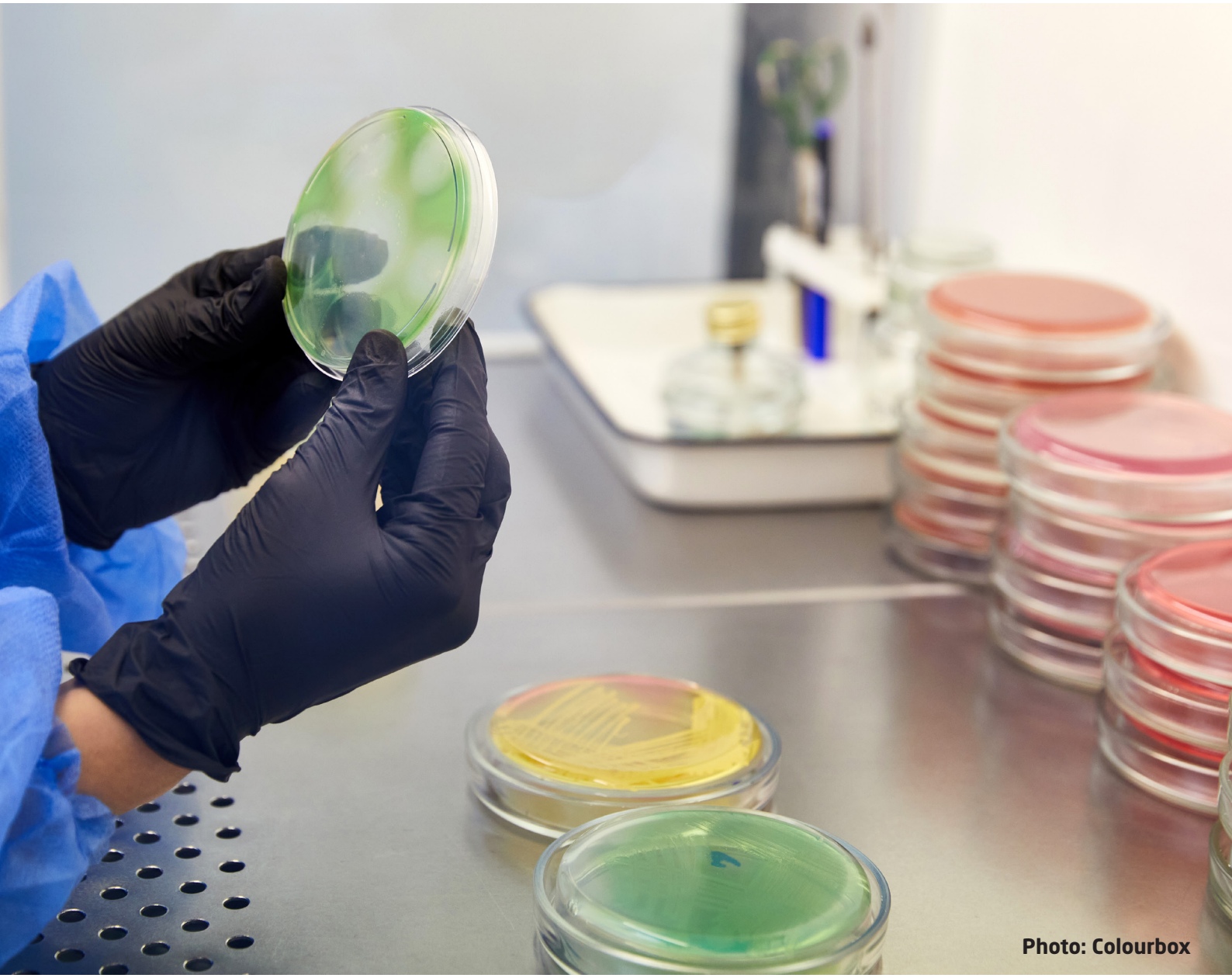
The 4th EQAsia External Quality Assessment trial: Klebsiella pneumoniae, Acinetobacter spp., and Staphylococcus aureus – 2022



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Executive Summary

This report summarizes the results of the 4th EQA trial of EQAsia, a Fleming Fund Regional Grant project 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 has been granted a 2nd phase (2023 to 2026) to continue to deliver the established EQA 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 2022 and included proficiency assessment for bacterial identification and antimicrobial susceptibility testing (AST) of *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Staphylococcus aureus*, as well as detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes (*K. pneumoniae*), the latter for surveillance purposes.

A total of 14 HH and 10 AH laboratories participated and submitted results for the EQA, corresponding to 19 participating laboratories in both the *K. pneumoniae* and *S. aureus* trials, and 16 in the *Acinetobacter* trial. These laboratories are from 12 countries situated in South and Southeast Asia (Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Malaysia, Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka and Timor-Leste).

The bacterial identification component consisted in identifying the five strains of the organism in focus (target organism) among a total of seven strains. For all three trials, one laboratory from each sector (HH and AH) reported all seven isolates as being the target organism. This observation suggests that these laboratories may not have performed bacterial identification and simply reported all seven strains as the target organism.

K. pneumoniae AST results revealed that apart from a few exceptions, the majority of the laboratories are proficient at testing amikacin, ampicillin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, levofloxacin, nalidixic acid, piperacillin/ tazobactam, sulfamethoxazole and tobramycin (median deviation of 0%). On the contrary, the median deviation was ≥ 10% for azithromycin, chloramphenicol, doripenem, imipenem, meropenem, trimethoprim and trimethoprim/ sulfamethoxazole. Tigecycline was tested by less than five laboratories, which presented varying deviations.

In the *Acinetobacter* trial, amikacin, ciprofloxacin, gentamicin and imipenem, as well as cefepime, levofloxacin, meropenem and tobramycin presented a median deviation around 0%, whereas cefotaxime, ceftazidime, colistin, doripenem, minocycline, piperacillin/ tazobactam and trimethoprim/sulfamethoxazole generated higher and more dispersed deviations. Doxycycline and tigecycline were tested by less than five laboratories.

Regarding the *S. aureus* trial, fusidate, kanamycin and linezolid presented no deviations, whereas ciprofloxacin, erythromycin, gentamicin, rifampicin, tetracycline and vancomycin had median deviations close to 0% with a few outliers. The remaining antimicrobials (cefoxitin, chloramphenicol, clindamycin, penicillin, quinupristin/dalfopristin, sulfamethoxazole and trimethoprim) presented higher median deviations and/or more dispersed deviations.

In general, the median deviation was above the acceptance level of 5% deviation from expected results in both the *K. pneumoniae* and *Acinetobacter* trials, whereas the *S. aureus* trial presented a median deviation below 5%.

A total of 16 laboratories submitted results concerning the quality strains relevant for the *K. pneumoniae* and *S. aureus* trials, and 13 laboratories for the *Acinetobacter* trial, meaning that in each trial, three laboratories did not submit results for the reference strains (one HH and four AH laboratories). For both *K. pneumoniae* and *Acinetobacter* trials the median

deviation was around 10%, whereas the median deviation for the *S. aureus* trial was 0%, even though the deviations observed were quite disperse in all of three trials.

Of the 19 participating laboratories in the *K. pneumoniae* trial, 13 (nine HH and four AH laboratories) reported results for detection and confirmation of presumptive ESBL-, AmpC-, or carbapenemase-producing *K. pneumoniae*, of which only three laboratories (two HH and one AH) correctly identified the five *K. pneumoniae* phenotypes, all five expected to be carbapenemase-producers. Correct classification of the carbapenemase phenotypes seems to be problematic among the participants, suggesting a need for further clarification and support.

1. Introduction

The EQAsia project was launched in 2020 aiming to strengthen the provision of External Quality Assessment (EQA) services across the One Health sector among National Reference Laboratories / Centres of Excellence in South and Southeast Asia. EQAsia is supported by the Fleming Fund and strives to increase the quality of laboratory-based surveillance of WHO GLASS pathogens [1] and FAO priority pathogens [2]. EQAsia has been granted a 2nd phase to continue to deliver the established EQA for both the HH sector and AH sector in the region from 2023 to 2026.

