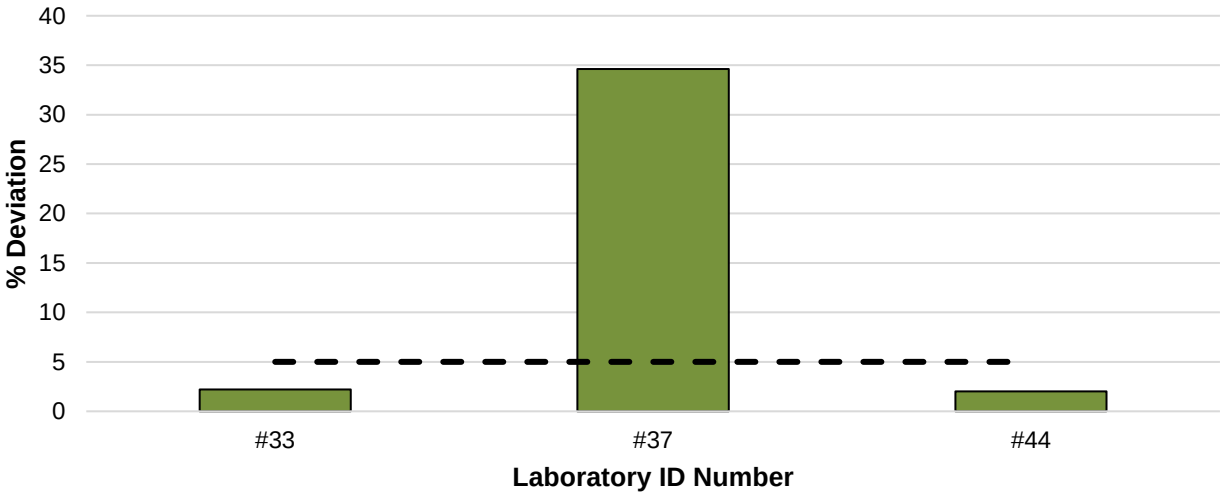


Figure 23. Percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by AH laboratories (n=3) participating in the 6th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strains *Pseudomonas aeruginosa* ATCC 27853

The quality control strain *P. aeruginosa* ATCC 27853 was sent to all participating laboratories free of charge (in previous trials) to be used as a reference strain for the *P. aeruginosa* panel. Among the three participating laboratories, only two submitted results for the reference strain *P. aeruginosa* ATCC 27853.

The highest proportion of test results outside of the expected range were observed for cefepime (1 out of 1), ceftazidime (1 out of 2) and meropenem (1 out of 2) (**Table 35**).

In terms of performance, laboratory #33 presented no deviation for the seven antimicrobials tested. Inversely, laboratories #44 had three deviations (**Figure 24**). Laboratory #44 presented deviations above the acceptance interval.

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

Antimicrobial	Proportion outside of range	
	Disk Diffusion	Total
AMK	0/1	0/1
FEP	1/1	1/1
TAZ	1/2	1/2
CIP	0/2	0/2
GEN	0/2	0/2
LEVO	0/1	0/1
MERO	1/2	1/2
PT4	0/1	0/1

Disk Diffusion – Inhibition Zone Diameter determination by Disk Diffusion

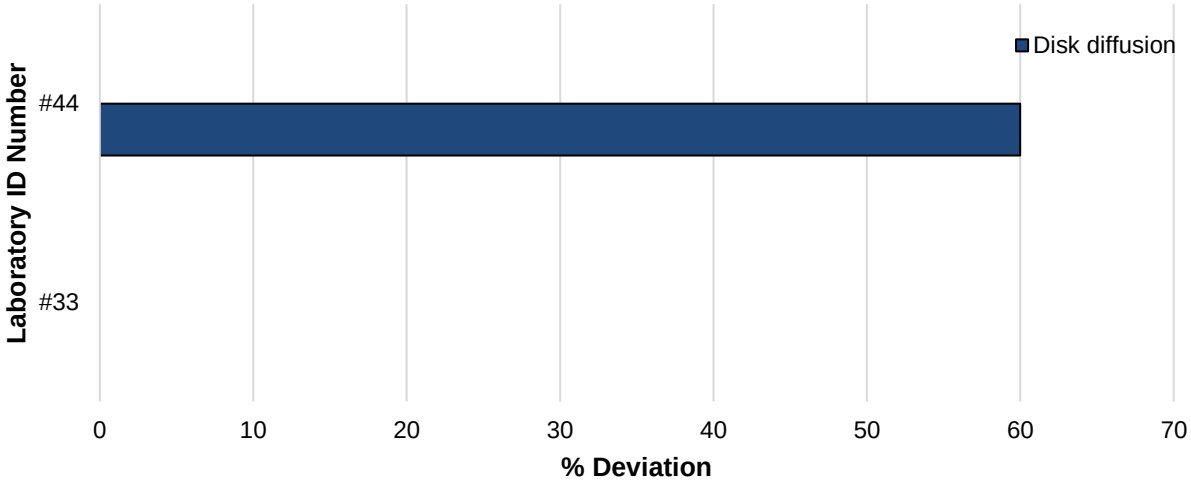


Figure 24. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *P. aeruginosa* panel by the AH laboratories.

4.5 *Staphylococcus aureus* panel

Eight laboratories from six countries uploaded results for the *S. aureus* panel.

4.5.1 Bacterial identification

All eight participating laboratories submitted results for bacterial identification (**Table 36**). All 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 within 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 23.1	*Staphylococcus aureus*	8/8
Sa EQAsia 23.2	*Staphylococcus aureus*	8/8
Sa EQAsia 23.3	*Staphylococcus aureus*	8/8
Sa EQAsia 23.4	*Staphylococcus aureus*	8/8
Sa EQAsia 23.5	Non-*Staphylococcus aureus*	8/8
Sa EQAsia 23.6	*Staphylococcus aureus*	8/8
Sa EQAsia 23.7	Non-*Staphylococcus aureus*	8/8

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 80.9% (strain Sa EQASIA 23.3) to 98.9% (strain Sa EQASIA 23.1) for each strain (**Table 37**). The results from two strains revealed more than 10% deviation (Sa EQASIA 23.3 and Sa EQASIA 23.4) (Table 18). This high deviation for Sa EQASIA 23.3 and Sa EQASIA 23.4 were mostly caused by the results submitted by laboratory #37, which reported a susceptible strain as resistant to several antimicrobials.

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 23.1	356	98.9
Sa EQAsia 23.2	356	94.7
Sa EQAsia 23.3	356	80.9
Sa EQAsia 23.4	356	87.6
Sa EQAsia 23.6	356	94.7

Sa, *S. aureus*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected results were sulfamethoxazole (21.7%), followed by vancomycin (20.0%), and rifampin (15.0%) (**Figure 25**).

Sulfamethoxazole was tested by only two laboratories. It means that even few incorrect results would result in a high percentage of deviation. In this case, the results reported by laboratory #44 were incorrect for three of the five strains.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for three out of the eight participants (**Figure 26**). In average, the deviation was 8.1% (ranging from 0.9% to 23.6%). Laboratory #37 presented the highest deviation, which can be explained by the already mentioned incorrect results reported for strain Sa EQASIA 23.3 and Sa EQASIA 23.4.