The EQAsia Consortium includes the National Food Institute, Technical University of Denmark (DTU Food) as the Lead Grantee, the International Vaccine Institute (IVI) in South Korea, the National Institute of Health (NIH), Department of Medical Sciences in Thailand 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 existing regional providers (NIH Thailand and CUVET Thailand). The program, referred to as a "One-Shop EQA 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 applied.

A total of five EQA trials are taking place during 2021-2022. As mentioned, the EQA trials have focused on the WHO GLASS pathogens [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 is offered twice (one in each year), 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 is to align with the scope of WHO Tricycle and suggested by FAO, to assess the veterinary laboratories' ability to detect multi-resistant bacteria from food matrices.

For a given organism, candidate strains are assessed and validated by DTU Food and the external partner (The Peter Doherty Institute for Infection and Immunity, Australia). The validation includes both phenotypic minimum inhibitory concentration (MIC) determination 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 fourth EQA trial of the EQAsia project (EQA4) carried out in April-June 2022. The trial encompasses the testing of a total of seven test strains of a given organism. Of these, five of the test strains are of the organism in focus (target organism), whereas two test strains are different from the targeted species (reported as non-[organism], e.g., non-*S. aureus*). For each of the seven test strains, participants are requested to report which five strains belong to the expected target organism. For the two organisms different from the expected, no further testing is required. For the remaining five test strains of the target organism, results in relation to AST are requested.

This fourth EQA trial includes identification and AST of *K. pneumoniae*, *Acinetobacter* spp. and *S. aureus*. The aim of this EQA trial is 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 EQA4 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), '1' (incorrect: major) or '3' (incorrect: minor), as explained in the EQA4 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 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 Human Health (HH) or the Food and Animal Health (AH) Sectors are presented separately. The laboratories are identified by codes and each code is known only by the corresponding laboratory and the organizers. The full list of laboratory codes is confidential and known only by the EQAsia Consortium.

This report 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) and Monica Lahra (WHO Collaborating Centre for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia).

2. Materials and Methods

2.1 Participants in EQAsia EQA4

A total of 24 laboratories participated in the fourth EQA survey of the EQAsia project: 14 laboratories belonging to the HH Sector and 10 belonging to the AH Sector, originating from: Bangladesh, Bhutan, Brunei Darussalam, Indonesia, Malaysia, Maldives, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka and Timor-Leste (**Figure 1**).

2.2 Strains

Participating laboratories could register for any of the trials. For each registration, the laboratory received seven bacterial strains of which only five strains were the targeted 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 EQA4, 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). Results could not be verified by the external partner for cefotaxime, cefotaxime / clavulanic acid, ceftazidime / clavulanic acid, ertapenem and sulfamethoxazole (*K. pneumoniae*); fusidate, kanamycin and sulfamethoxazole (*S. aureus*). Expected MIC values (**Appendix 2a-c**) of the selected strains for this EQA were further confirmed by NIH and CUVET.

The reference strains *E. coli* ATCC 25922, *E. coli* NCTC 13846, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were provided to all participants (in this trial or in previous trials) free of charge with instructions for storage and maintenance for quality assurance purposes and 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], and from EUCAST in document "Routine and extended internal quality control for MIC determination and disk diffusion" [4].

Figure 1: Countries participating in the 4th EQA of the EQAsia 2021-22 project on antimicrobial susceptibility testing. Colour indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all three organisms are listed in the EQA4 protocol (**Appendix 1**) and summarized 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.) [3]. 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 (*K. pneumoniae* trial) 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] recommendations for surveillance, also included in the EQA4 protocol (**Appendix 1**).

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

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA4 2022. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

K. pneumoniae	*Acinetobacter*	*S. aureus*
Amikacin (AMK)	Amikacin (AMK)	Cefoxitin (FOX)
Ampicillin (AMP)	Cefepime (FEP)	Chloramphenicol (CHL)
Azithromycin (AZI)	Cefotaxime (FOT)	Ciprofloxacin (CIP)
Cefepime (FEP)	Ceftazidime (TAZ)	Clindamycin (CLI)
Cefotaxime (FOT)	Ciprofloxacin (CIP)	Erythromycin (ERY)
Cefotaxime/clavulanic acid (F/C)	Colistin (COL)	Fusidate (FUS)
Cefoxitin (FOX)	Doripenem (DOR)	Gentamicin (GEN)
Ceftazidime (TAZ)	Doxycycline (DOX)	Kanamycin (KAN)
Ceftazidime/clavulanic acid (T/C)	Gentamicin (GEN)	Linezolid (LZD)
Chloramphenicol (CHL)	Imipenem (IMI)	Penicillin (PEN)
Ciprofloxacin (CIP)	Levofloxacin (LEVO)	Quinupristin/dalfopristin (SYN)
Colistin (COL)	Meropenem (MERO)	Rifampin (RIF)
Doripenem (DOR)	Minocycline (MIN)	Sulfamethoxazole (SMX)
Ertapenem (ETP)	Piperacillin/tazobactam (P/T4)	Tetracycline (TET)
Gentamicin (GEN)	Tigecycline (TGC)	Trimethoprim (TMP)
Imipenem (IMI)	Tobramycin (TOB)	Vancomycin (VAN)
Levofloxacin (LEVO)	Trimethoprim/sulfamethoxazole (SXT)	
Meropenem (MERO)		
Nalidixic Acid (NAL)		
Piperacillin/tazobactam (P/T4)		
Sulfamethoxazole (SMX)		
Tetracycline (TET)		
Tigecycline (TGC)		
Tobramycin (TOB)		
Trimethoprim (TMP)		
Trimethoprim/sulfamethoxazole (SXT)		

2.4 Distribution

The bacterial strains were dispatched as lyophilized strains in April 2022 by NIH and CUVET to the HH and AH laboratories, respectively. The shipment (UN3373, biological substances category B) was sent according to International Air Transport Association (IATA) regulations. Participating laboratories received information on how to open, revive and store these lyophilized cultures.

2.5 Procedure

Protocols and all relevant information were 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 *K. pneumoniae*.

Procedures as disk diffusion, gradient test, agar dilution and broth dilution were all valid. 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 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 a number of 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)' 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). **Table 2** summarizes all the situations where this adaptation in the informatics module was applied.

Table 2. Adjusted scores for reported MIC values for *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 reference strains. Adjustments were made due to the limitation of the broth microdilution (automated) method applied.

***E. coli* ATCC 25922**		
Antimicrobial	**MIC Quality Control Range**	**MIC reported**
Cefepime	0.016-0.12	≤ 1
Ceftazidime	0.06-0.5	≤ 1
Ciprofloxacin	0.004-0.016	≤ 0.25
Doripenem	0.016-0.06	≤ 0.5
Ertapenem	0.004-0.016	≤ 0.5
Levofloxacin	0.008-0.06	≤ 0.12
Meropenem	0.008-0.06	≤ 0.25
Tigecycline	0.03-0.25	≤ 0.5
***S. aureus* ATCC 29213**		
Antimicrobial	**MIC Quality Control Range**	**MIC reported**
Rifampin	0.004-0.016	≤ 0.5

3. Results – Human Health Laboratories

3.1 Overall participation

Of the 14 Human Health laboratories participating in the 4th EQA of the EQAsia Programme, 13 laboratories submitted results for the *K. pneumoniae* trial, and 11 for the *Acinetobacter* and *S. aureus* trials. The methodologies applied by the laboratories varied greatly and are summarized in **Figure 2**. Most of the laboratories opted for only disk diffusion or broth microdilution (automated), or a mixture of the two methodologies, reporting both Inhibition Zone Diameters and MIC depending on the antimicrobial drug tested. The remaining laboratories applied up to five different methodologies, including the abovementioned, as well as gradient test, agar dilution and broth microdilution (conventional).

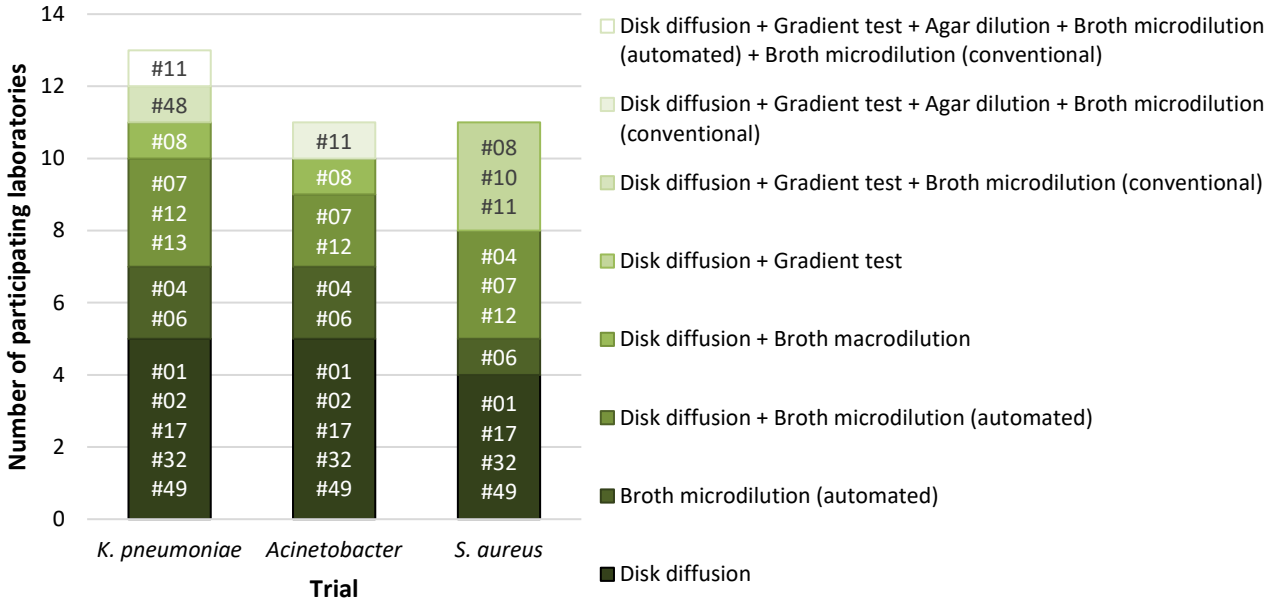

Figure 2. Methodologies applied by the HH laboratories participating in each of the trials.

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

The EQA set-up allowed laboratories to choose not only the bacterial pathogens, but also the antimicrobials among the panel of suggested drugs (**Table 1**). For the Gram-negative bacteria *K. pneumoniae* and *Acinetobacter*, amikacin, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin/tazobactam and trimethoprim/sulfamethoxazole were tested by at least 90% of the participating laboratories (**Table 3**). In contrast, the last resort antibiotics such as colistin and tigecycline, as well as doripenem and tobramycin were tested by less than half of the laboratories (**Table 3**). For the *K. pneumoniae* trial in particular, antibiotics such as sulfamethoxazole and trimethoprim were tested by only one HH laboratory, as the combination drug trimethoprim/sulfamethoxazole was tested instead. For the Gram-positive bacteria, the most tested antimicrobials were cefoxitin, erythromycin and penicillin (tested by all participating laboratories), as well as ciprofloxacin and tetracycline (tested by 10 out of the 11 laboratories). In contrast, fusidate, kanamycin, quinupristin/dalfopristin and sulfamethoxazole were tested by less than 30%

of the laboratories submitting results for the *S. aureus* trial (**Table 3**).

Scattering of missing data or incomplete AST results entries were observed in all three trials (**Tables 4-6**). Nine of the 13 laboratories selecting *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* trial were seen for laboratories #04, #13 and #17 (**Table 4**) In the case of laboratory #13, for example, it was noticed that results were reported, but no interpretation was submitted. As only the categorisation as R, I or S is evaluated, the results for these antimicrobials could not be scored.

Similarly, more than half of the laboratories selecting *Acinetobacter* (n=6) submitted incomplete results of the selected antimicrobials (**Table 5**). The highest number of incomplete results in the *Acinetobacter* trial was seen for laboratories #01 and #07 (**Table 5**). As for the *K. pneumoniae* trial, one of the laboratories (#01) did not provide interpretation of the obtained results for the antimicrobials presented in **Table 5**, which prevented the assessment of their performance for those drugs.

Regarding the *S. aureus* trial, two out of 11 participating laboratories revealed incomplete results of their own available antimicrobial agents (**Table 6**). It is crucial that participants are careful when entering results in the informatics system, as these mistakes/missing data will lead to a wrong assessment of their performance.

Table 3. Antimicrobial agents tested by the HH laboratories for each trial. For a given trial (Kp, Aci,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 trial are represented as --.

Antimicrobial	Laboratories in total: n (% of n/N)		
	Kp	Aci	Sa
AMK	13 (100.0)	10 (90.9)	--
AMP	11 (84.6)	--	--
AZI	7 (53.8)	--	--
FEP	8 (61.5)	8 (72.7)	--
FOT	8 (61.5)	6 (54.5)	--
FOX	9 (69.2)	--	11 (100.0)
TAZ	12 (92.3)	10 (90.9)	--
CHL	8 (61.5)	--	9 (81.8)
CIP	13 (100.0)	11 (100.0)	10 (90.9)
CLI	--	--	9 (81.8)
COL	6 (46.2)	4 (36.4)	--
DOR	4 (30.8)	4 (36.4)	--
DOX	--	2 (18.2)	--
ETP	10 (76.9)	--	--
ERY	--	--	11 (100.0)
FUS	--	--	2 (18.2)
GEN	13 (100.0)	11 (100.0)	9 (81.8)
IMI	11 (84.6)	9 (81.8)	--
KAN	--	--	3 (27.3)
LEVO	9 (69.2)	8 (72.7)	--
LZD	--	--	9 (81.8)
MERO	12 (92.3)	10 (90.9)	--
MIN	--	3 (27.3)	--
NAL	4 (30.8)	--	--
PEN	--	--	11 (100.0)
P/T4	13 (100.0)	10 (90.9)	--
SYN	--	--	3 (27.3)
RIF	--	--	5 (45.5)
SMX	1 (7.7)	--	3 (27.3)
TET	7 (53.8)	--	10 (90.9)
TGC	2 (15.4)	1 (9.1)	--
TOB	6 (46.2)	5 (45.5)	--
TMP	1 (7.7)	--	5 (45.5)
SXT	13 (100.0)	11 (100.0)	--
VAN	--	--	7 (63.6)
Total (N)	**13**	**11**	**11**

Kp, *K. pneumoniae*; Aci, *Acinetobacter*; Sa, *S. aureus*
(n) number of laboratories that reported results for the antimicrobial; (N) total number of participating laboratories for the trial

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by HH laboratories (n=13) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Kp EQAsia 22.1	Kp EQAsia 22.2	Kp EQAsia 22.4	Kp EQAsia 22.5	Kp EQAsia 22.7
#02	--	FOT	--	--	--
#04	CHL; TOB	CHL		IMI	CHL; IMI; TOB
#06	--	ETP	--	--	--
#08	--	--	--	--	FOX
#13	--	AZI; TAZ; PT4	AZI	--	PT4
#17	COL; TMP	COL; ETP	COL; DOR	COL	ETP
#32		--	--	PT4	--
#48	--	--	--	--	PT4
#49	TGC	TGC	--	TGC	TGC

Kp, *K. pneumoniae*; blue shade, strain not tested

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter* strains reported by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Aci EQAsia 22.1	Aci EQAsia 22.3	Aci EQAsia 22.4	Aci EQAsia 22.5	Aci EQAsia 22.6
#01	--	AMK; FEP; FOT; TAZ; CIP	--	--	--
#06	--	COL	COL	COL	COL
#07	--	--	FEP; TAZ; CIP; GEN; MERO; PT4; SXT	--	--
#08	--	AMK	--	--	--
#17	DOR	--	--	--	--
#49	FEP	--	FEP	FEP	

Aci, *Acinetobacter*; blue shade, strain not tested

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Sa EQAsia 22.1	Sa EQAsia 22.2	Sa EQAsia 22.3	Sa EQAsia 22.6	Sa EQAsia 22.7
#17	--	--	--	ERY	--
#32	CHL	--	--	--	PEN

Sa, *S. aureus*

3.2 *Klebsiella pneumoniae* trial

Thirteen laboratories from 11 countries uploaded results for the *K. pneumoniae* trial.