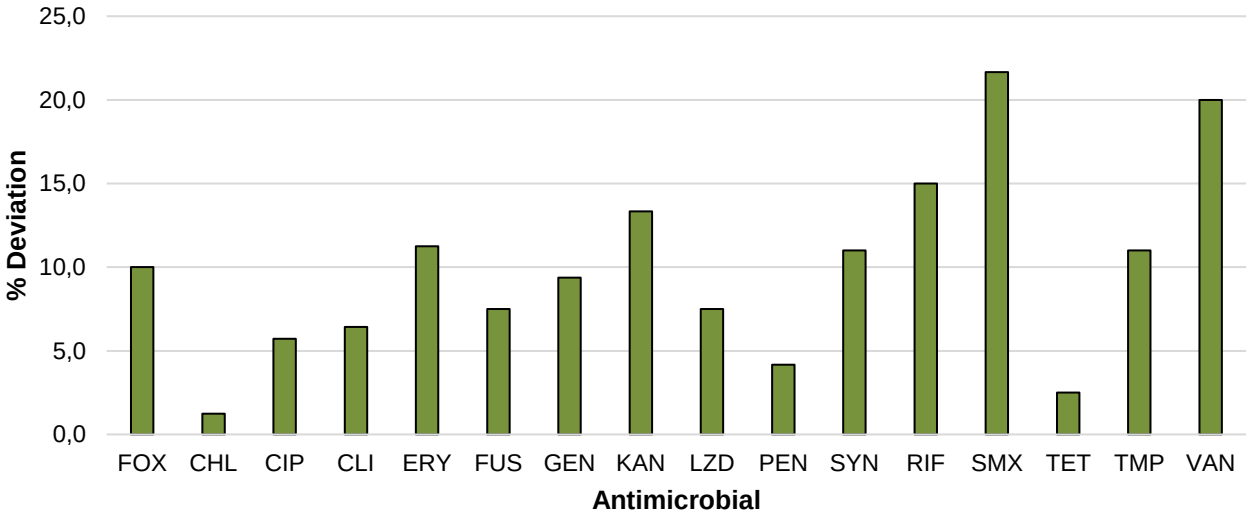


Figure 25. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

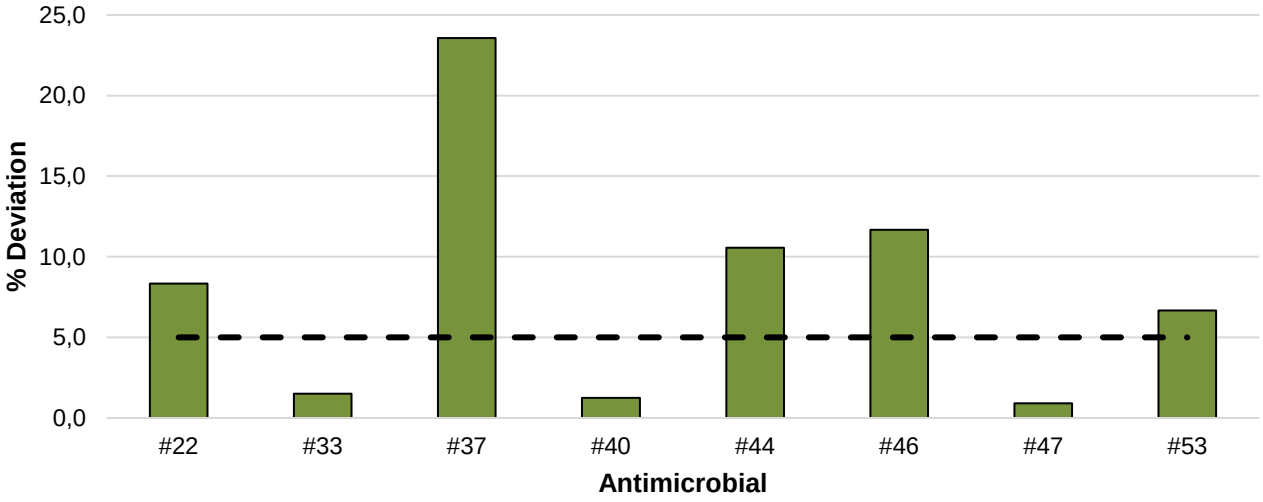


Figure 26. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 6th EQA of the EQAsia project. Results are categorized by laboratory ID number.

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 free of charge (in previous trials) to all participating laboratories to be used as reference strains for the *S. aureus* panel.

All eight participating laboratories submitted AST results for the reference strains: seven laboratories reported data for *S. aureus* ATCC 25923 reference strain as disk diffusion was the methodology applied (**Table 38,** *).

Laboratories #37 and #40 selected *S. aureus* ATCC 29213 to test vancomycin by broth microdilution method (**Table 38,** **). Laboratory #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 vancomycin (2 out of 4) and sulfamethoxazole (1 out of 3) (**Table 38**).

A closer look at the laboratories' performance (**Figure 27**) shows that three laboratories had no deviation from the expected range (#22, #33 and #47). Inversely, laboratory #40 presented a 36.4% deviation, corresponding to incorrect results for all 12 tested antimicrobials. The remaining four laboratories (#37, #44, #46 and #53) had three, two, two and one deviations each, respectively.

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 Diff. *	MIC **	Total
FOX	1/5	--	**1/5**
CHL	2/7	0/1	**2/8**
CIP	0/6	0/1	**0/7**
CLI	0/6	0/1	**0/7**
ERY	0/7	0/1	**0/8**
FUS	0/2	--	**0/2**
GEN	1/7	0/1	**1/8**
KAN	0/3	--	**0/3**
LZD	1/5	0/1	**1/6**
PEN	1/5	0/1	**1/6**
SYN	1/4	--	**1/4**
RIF	0/3	--	**0/3**
SMX	1/3	--	**1/3**
TET	2/7	0/1	**2/8**
TMP	1/5	--	**1/5**
VAN	1/1	1/3	**2/4**

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

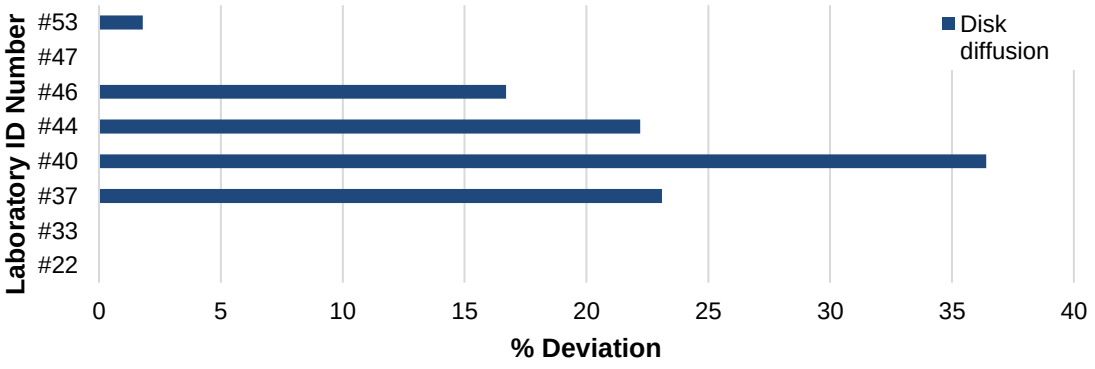


Figure 27. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* panel by the AH laboratories.