3.2.1 Bacterial identification

Of the 13 participating laboratories, 11 correctly identified the tested *K. pneumoniae* and non-*K. pneumoniae* strains (**Table 7**).

Table 7. Bacterial identification of each of the seven test strains provided related to the *K. pneumoniae* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Kp EQASIA 22.1	*K. pneumoniae*	12/12
Kp EQASIA 22.2	*K. pneumoniae*	13/13
Kp EQASIA 22.3	Non-*K. pneumoniae* (*Escherichia coli*)	12/13
Kp EQASIA 22.4	*K. pneumoniae*	12/13
Kp EQASIA 22.5	*K. pneumoniae*	13/13
Kp EQASIA 22.6	Non-*K. pneumoniae* (*Shigella flexneri*)	11/12
Kp EQASIA 22.7	*K. pneumoniae*	13/13

Kp, *K. pneumoniae*

Laboratories #32 and #49 did not test strains Kp EQASIA 22.1 and Kp EQASIA 22.6, respectively. The non-*K. pneumoniae* strains Kp EQASIA 22.3 (*Escherichia coli*) and Kp EQASIA 22.6 (*Shigella flexneri*) were reported by laboratory #32 as *K. pneumoniae*, suggesting that the laboratory did not perform bacterial identification and simply reported all seven strains as *K. pneumoniae*. Laboratory #04 additionally misidentified strain Kp EQASIA 22.4 as non-*K. pneumoniae* (**Table 7**).

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 of the trial.

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 91.1% (strain Kp EQASIA 22.2) to 96.7% (strain Kp EQASIA 22.1) for each strain (**Table 8**). All five strains revealed deviations below 10%, yet four of them presented more than 5% deviation. For instance, Kp EQASIA 22.2, the strain with the highest deviation, was reported by laboratory #32 as resistant to all tested antimicrobials, though a quite susceptible profile would be expected (**Appendix 2a**); on the contrary, the same laboratory reported strain Kp EQASIA 22.7 as susceptible to several antimicrobials, where the outcome as resistant would be expected. An identical situation was observed for strain Kp EQASIA 22.5, which was reported as susceptible or intermediate to some antimicrobials by laboratory #12, where it was

expected to be resistant (**Appendix 2a**).

Table 8. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S) from 13 HH laboratories for the *K. pneumoniae* trial.

Strain	AST in total	% Correct
Kp EQASIA 22.1	188	96.7
Kp EQASIA 22.2	192	91.1
Kp EQASIA 22.4	184	92.7
Kp EQASIA 22.5	197	94.4
Kp EQASIA 22.7	193	92.4

Kp, *K. pneumoniae*

Antimicrobial-based analysis

Of the 24 tested and scored antimicrobial agents, seven revealed to exceed a 10% deviation: tigecycline (41.7%), azithromycin (18.9%), doripenem (17.1%), chloramphenicol (13.9%), imipenem (13.9%), meropenem (12.5%) and trimethoprim/sulfamethoxazole (11.9%). On the contrary, ampicillin, piperacillin/tazobactam, sulfamethoxazole and trimethoprim revealed no deviation from the expected results (**Figure 3**).

Tigecycline was tested by only two laboratories, even though it seems that laboratory #49, which submitted results for only one strain, did it by mistake. It means that even few incorrect results would result in a high percentage of deviation. In this case, the results reported by laboratory #12 were incorrect for two of the five strains.

Regarding azithromycin deviation, some of the incorrect reported results were close to the threshold for categorising the strain as susceptible or resistant (intermediate option is not available), which resulted in a heavy score penalty (score of 0 or 1 out of the possible full score 4).

Doripenem's deviation can in part be explained by laboratory #01 results, which reported all five strains as resistant to the drug (inhibition zone diameters of 6 mm), even though only two were in fact resistant.

The remaining deviations do not appear to have occurred for a particular strain or caused by a specific laboratory.

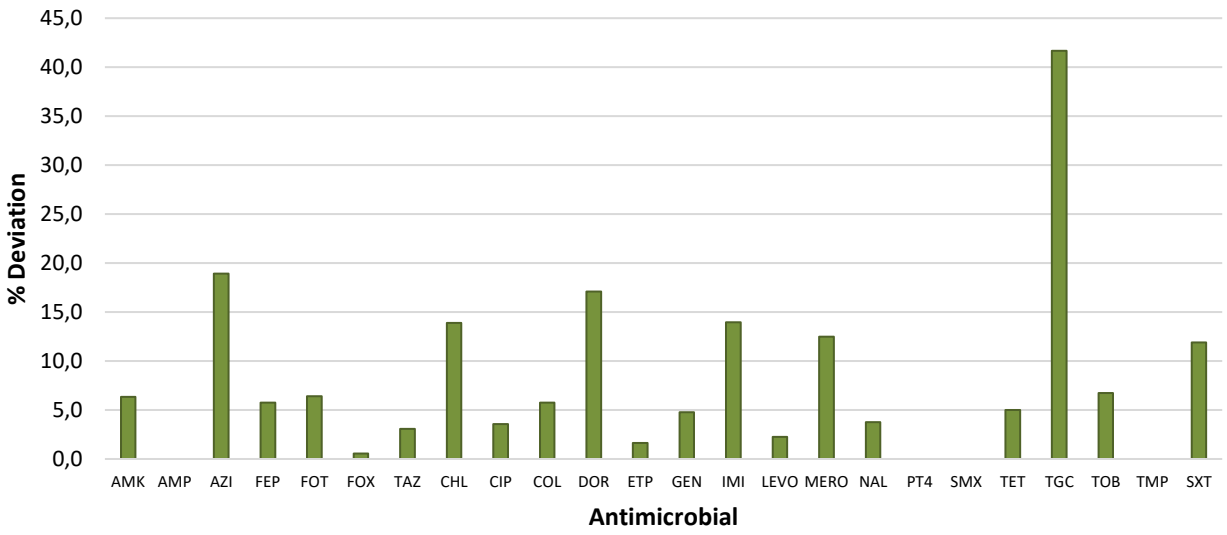

Figure 3. Percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by HH laboratories (n=13) participating in the 4th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

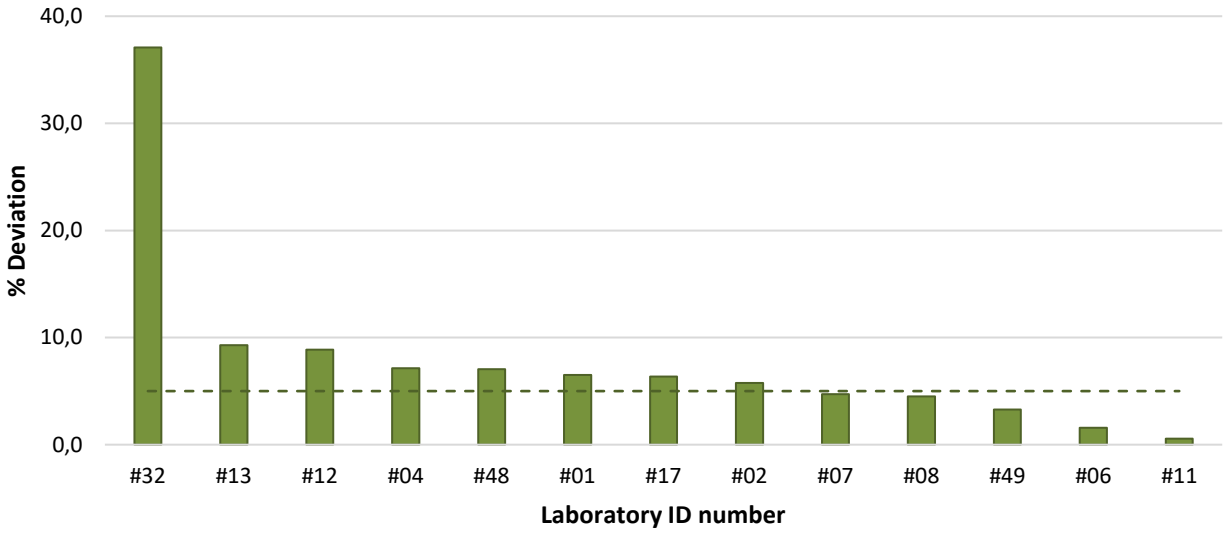

Figure 4. Percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by HH laboratories (n=13) participating in the 4th EQA in the EQAsia project. Results are categorized by laboratory ID number.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for laboratories # 07, #08, #49, #06 and #11 (**Figure 4**). In average, the deviation was 7.9% (ranging from 0.6 to 37.1%). As the acceptance level was set to 5% deviation, eight laboratories (#32, #13, #12, #04, #48, #01, #17 and #02) did not perform within the expected range for the *K. pneumoniae* trial.

The highest deviation was observed for laboratory #32 and can be explained by the incorrect results reported for strains Kp EQASIA 22.2 and Kp EQASIA 22.7, as mentioned before; a deeper analysis suggests that it may be possible that the laboratory switched around these two strains. Laboratory #12 deviation seems to have also originated from the abovementioned incorrect results reported for strain Kp EQASIA 22.5, along with other occasional misinterpretation of cefepime results.

Laboratory #13 deviation was caused by misinterpretation of obtained results, in particular colistin results, which were interpreted as susceptible, when intermediate or resistant are the only possible options, as recommended in the CLSI guidelines and as stated in the EQA4 protocol (**Appendix 1**).

The remaining laboratories with deviations slightly above 5% presented random incorrect and/or misinterpreted results, not particularly associated with a specific strain or antimicrobial.

3.2.3 β-lactamase producing *K. pneumoniae*

Nine out of the 13 participating laboratories uploaded results for this component of the *K. pneumoniae* trial. Yet, for strains Kp EQASIA 22.2 and Kp EQASIA 22.4 only seven and eight laboratories, respectively, tested for ESBL-production (**Table 9**). Of all nine laboratories, only two laboratories (#08 and #11) correctly identified all the carbapenemase phenotypes among the five *K. pneumoniae* strains. Strain Kp EQASIA 22.5 was correctly identified by all laboratories (**Table 9**), followed by strain Kp EQASIA 22.1, which was wrongly classified by only one laboratory (#12); this laboratory classified this strain and Kp EQAsia 22.7 as ESBL+AmpC-producing *K. pneumoniae* strains instead of carbapenemase-producers, even though the meropenem MIC reported was > 0.12 µg/mL. Strain Kp EQAsia 22.7 was also reported as ESBL+AmpC-producer by two other laboratories (#07, #17) and AmpC-producer by

another (#01), even though the laboratories obtained values for meropenem > 0.12 µg/mL or < 25 mm. A similar situation was identified for strain Kp EQAsia 22.4 and laboratories #07 and #48. Lastly, strain Kp EQAsia 22.2 was identified as susceptible by laboratories #06 and #12.

Table 9. 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 9 HH laboratories.

Strain code		Kp EQASIA 22.1	Kp EQASIA 22.2	Kp EQASIA 22.4	Kp EQASIA 22.5	Kp EQASIA 22.7
Expected results		Carbapenemase	Carbapenemase	Carbapenemase	Carbapenemase	Carbapenemase
Obtained results (n/N)	ESBL	--	--	--	--	--
	AmpC	--	--	--	--	1/9 (11.1%)
	ESBL + AmpC	1/9 (11.1%)	--	2/8 (25.0%)	--	3/9 (33.3%)
	Carbapenemase	**8/9 (88.9%)**	**5/7 (71.4%)**	**6/8 (75.0%)**	**9/9 (100.0%)**	**5/9 (55.6%)**
	Other	--	--	--	--	--
	Susceptible*	--	2/7 (28.6%)	--	--	--

Kp, *K. pneumoniae*
*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 (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *K. pneumoniae* trial.

Among the 13 participating laboratories, 12 submitted results for the reference strain *E. coli* ATCC 25922 and only five 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 dilution, broth macro or microdilution (automated and conventional) (**Table 10**). For testing *E. coli* NCTC 13846, MIC was determined by either broth macro or microdilution methods.

The highest proportion of test results outside of the expected range was observed for trimethoprim (1 out of 2), doripenem, ertapenem, piperacillin/tazobactam and tobramycin (1 out of 3 or 3 out of 9) (**Table 10**).

Regarding the laboratories' performance (**Figure 5**), laboratories #11 and #17 presented no deviation. While laboratory #17 applied disk diffusion, laboratory #11 used a mixture of disk diffusion, gradient test, broth microdilution and agar dilution. The remaining 10 laboratories presented deviations that ranged from 6.3% to 75.0% (**Figure 5**). Laboratories #06 and #08 presented one deviation each, laboratory #07 two deviations and laboratory #04 four deviations, when applying broth microdilution. Some of the remaining deviations were seen when disk diffusion was the methodology applied; laboratories #49 and #02 presented two and four deviations, respectively, where the Inhibition Zone Diameters reported were slightly below the acceptance interval; laboratory #01 presented a total of seven deviations, both above and below the expected range.

Laboratory #48 tested some antimicrobial agents by gradient test (ertapenem, imipenem and meropenem); however, the values reported regarding the reference strain appear to be Inhibition Zone Diameters and not MIC values, which led to evaluating such results as incorrect. Similar situations were observed for laboratories #12 and #13, which tested the test strains and the reference strain by different methodologies; when a methodology is selected for the test strains for certain antimicrobials, the same methodology is expected to be used for the reference strains. Using different methodologies will result in score penalties.

Table 10. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *K. pneumoniae* trial. Proportion of test results outside of expected range is presented by methodology used.

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

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

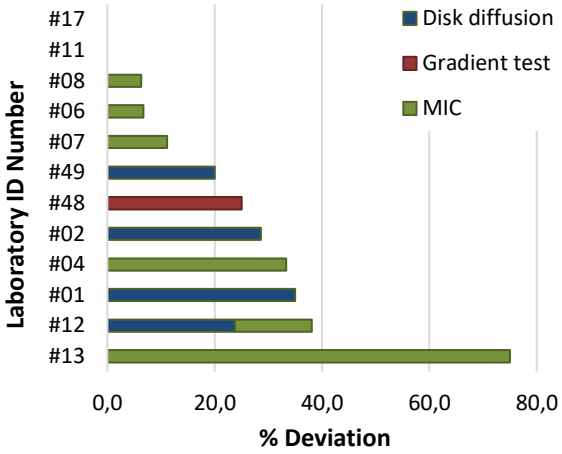

Figure 5. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *K. pneumoniae* trial by the HH laboratories.

3.3 *Acinetobacter* trial

A total of 11 laboratories from nine countries uploaded results for the *Acinetobacter* trial.

3.3.1 Bacterial identification

Nine out of 11 participating laboratories correctly identified all seven test strains provided (**Table 11**). The *A. pittii* strain Aci EQASIA 22.6 was misidentified as non-*Acinetobacter* by laboratory #49, whereas the non-*Acinetobacter* strain Aci EQASIA 22.7 was reported as *Acinetobacter*. Laboratory #32 reported the two non-*Acinetobacter* strains as *Acinetobacter*, suggesting that no bacterial identification was performed by the laboratory.

Table 17. Bacterial identification of each of the seven test strains provided related to the *Acinetobacter* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Aci EQASIA 22.1	*A. baumannii*	11/11
Aci EQASIA 22.2	Non-*Acinetobacter* (*P. aeruginosa*)	10/11
Aci EQASIA 22.3	*A. baumannii*	11/11
Aci EQASIA 22.4	*A. lowffii*	11/11
Aci EQASIA 22.5	*A. baumannii*	11/11
Aci EQASIA 22.6	*A. pittii*	10/11
Aci EQASIA 22.7	Non-*Acinetobacter* (*P. aeruginosa*)	9/11

Aci, *Acinetobacter*

3.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 90.7% (strain Aci EQASIA 22.6) to 97.6% (strain Aci EQASIA 22.3) for each strain (**Table 12**). The deviation for strain Aci EQASIA 22.6 (almost 10%) seems to have been caused by some instances of results' misinterpretation, as well as being reported resistant to some antimicrobials by laboratories #04 and #06, opposite to what would be expected.