5. Results – Overall

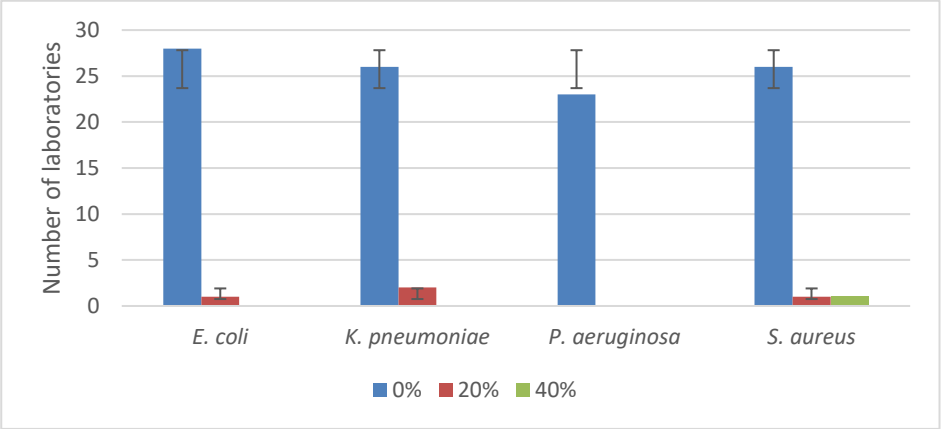
5.1 Bacterial identification

A total of 23 HH and nine AH laboratories participated in this EQA trial. One HH laboratory 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 29 laboratories for the *E. coli* panel, 28 laboratories for the *K. pneumoniae* panel, 23 – for *P. aeruginosa*, and 28 – 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 trials, 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 component of *E. coli, K. pneumoniae,* and *S. aureus*. There were no deviations in the bacterial identification of the *P. aeruginosa* panel.

Figure 28. Percentage of deviation in the bacterial identification of *E. coli*, *K. pneumoniae*, *P. aeruginosa* 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 trials, and for each trial in general, is presented in this section.

5.2.1 Antimicrobials

In each of the panels, the antimicrobials were tested by a varying number of laboratories. **Figures 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 tobramycin and tigecycline (37.8% and 35.6%, respectively), likely since these antimicrobials were tested by fewer laboratories compared to most of the other agents (**Figure 29**). All other antimicrobials showed deviations below 20%.

The results submitted for the *K. pneumoniae* panel showed most deviations for tigecycline (68.2%) mainly because of fewer tests being done (n=22) (**Figure 30)**. Overall, compared to the *E. coli* panel, there were fewer deviations for all other antibiotics except for tetracycline and ciprofloxacin.

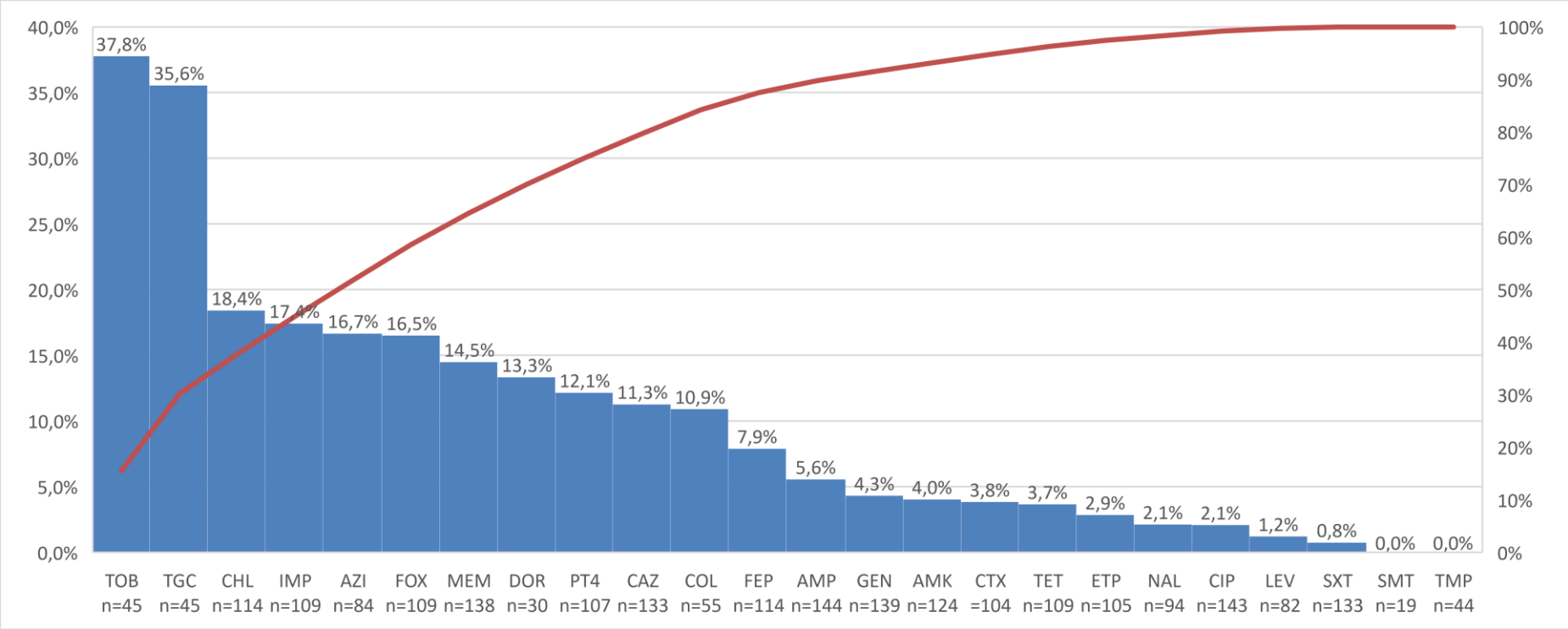


Figure 29. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *E. coli* strains by the participating laboratories (n=29) in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

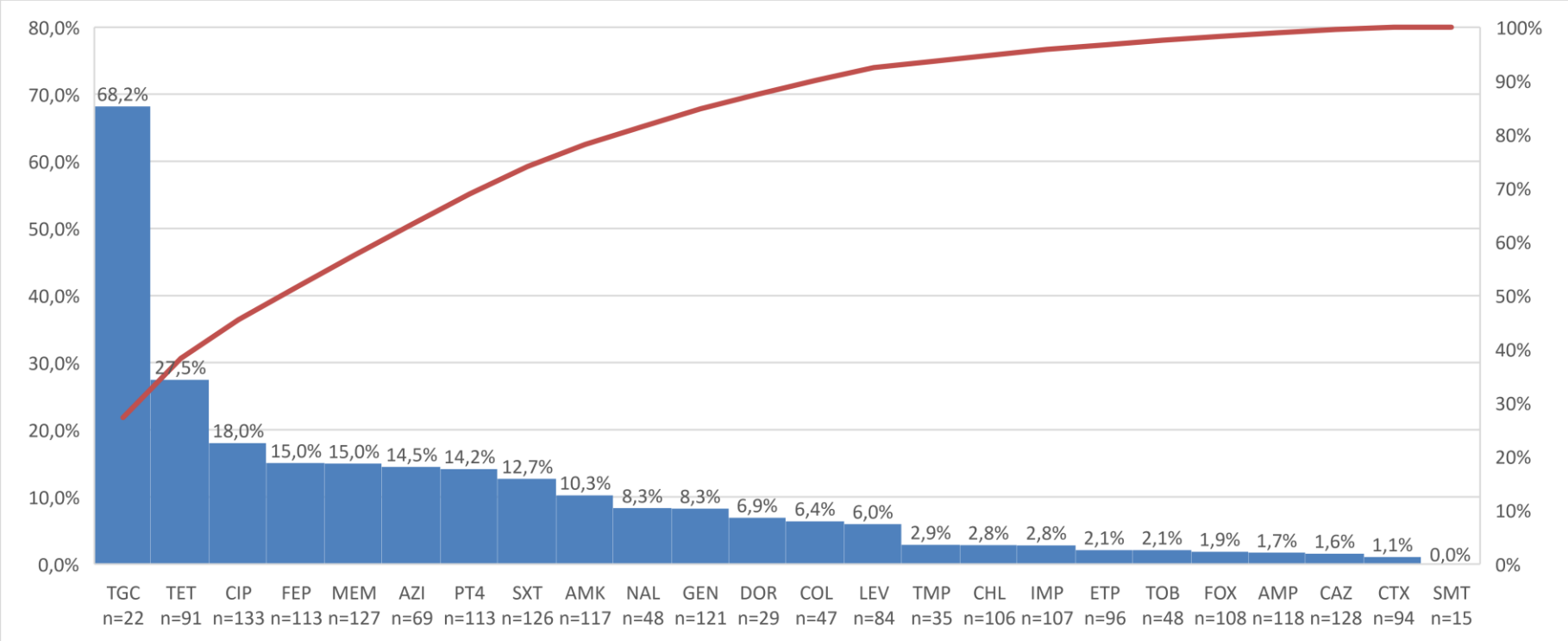


Figure 30. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by the participating laboratories (n=28) in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

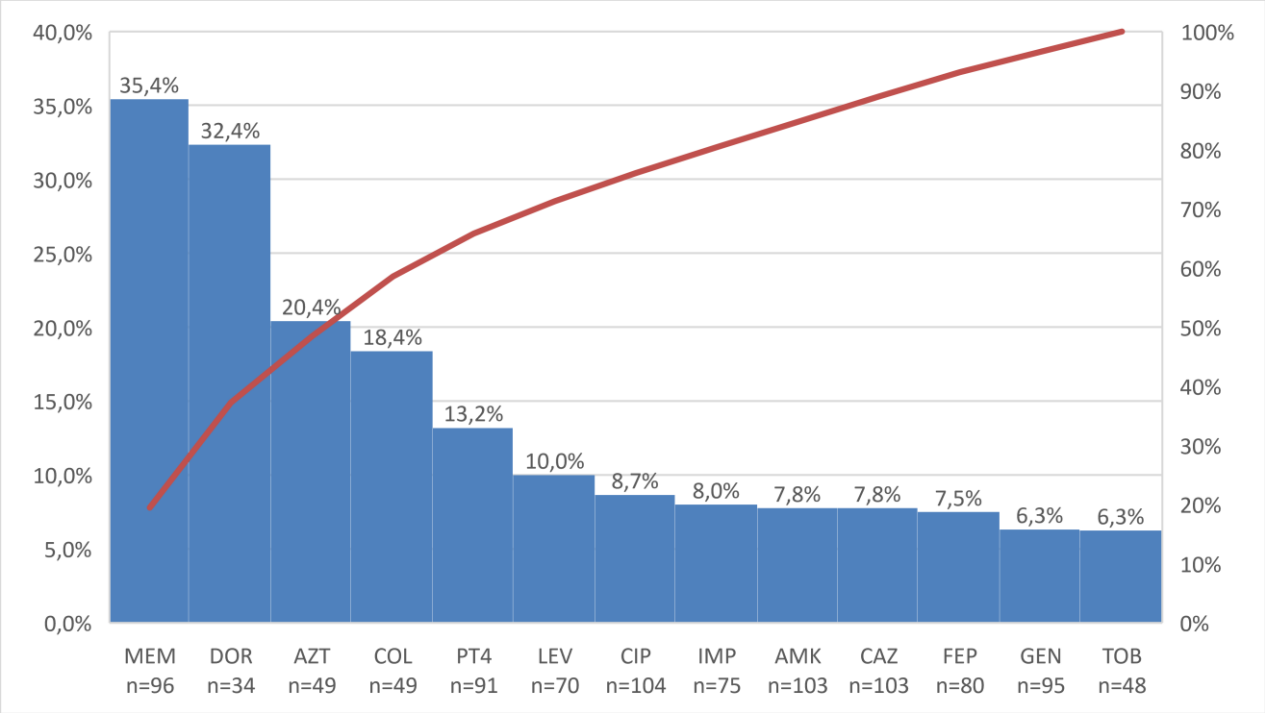


Figure 31. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *P. aeruginosa* strains by the participating laboratories (n=23) in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

The results submitted for the *P. aeruginosa* panel showed deviations for all reported antimicrobials, mostly for meropenem (35.4%) and doripenem (32.4%) (**Figure 31)**. All other results showed deviations of 20% or less.