Antimicrobial-based analysis

All 17 antimicrobials presented deviations from the expected results, where tigecycline (15.0%), doripenem (11.8%) and piperacillin/tazobactam (11.5%) presented the highest deviation (**Figure 6**). Tigecycline was only tested by laboratory #12, and a single incorrect result caused the observed deviation; doripenem's deviation was mostly caused by the results submitted by laboratory #01, which reported all five *Acinetobacter* strains as resistant to doripenem (Inhibition Zone Diameters of 6mm), even though only three were in fact resistant; regarding piperacillin/tazobactam, the majority of the incorrect results were minor errors, meaning that the deviation observed was within the methodology variation, which resulted in different interpretations and slight score penalties.

Table 12. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 11 HH laboratories for the *Acinetobacter* trial.

Strain	AST in total	% Correct
Aci EQASIA 22.1	121	95.5
Aci EQASIA 22.3	116	97.6
Aci EQASIA 22.4	114	93.4
Aci EQASIA 22.5	121	93.6
Aci EQASIA 22.6	113	90.7

Aci, *Acinetobacter*

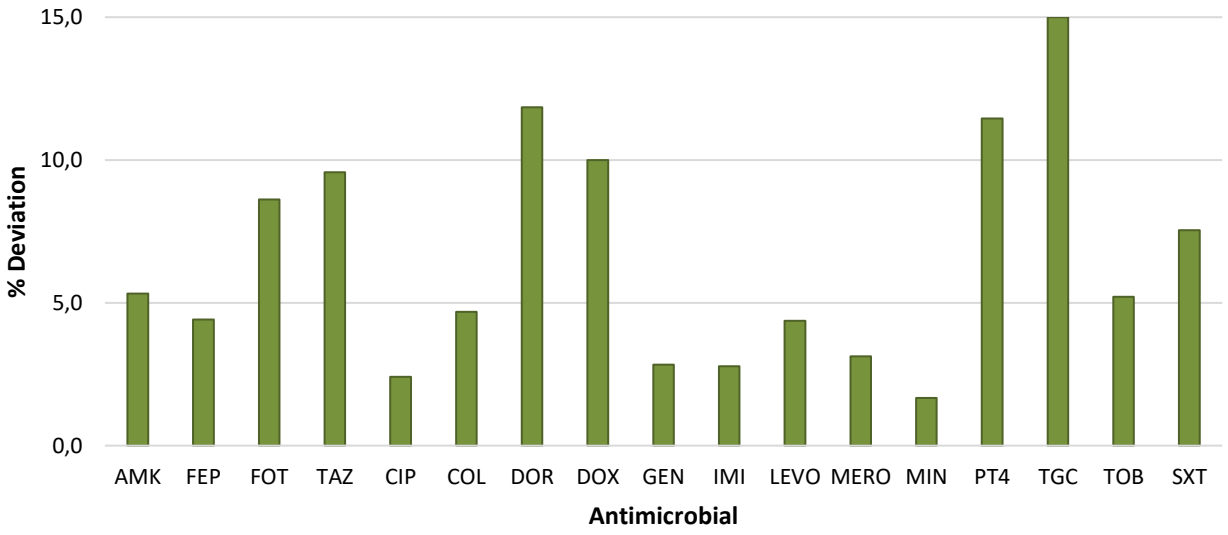

Figure 6. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* strains by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

For the *Acinetobacter* trial, eight out of the 11 HH laboratories presented a deviation above the acceptance level of 5% (laboratories #32, #12, #06, #04, #01, #02, #17 and #49). The average deviation was 5.9% (ranging from 0.8 to 11.7%) (**Figure 7**). Laboratories #01, #02, #17 and #49 presented deviations only slightly above the acceptance level; on the opposite, laboratories #32 and #12 had deviations above 10% (**Figure 7**). Laboratory #32 deviations were due to misinterpretation of results for several antimicrobials, such as amikacin, ciprofloxacin, meropenem and trimethoprim/ sulfamethoxazole); laboratory #12 reported cefotaxime incorrect results for three of the five strains, and additionally reported several incorrect results for strain Aci EQAsia 22.4, which was found to be resistant to some antimicrobials, where it was expected to be susceptible; as mentioned in the sub-section '*Strain-based analysis*', laboratories #04 and #06 reported a few incorrect results for strain Aci EQASIA 22.6, which is the main contributor for the deviation observed for these two laboratories.

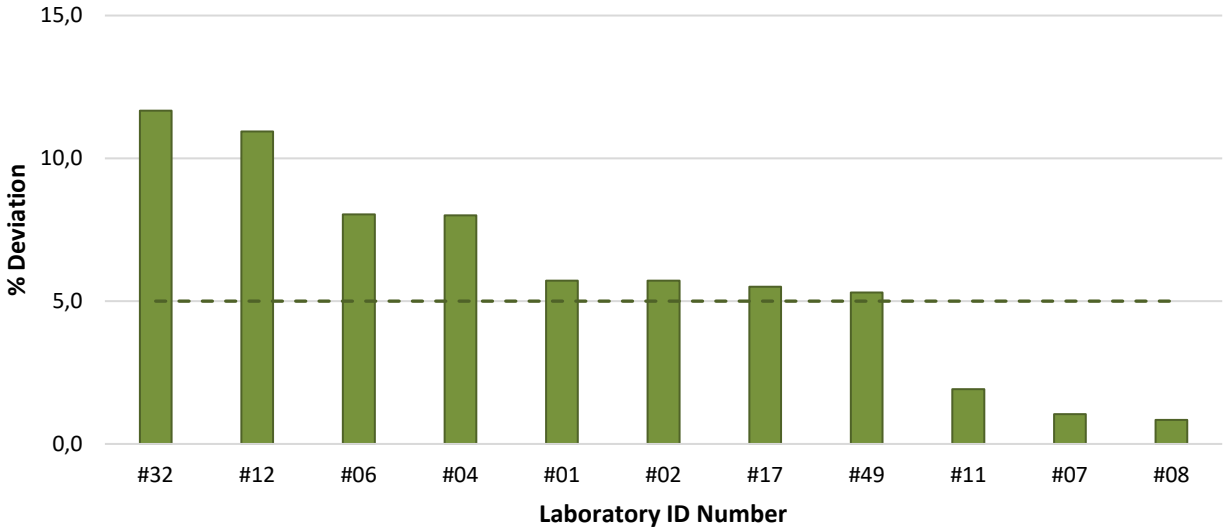

Figure 7. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* strains by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.3.3 Quality control strain *P. aeruginosa* ATCC 27853

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

Among the 11 participating laboratories, 10 submitted results for the reference strain *P. aeruginosa* ATCC 27853. The laboratories used different methodologies for testing the reference strain *P. aeruginosa* ATCC 27853: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, agar dilution, broth macro or microdilution (**Table 13**). The highest proportion of test results outside of the expected range was observed for trimethoprim/sulfamethoxazole (2 out of 3), cefotaxime (2 out of 5), amikacin (3 out of 10) and doripenem (1 out of 4) (**Table 13**).

Considering the deviations, the laboratories' performance seems to be independent of the methodology applied for AST of the quality control strain (**Figure 8**). Laboratories #02, #06, #11 and #17 presented no deviation. While laboratories #02 and #17 applied disk diffusion and laboratory #06 applied broth microdilution (automated) as the sole method, laboratory #11

applied a mixture of methodologies (disk diffusion, gradient test, agar dilution and conventional broth microdilution).

Table 13. AST of the reference strain *P. aeruginosa* ATCC 27853 in the *Acinetobacter* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range			
	Disk Diff.	Gradient	MIC	Total
AMK	3/7	0/1	0/2	**3/10**
FEP	0/3	0/1	0/4	**0/8**
FOT	1/3	--	1/2	**2/5**
TAZ	2/5	0/1	0/4	**2/10**
CIP	0/5	0/1	0/4	**0/10**
COL	--	--	0/4	**0/4**
DOR	1/2	--	0/2	**1/4**
GEN	1/6	--	0/4	**1/10**
IMI	1/4	0/1	0/3	**1/8**
LEVO	0/3	0/1	0/3	**0/7**
MERO	1/4	0/1	0/4	**1/9**
P/T4	0/5	0/1	0/4	**0/10**
TGC	--	--	--	**--**
TOB	0/3	0/1	--	**0/4**
SXT	--	0/1	2/2	**2/3**

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

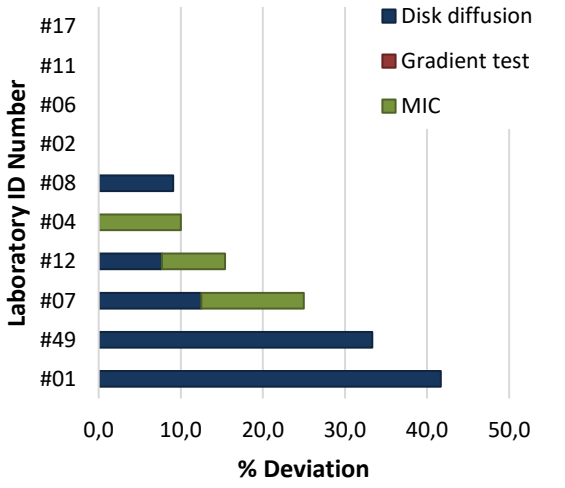

Figure 8. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *Acinetobacter* trial by the HH laboratories.