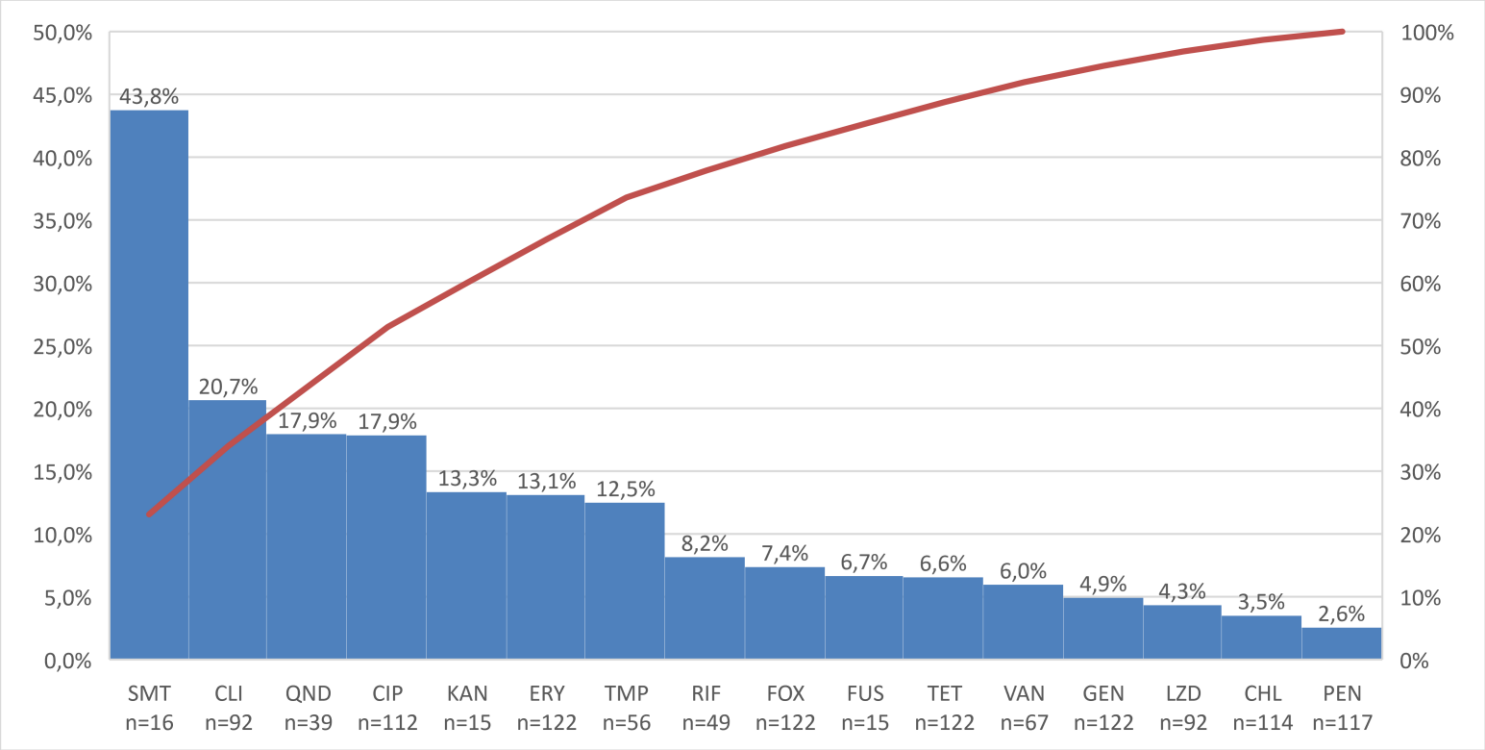


Figure 32. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by the participating laboratories (n=28) in the 6th EQA of the EQAsia project. Results are categorized according to antimicrobial agent by decreasing percentage of deviations. The number of tests performed is indicated below each antimicrobials' abbreviation. The red line represents the cumulative percentage of deviation.

The results submitted in the *S. aureus* panel showed deviations for all reported antimicrobials, mostly for sulfamethoxazole (43.8%). However, this antimicrobial was tested by very few (predominantly AH) laboratories (**Figure 32)**. Of the other tested antimicrobials, clindamycin, quinupristin/dalfopristin and ciprofloxacin showed the most deviations.

5.2.2 Laboratories performance

In each of the panels, the laboratories' performance varied based on their performance score. However, the performance rate was not substantially different between the four panels included in this EQA round (**Figure 33**).

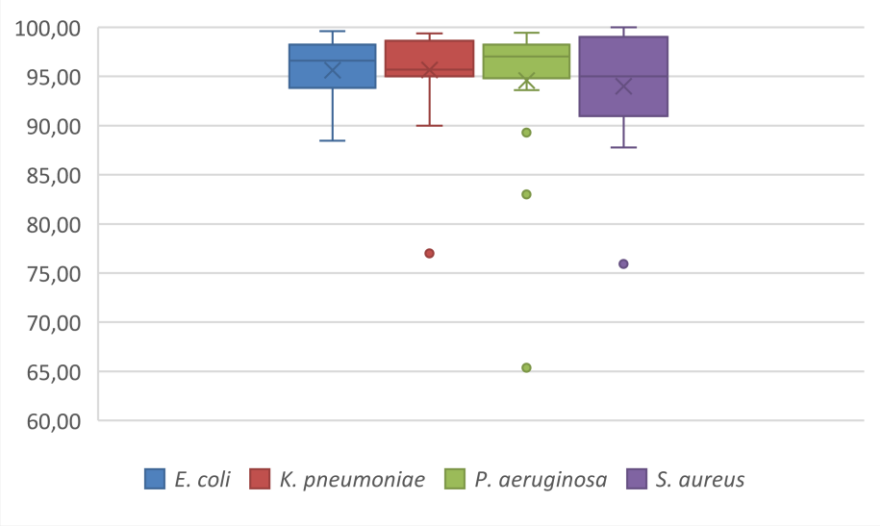


Figure 33. Distribution of the performance rate according to the obtained AST results by laboratories participating in the 6th EQA of the EQAsia project. Most laboratories' performance rate was clustered between 87.8% and 100%, being more homogenous for *P. aeruginosa* panel with just a few outliers.

Out of the four panels included in this trial, the obtained results were the best for the *E. coli* and *K. pneumoniae* panels (average score 95.5% and 95.9%, respectively). The labs with minimum score in these two panels had a performance rate of 88.5% and 77%, respectively. The performance score of the participating laboratories in the *P. aeruginosa* panel were mostly clustered between 90% and 100%, with just three laboratories having a score below 90%. The average score for this panel was 94.5%. The results submitted for the *S. aureus* panel were more heterogenous and generated scores of 75.9% and 100%, with an average of 93.9%.

Laboratories were ranked (#1 to #31) based on their average score across the panels in which they participated. The average score varied between 81.8% (rank #31) and 99.3% (rank #1). The total average score among all 31 laboratories that submitted results was 93.6%, while the median was 94.1%.

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

5.3 Quality control strains

Relevant quality control strains were tested for each of the panels: *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were used as reference strains for the *E. coli* and *K. pneumoniae* panels, *P. aeruginosa* ATCC 27853 for the *P. aeruginosa* panel, and *S. aureus* ATCC 25923 and *S. aureus* ATCC 29212 for testing when disk diffusion or MIC determination methodologies were applied, respectively, for the *S. aureus* panel. As with previous EQAsia EQAs, many of the laboratories were struggling the most with the results obtained when testing quality control strains. Several laboratories (3 in the *P. aeruginosa* panel and 5 in the *S. aureus* panel) did not submit results from reference strain testing at all. For the *E. coli* EQA round, there were ten laboratories (7 HH and 3 AH) that did not have deviation in their quality control results. However, all the other laboratories (n=19) presented deviations between 5.6% and 63.2%. Since the same quality control strains were used also for the *K. pneumoniae* panel, the submitted results were similar. Nine laboratories (7 HH and 2 AH) showed no deviations, while the results from the other 18 laboratories deviated

ranging between 5.6% to 65%. There was much less heterogeneity in the *P. aeruginosa* panel where the deviations were between 9.1% and 42.9%. 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 40%.

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 and partly also in the *K. pneumoniae* panel since the quality control strains used were the same for both panels (**Figure 34**). The minimum score in the *E. coli* panel was 36.8%, while in the *K. pneumoniae* panel it was 35%. The testing of the *P. aeruginosa* ATCC 27853 quality control strain was more straightforward and the deviations there from the expected ranges were observed less. Contrary to the test strains AST results in this panel, most of the QC results generated a clustered range of scores between 84.6% and 100%, with just two outliers outside of this range. Partly because fewer laboratories tested the *S. aureus* quality control strains, the performance scores here were more diverse ranging from 60% to 100%.

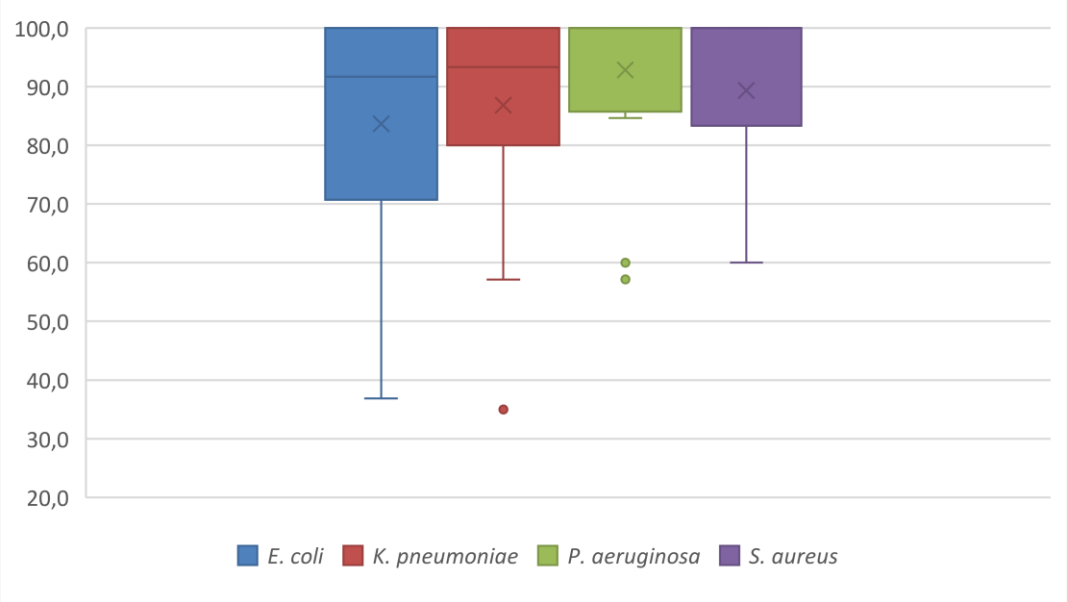


Figure 34. Distribution of the performance rate according to the obtained AST results for the reference strains by laboratories participating in the 6th EQA of the EQAsia project.

6. Discussion

6.1 Human Health Laboratories

Of 23 Human Health laboratories participating in the 6th EQA of the EQAsia project, 22 laboratories have 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 *S. aureus* panel. It would be expected that the isolates of each trial would be tested against the same panel of antimicrobials, allowing for a solid assessment of the laboratories' performance and capacity.

Regarding the bacterial identification component of this EQA, the participants showed high proficiency in correctly identifying the *E. coli, K. pneumoniae* and *P. aeruginosa* species among the provided test strains. In the *S. aureus* 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 (42.3% and 60.0% in the *E. coli* and *K. pneumoniae* panels, respectively). This was likely due to the fact that there is 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* trial), clindamycin revealed a rather high deviation (28.1%).

Regarding the HH laboratories' AST performance, on average, the deviation was 8.1% in the *E. coli* panel, 9.3% in the *K. pneumoniae* panel, 11.0% in the *P. aeruginosa* panel and 8.0% 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 and 19 participating laboratories submitted results for *E. coli* and *K. pneumoniae*, respectively, and, in most of the cases, were able to differentiate the carbapenemase-producers from susceptible (no ESBL, AmpC or carbapenemase)/ESBL/AmpC-producers. Two laboratories correctly identified all the carbapenemase phenotypes among the 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 support on capacity building.

Among all laboratories, there were five laboratories that did not submit antimicrobial susceptibility testing results for the quality control strains: laboratory #16 did not submit results for the reference strain in the *P. aeruginosa* panel, while laboratories #17, #32, #51, and #52 – for the *S. aureus* panel. 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, nine laboratories participated in the 6th EQA of the EQAsia project. The participating laboratories mostly applied disk diffusion alone for determining Inhibition Zone Diameters, others opted for broth microdilution (automated) or a mixture of the two methodologies.

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

As mentioned above, bacterial identification was the first component in each of the panels. There were no major issues with bacterial identification of the five target strains among the seven isolates provided (except for two laboratories in the *K. pneumoniae* panel). Strain Kp EQAsia 23.3 was misidentified as non- *K. pneumoniae* by laboratory #18, whereas the non- *K. pneumoniae* strain Kp EQAsia 23.4 was reported as *K. pneumoniae* by laboratories #18 and #37. Strain Kp EQAsia 23.5 was misidentified as non- *K. pneumoniae* by laboratory #37.

For the antimicrobial susceptibility testing performance, and as seen for the HH laboratories, tigecycline presented quite high deviations in the *E. coli* and *K. pneumoniae* panels (19.7% and 56.3%, respectively), which can be explained by the fact that tigecycline was tested by very few laboratories.

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 four out of the eight participants in the *E. coli* panel and for only one participant in the *K. pneumoniae* panel. Almost all (two out of the three laboratories) showed a deviation below 5% in the *P. aeruginosa* panel. For the *S. aureus* panel, the ratio was three out of eight participants.

Five out of the eight participating laboratories in the *E. coli* panel and four out of the five in the *K. pneumoniae* panel submitted results for the detection and confirmation of presumptive beta-lactamase producing bacteria. Three laboratories (#22, #33 and #37) correctly identified all the *E. coli* carbapenemase phenotypes. The laboratories were divided in their results submitted for the *K. pneumoniae* panel regarding carbapenemase production. As seen in some of the HH laboratories, classification of the carbapenemase phenotypes seems to be problematic. This observation suggests that further clarification on the classification of the different phenotypes is still required.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the panels. All participating laboratories submitted results for the reference strains in the *E. coli*, *K. pneumoniae* and *S. aureus* panels. One laboratory did not submit results for the *P. aeruginosa* reference strain. Testing the recommended reference strains is required in terms of quality control and reliability of AST results and performance. For the laboratories reporting data, the deviations in this component were defined as AST results of the reference strain that were outside the quality control acceptance intervals. The deviations originated mostly from disk diffusion, where the Inhibition Zone Diameters determined were either above or below the expected range, which suggests that handling of reference strains needs to be strengthened to ensure the laboratories' good performance.

7. Conclusions

This report presents the results of the EQAsia 6th EQA trial, which was carried out in April – June 2023 and included bacterial identification and antimicrobial susceptibility testing (AST) of four prominent WHO and FAO priority pathogens: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The goal of EQAsia EQAs is to have all participating Human and Food and Animal Health laboratories performing accurate bacterial identification and antimicrobial susceptibility testing of the offered pathogens with a result deviation level below 5%, and to address underperformance by supporting the laboratories with 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* and enterococci).

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 and encouraged. 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.

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. 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 would allow laboratories to generate reliable results.

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

8. References

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

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

[4] The European Committee on Antimicrobial Susceptibility Testing. *Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST*. Version 12.0, 2022. 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: EQA6 Protocol

EQAsia EQA6 trial

Protocol

Identification and antimicrobial susceptibility testing (AST) of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* test strains

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1 INTRODUCTION

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

The **EQAsia EQA6 trial** includes four EQA panels each composed of seven test strains – *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. Each of the four panels includes five strains of the targeted species and two non-target strains. Participating laboratories are asked to perform identification of all seven test strains from the panels they signed up for, as well as antimicrobial susceptibility testing (AST) only on the five target strains in each panel.