Six laboratories (#08, #04, #12, #07, #49 and #01) presented deviations that ranged from 9.1% to 41.7% (**Figure 8**). Laboratories #04 and #08 presented one deviation each, whereas laboratories #12 and #07 had two deviations each, one that occurred when disk diffusion was applied and the other when broth microdilution was used. Laboratory #49 had only two deviations as well, but as the laboratory tested a total of six antimicrobials, the overall deviation was high. Laboratory #01 presented five deviations, three of them above the acceptance interval (amikacin, cefotaxime and gentamicin) and two below (doripenem and imipenem).

3.4 *Staphylococcus aureus* trial

Eleven laboratories from nine countries uploaded results for the *S. aureus* trial.

3.4.1 Bacterial identification

Of the 11 participating laboratories, 10 correctly identified the tested *S. aureus* and non-*S. aureus* strains (**Table 14**). Laboratory #01 did not test strains Sa EQASIA 22.3 and Sa EQASIA 22.4; the two non-*S. aureus* strains (strains Sa EQASIA 22.4 and Sa EQASIA 22.5) were reported as *S. aureus* by laboratory #32, suggesting that no bacterial identification was in fact performed.

Table 14. Bacterial identification of each of the seven test strains provided related to the *S. aureus* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Sa EQASIA 22.1	*S. aureus*	11/11
Sa EQASIA 22.2	*S. aureus*	11/11
Sa EQASIA 22.3	*S. aureus*	10/10
Sa EQASIA 22.4	Non-*S. aureus* (*S. epidermis*)	9/10
Sa EQASIA 22.5	Non-*S. aureus* (*S. chromogenes*)	10/11
Sa EQASIA 22.6	*S. aureus*	11/11
Sa EQASIA 22.7	*S. aureus*	11/11

Sa, *S. aureus*

3.4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 92.5% (strain Sa EQASIA 22.2) to 97.8% (strain Sa EQASIA 22.6) for each strain (**Table 15**).

Table 15. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 11 HH laboratories for the *S. aureus* trial.

Strain	AST in total	% Correct
Sa EQASIA 22.1	116	96.3
Sa EQASIA 22.2	117	92.5
Sa EQASIA 22.3	104	96.4
Sa EQASIA 22.6	116	97.8
Sa EQASIA 22.7	116	96.3

Sa, *S. aureus*

Even though none of the strains revealed more than 10% deviation, the highest deviation seen for strain Sa EQASIA 22.2 can be explained by the testing results for penicillin, where some laboratories found the strain to be resistant to the drug when it was expected to be susceptible, as well as by few incorrect results reported by laboratory #32.

Antimicrobial-based analysis

Antimicrobials with highest deviation from the expected result were clindamycin (16.5%) and sulfamethoxazole (10.0%), whereas fusidate, kanamycin and linezolid revealed no deviation from the expected results (**Figure 9**).

The deviation observed for clindamycin was mostly due to laboratory #12, which reported all five strains as resistant to the drug, when only two were in fact resistant. Sulfamethoxazole was tested by only three laboratories, meaning that even few incorrect results would result in a high percentage of deviation. In this case, two laboratories reported strain Sa EQASIA 22.2 as resistant to the drug, even though it was expected to be susceptible, resulting in a high score penalty (score of 1).

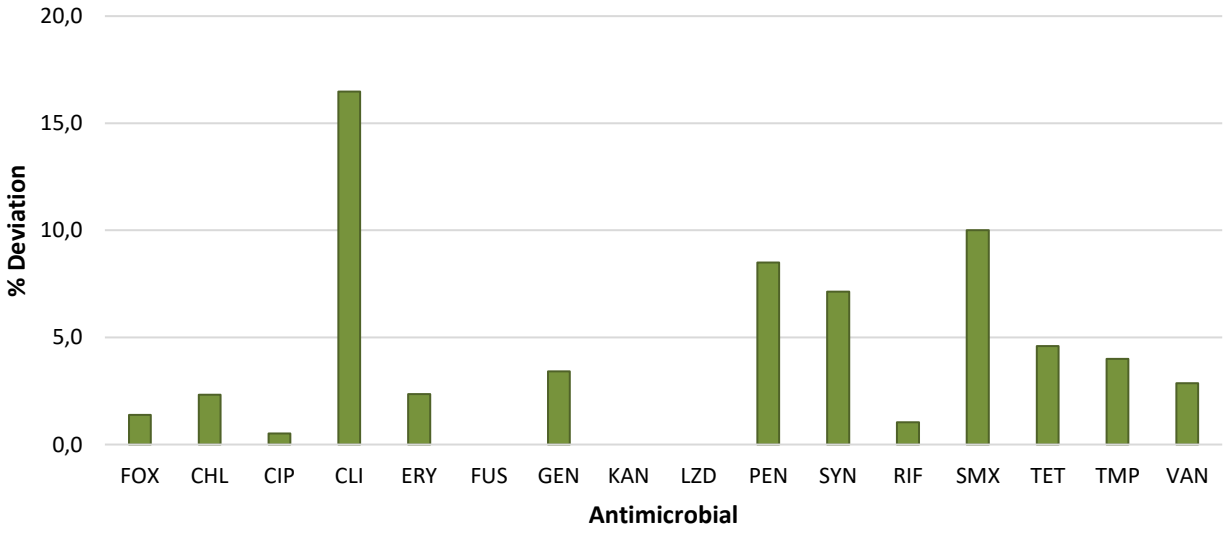

Figure 9. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

For the *S. aureus* trial, four out of the 11 HH laboratories presented a deviation above the acceptance level of 5% (#32, #12, #04 and #01). The average deviation was 4.9% (ranging from 0.0 to 17.9%) (**Figure 10**). For laboratories #01, #04 and #12, the deviations were only a bit above the acceptance level (**Figure 10**). Laboratory #32, however, presented a quite high deviation, which was largely caused by the already mentioned incorrect results for strain Sa EQASIA 22.2, in addition to misinterpretation of the results obtained for tetracycline.

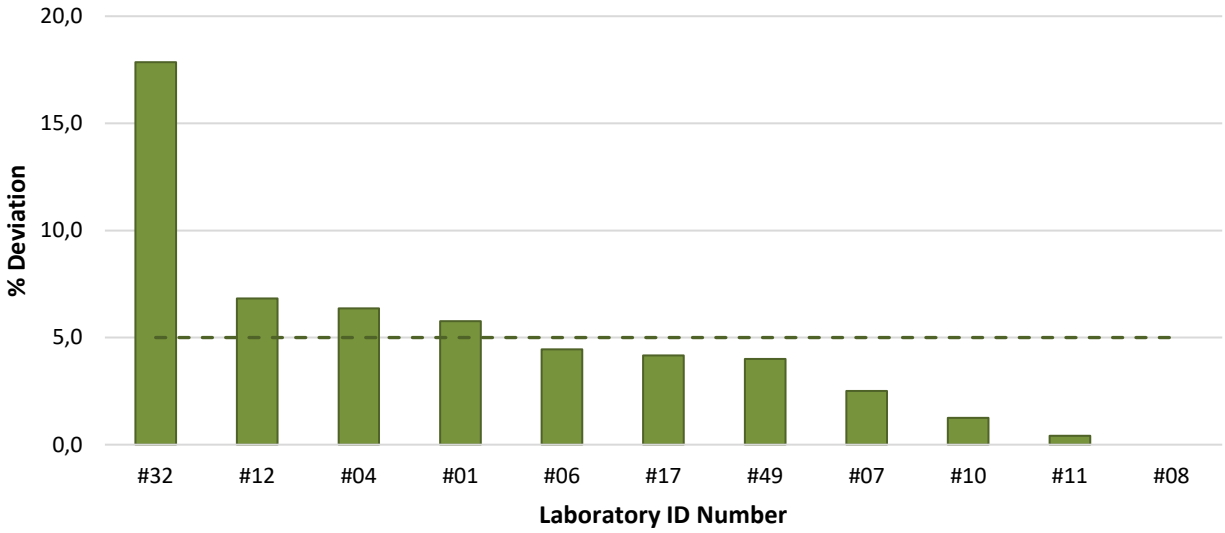

Figure 10. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by HH laboratories (n=11) participating in the 4th EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.4.3 Quality control strains *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213

The quality control strains *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 for testing when disk diffusion or MIC determination methodologies are applied, respectively, were sent free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *S. aureus* trial.