Additionally, AST of the relevant reference strains for quality control (QC) is also part of each EQA trial round. The QC reference strains supplied during previous EQA rounds are *Escherichia coli* ATCC 25922/CCM 3954, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC). 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.

2 OBJECTIVES

The main objective of this EQA is to support laboratories to assess and, if necessary, improve the identification and antimicrobial susceptibility testing of pathogens, specifically *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3 EQA6 OUTLINE

3.1 Shipping and receipt of strains

Your laboratory is one of the 32 human health and animal health laboratories from South and Southeast Asia participating in EQA6. In April 2023, 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.

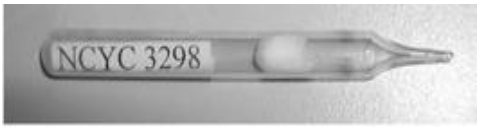
- ***Klebsiella pneumoniae* panel** - seven test strains of which five are *K. pneumoniae* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) reference strains have been provided in previous EQA rounds.

- ***Pseudomonas aeruginosa* panel** - seven test strains of which five are *P. aeruginosa* and two are non-target species. The *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 reference strain 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.!!! The *E. coli* and *S. aureus* panel strains are shipped lyophilized. The *K. pneumoniae* and *P. aeruginosa* strains are shipped on media in transport tubes (swabs).



3.2 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.

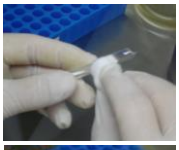
- Needed material:
  - An ampoule cutter or a file
  - Sterile Luria Bertani (LB) broth
  - Agar plates (5 to 6 plates per one strain)
  - Autopipette with tips or Pasteur pipettes
  - Inoculating loop

1. Carefully take the ampoule out of the wrap.

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

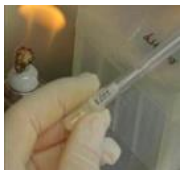
   Note: The ampoule can be cut in the middle or below the cotton wool plug.

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



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

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



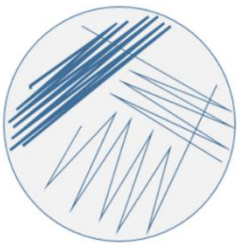
6. Transfer one drop of each strain onto one LB agar plate using autopipette or Pasteur pipette. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.

7. Incubate the inoculated plates and the suspension tubes at 370C overnight and observe the bacterial growth.

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

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

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



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

It is furthermore recommended that the strains are stored in your strain collection (e.g., in a -80°C freezer), at least until you have reviewed your results from this EQA trial. The stored test strains should serve as reference if discrepancies are detected during the testing (e.g., they can be used to detect errors such as mislabelling or contamination), and they can also serve as reference material available at a later stage, when needed.

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

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

3.3 Identification of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* test strains

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

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

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

The strains identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* 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, 32nd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 12.0, 2022) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* can be found in **Tables 1-4**, respectively. **Please make sure to use the correct table for the interpretation**.

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

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

Antimicrobials	Reference values MIC (µg/mL)			Reference values Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
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	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
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	≤ 4	8	≥ 16	≥ 15	13-14	≤ 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, 32st Ed.
*Reference values are based on Enterobacterales clinical breakpoints from www.eucast.org (Tables v. 12.0, 2022)

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	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
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	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
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	≤ 4	8	≥ 16	≥ 15	13-14	≤ 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, 32nd Ed.
*Reference values are based on *K. pneumoniae* epidemiological cut off values from https://mic.eucast.org/ on January 2022.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
Aztreonam, AZT	≤ 8	16	≥ 32	≥ 22	16-21	≤ 15
Cefepime, FEP	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ciprofloxacin, CIP	≤ 0.5	1	≥ 2	≥ 25	19-24	≤ 18
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Imipenem, IMI	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Levofloxacin, LEVO	≤ 1	2	≥ 4	≥ 22	15-21	≤ 14
Meropenem, MERO	≤ 2	4	≥ 8	≥19	16-18	≤ 15
Piperacillin/tazobactam, PT4	≤ 16/4	32/4-64/4	≥ 128/4	≥ 21	15-20	≤ 14
Tobramycin, TOB	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12

Reference values are based on *P. aeruginosa* breakpoints from CLSI M100, 32nd Ed.

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
Fusidate, 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, 32nd Ed.
*Reference values are based on *Staphylococcus aureus* clinical breakpoints from www.eucast.org (Tables v. 12.0, 2022).

4 SUBMISSION OF RESULTS VIA THE INFORMATICS MODULE

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

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

Login to the Informatics Module:

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

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

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

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

Results must be submitted no later than June 2nd, 2023.

If you have troubles entering your results or if you experience technical problems with the informatics module, please contact the DTU team directly, explaining the issues that you encountered:

Tomislav Kostyanev email: tokos@food.dtu.dk

Hiba Al Mir email: hiami@food.dtu.dk

National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK

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

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

5 EVALUATION OF RESULTS

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

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

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

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 23.2	≤ 4	S	> 32	R	32	R	> 32	R	> 64	R	> 64/4	
Ec EQAsia 23.4	≤ 4	S	> 32	R	16	S	> 16	R	> 32	R	4/4	
Ec EQAsia 23.5	≤ 4	S	> 32	R	8	S	16	R	> 64	R	≤ 0.06/4	
Ec EQAsia 23.6	≤ 4	S	4	S	8	S	≤ 0.06	S	≤ 0.25	S	0.12/4	
Ec EQAsia 23.7	≤ 4	S	> 32	R	4	S	≤ 0.06	S	≤ 0.25	S	≤ 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 23.2	> 64	R	> 128	R	> 128/4		≤ 8	S	> 8	R	≤ 0.25	I
Ec EQAsia 23.4	16	I	> 16	R	1/4		> 64	R	> 8	R	> 4	R
Ec EQAsia 23.5	4	S	4	S	0.25/4		≤ 8	S	≤ 0.015	S	≤ 0.25	I
Ec EQAsia 23.6	8	S	≤ 0.25	S	≤ 0.12/4		16	I	≤ 0.0.15	S	≤ 0.25	I
Ec EQAsia 23.7	4	S	≤ 0.25	S	≤ 0.12/4		32	R	8	R	> 4	R

R, Resistant; I, Intermediate; S, Susceptible

	Doripenem (DOR)		Ertapenem (ETP)		Gentamicin (GEN)		Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Nalidixic acid (NAL)	
Ec EQAsia 23.2	> 2	R	> 4	R	≤ 0.5	S	16	R	> 8	R	> 16	R	> 64	R
Ec EQAsia 23.4	1	S	> 4	R	> 16	R	1	S	> 8	R	2	I	> 64	R
Ec EQAsia 23.5	≤ 0.12	S	≤ 0.015	S	≤ 0.5	S	≤ 0.12	S	≤ 1	S	≤ 0.03	S	≤ 4	S
Ec EQAsia 23.6	≤ 0.12	S	≤ 0.015	S	≤ 0.5	S	≤ 0.12	S	≤ 1	S	≤ 0.03	S	≤ 4	S
Ec EQAsia 23.7	≤ 0.12	S	≤ 0.015	S	> 16	R	≤ 0.12	S	8	R	≤ 0.03	S	> 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)	
Ec EQAsia 23.2	> 64	R	> 512	R	> 32	R	≤ 0.25	S	> 8	R	> 16	R	> 4/76	R
Ec EQAsia 23.4	> 64	R	> 512	R	> 32	R	0.5	S	4	S	> 16	R	> 4/76	R
Ec EQAsia 23.5	≤ 8	S	≤ 8	S	≤ 2	S	≤ 0.25	S	≤ 1	S	≤ 0.25	S	≤ 0.5/9.5	S
Ec EQAsia 23.6	≤ 8	S	≤ 8	S	≤ 2	S	0.125	S	≤ 1	S	≤ 0.25	S	≤ 0.5/9.5	S
Ec EQAsia 23.7	≤ 8	S	> 512	R	> 32	R	≤ 0.25	S	8	I	≤ 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 23.1	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	
Kp EQAsia 23.3	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	
Kp EQAsia 23.5	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	2/4	
Kp EQAsia 23.6	≤ 4	S	> 32	R	16	S	2	S	8	R	≤ 0.06/4	
Kp EQAsia 23.7	≤ 4	S	32	R	8	S	≤ 0.06	S	≤ 0.25	S	≤ 0.06/4	

R, Resistant; I, Intermediate; S, Susceptible

	Cefoxitin (FOX)		Ceftazidime (TAZ)		Ceftazidime+clav (T/C)		Chloramphenicol (CHL)		Ciprofloxacin (CIP)		Colistin (COL)	
Kp EQAsia 23.1	> 64	R	32	R	16/4		> 64	R	> 8	R	≤ 0.25	I
Kp EQAsia 23.3	> 64	R	> 128	R	> 128/4		> 64	R	> 8	R	0.5	I
Kp EQAsia 23.5	> 64	R	> 128	R	2/4		> 64	R	> 8	R	≤ 0.25	I
Kp EQAsia 23.6	4	S	0.5	S	≤ 0.12/4		≤ 8	S	0.5	I	≤ 0.25	I
Kp EQAsia 23.7	2	S	≤ 0.25	S	≤ 0.12/4		≤ 8	S	0.03	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)	
Kp EQAsia 23.1	> 2	R	> 4	R	> 16	R	16	R	> 8	R	> 16	R	> 64	R
Kp EQAsia 23.3	> 2	R	> 4	R	> 16	R	> 16	R	> 8	R	> 16	R	> 64	R
Kp EQAsia 23.5	1	S	> 4	R	> 16	R	0.25	S	> 8	R	2	I	> 64	R
Kp EQAsia 23.6	≤ 0.12	S	≤ 0.015	S	16	R	0.25	S	≤ 1	I	≤ 0.03	S	8	S
Kp EQAsia 23.7	≤ 0.12	S	≤ 0.015	S	≤ 0.5	S	0.25	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)	
Kp EQAsia 23.1	> 64	R	> 512	R	8	I	0.5	S	> 8	R	> 16	R	> 4/76	R
Kp EQAsia 23.3	> 64	R	> 512	R	> 32	R	0.5	S	> 8	R	> 16	R	> 4/76	R
Kp EQAsia 23.5	> 64	R	> 512	R	8	I	0.5	S	> 8	R	1	S	2/38	S
Kp EQAsia 23.6	≤ 8	S	> 512	R	> 32	R	0.5	S	2	S	> 16	R	> 4/76	R
Kp EQAsia 23.7	≤ 8	S	≤ 8	S	≤ 2	S	≤ 0.25	S	≤ 1	S	0.5	S	≤ 0.5/9.5	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Amikacin (AMK)		Aztreonam (AZT)		Cefepime (FEP)		Ceftazidime (TAZ)		Ciprofloxacin (CIP)		Colistin (COL)	
Pa EQAsia 23.1	8	S	> 16	R	> 16	R	> 16	R	> 2	R	2	I
Pa EQAsia 23.3	> 32	R	≤4	S	> 16	R	> 16	R	> 2	R	2	I
Pa EQAsia 23.4	≤ 4	S	16	I	4	S	4	S	0.5	S	0.5	I
Pa EQAsia 23.5	≤ 4	S	4	S	8	S	2	S	> 2	R	1	I
Pa EQAsia 23.7	≤ 4	S	≤ 2	S	≤ 2	S	≤ 1	S	≤ 0.25	S	1	I

R, Resistant; I, Intermediate; S, Susceptible

	Doripenem (DOR)		Gentamicin (GEN)		Imipenem (IMI)		Levofloxacin (LEVO)		Meropenem (MERO)		Piperacillin/tazo (PT/4)		Tobramycin (TOB)	
Pa EQAsia 23.1	> 2	R	4	S	> 8	R	> 8	R	> 8	R	> 64	R	≤ 1	S
Pa EQAsia 23.3	> 2	R	> 8	R	> 8	R	> 8	R	> 8	R	> 64	R	> 8	R
Pa EQAsia 23.4	1	S	≤ 1	S	≤ 1	S	2	I	4	I	32	I	≤ 1	S
Pa EQAsia 23.5	4	I	≤ 1	S	> 8	R	> 8	R	4	I	≤ 8	S	≤ 1	S
Pa EQAsia 23.7	≤ 0.12	S	≤ 1	S	≤ 1	S	≤ 1	S	≤ 1	S	≤ 8	S	≤ 1	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)		Fusidate (FUS)	
Sa EQAsia 23.1	8	R	8	S	≤ 0.25	S	≤ 0.12	S	0.5	S	≤ 0.25	S
Sa EQAsia 23.2	4	S	8	S	≤ 0.25	S	≤ 0.12	S	> 8	R	≤ 0.25	S
Sa EQAsia 23.3	2	S	≤ 4	S	≤ 0.25	S	≤ 0.12	S	≤ 0.25	S	≤ 0.25	S
Sa EQAsia 23.4	> 16	R	8	S	> 8	R	> 4	R	> 8	R	≤ 0.25	S
Sa EQAsia 23.6	4	S	8	S	2	I	≤ 0.12	S	> 8	R	> 4	R

R, Resistant; I, Intermediate; S, Susceptible

	Gentamicin (GEN)		Kanamycin (KAN)		Linezolid (LZD)		Penicillin (PEN)		Quinupristin/Dalfo (SYN)	
Sa EQAsia 23.1	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 23.2	≤ 0.5	S	≤ 4	S	2	S	> 1	R	≤ 0.5	S
Sa EQAsia 23.3	≤ 0.5	S	≤ 4	S	2	S	0.5	R	≤ 0.5	S
Sa EQAsia 23.4	> 16	R	> 32	R	2	S	> 1	R	1	S
Sa EQAsia 23.6	≤ 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 23.1	≤ 0.015	S	≤ 64	S	≤ 0.5	S	> 16	R	≤ 1	S
Sa EQAsia 23.2	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQAsia 23.3	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQAsia 23.4	≤ 0.015	S	256	S	> 16	R	> 16	R	2	S
Sa EQAsia 23.6	≤ 0.015	S	≤ 64	S	> 16	R	≤ 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 32nd edition, Tables 4A-1 and 5A-1

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

MIC range in accordance to "The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. 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 32nd 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
Fusidate, 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 32nd edition, Tables 4A-1 and 5A-1

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