Among the 11 participating laboratories in the trial, 10 laboratories submitted results for the reference strains. Different methodologies for testing the reference strain *S. aureus* ATCC 29213 were applied: MIC was determined by either gradient test or broth microdilution (**Table 16**, **). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine susceptibility by disk diffusion (**Table 16**, *). Laboratory #04, however, reported results for strain *S. aureus* ATCC 25923, even though only one antimicrobial (tetracycline) was tested by disk diffusion. For that reason, the MIC values reported for the other antimicrobials could not be assessed, since strain *S. aureus* ATCC 29213 should have been used instead.

The highest proportion of test results outside of the expected range were observed for quinupristin/dalfopristin (1 out of 3) and penicillin (2 out of 9) (**Table 16**). All deviations occurred when disk diffusion was applied.

Table 16. AST of the reference strain *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial. Proportion of test results outside of expected range is presented by methodology used.

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

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

A closer look at the laboratories' performance (**Figure 11**) shows that laboratories #04, #06 #08, #10, #11, #12 and #17 had no deviations. Of those, laboratories #06 and #12 opted for broth microdilution as the sole methodology, laboratories #04 and #17 opted for disk diffusion, whereas laboratories #08, #10 and #11 applied both disk diffusion and gradient test.

In reverse, the other three laboratories had deviations ranging from 7.1 to 28.6% (**Figure 11)**. Laboratories #07, #49 and #01 presented one, two and three deviations, respectively. Laboratory #07 reported an Inhibition Zone Diameter for cefoxitin 2mm above the acceptance interval; laboratories #01 and #49 deviations were all below the expected range.

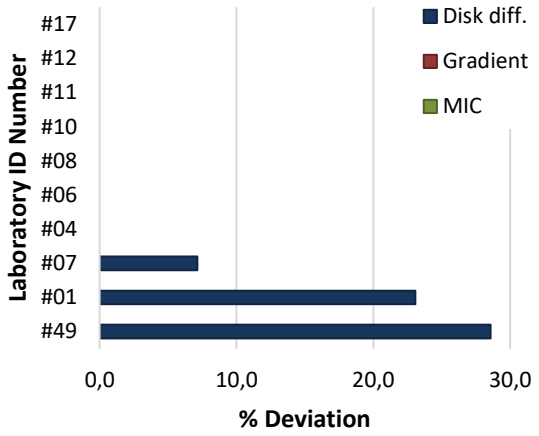

Figure 11. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the 10 Animal Health laboratories participating in the 4th EQA of the EQAsia Programme, six laboratories submitted results for the *K. pneumoniae* trial, five for the *Acinetobacter* trial and eight laboratories submitted results for the *S. aureus* trial (**Figure 12**). Applied AST methodologies for the three trials are presented in **Figure 12**.

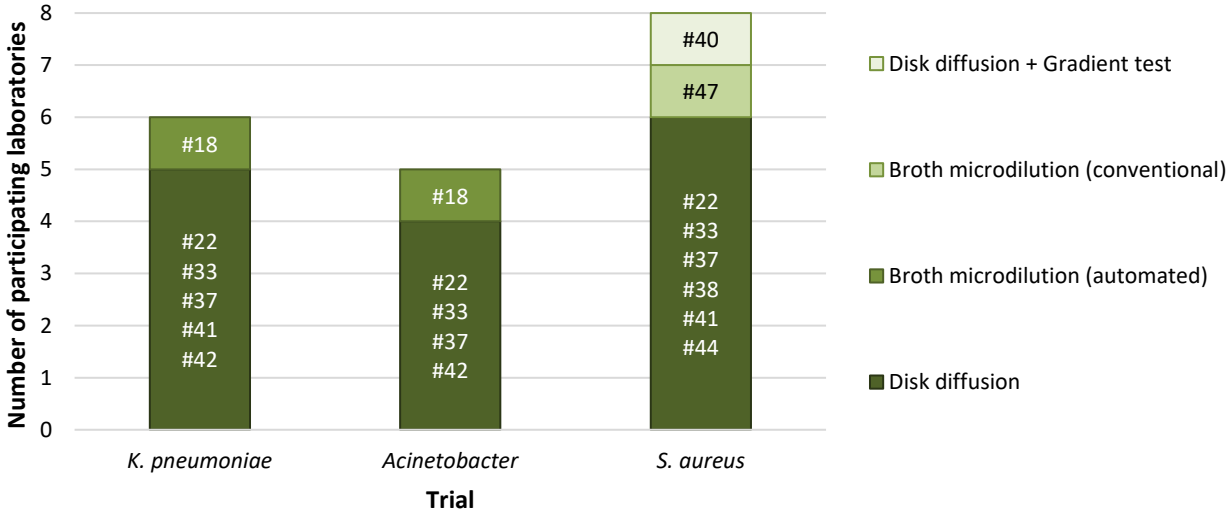

Figure 12. Methodologies applied by the AH laboratories participating in each of the trials.

Disk diffusion as the sole method was the preferred choice for seven of the participating laboratories. Laboratories #18 and #47 reported MIC values obtained by either automated or conventional broth microdilution method, respectively. Laboratory #40 was the only participant that used a mixture of disk diffusion and gradient test (**Figure 12**).

Table 17. Antimicrobial agents tested by the AH laboratories for each trial. For a given trial (Kp, Aci, 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 trial are represented as --.

Antimicrobial	Laboratories in total: n (% of n/N)		
	Kp	Aci	Sa
AMK	4 (66.7)	3 (60.0)	--
AMP	6 (100.0)	--	--
AZI	4 (66.7)	--	--
FEP	4 (66.7)	3 (60.0)	--
FOT	5 (83.3)	3 (60.0)	--
FOX	5 (83.3)	--	5 (62.5)
TAZ	4 (66.7)	3 (60.0)	--
CHL	4 (66.7)	--	8 (100.0)
CIP	6 (100.0)	5 (100.0)	8 (100.0)
CLI	--	--	7 (87.5)
COL	2 (33.3)	2 (40.0)	--
DOR	2 (33.3)	3 (60.0)	--
DOX	--	1 (20.0)	--
ETP	3 (50.0)	--	--
ERY	--	--	8 (100.0)
FUS	--	--	2 (25.0)
GEN	5 (83.3)	4 (80.0)	8 (100.0)
IMI	5 (83.3)	4 (80.0)	--
KAN	--	--	2 (25.0)
LEVO	2 (33.3)	4 (80.0)	--
LZD	--	--	6 (75.0)
MERO	5 (83.3)	4 (80.0)	--
MIN	--	3 (60.0)	--
NAL	5 (83.3)	--	--
PEN	--	--	7 (87.5)
P/T4	3 (50.0)	4 (80.0)	--
SYN	--	--	5 (62.5)
RIF	--	--	4 (50.0)
SMX	4 (66.7)	--	4 (50.0)
TET	5 (83.3)	--	8 (100.0)
TGC	1 (16.7)	1 (20.0)	--
TOB	2 (33.3)	2 (40.0)	--
TMP	4 (66.7)	--	5 (62.5)
SXT	5 (83.3)	4 (80.0)	--
VAN	--	--	2 (25.0)
Total (N)	**6**	**5**	**8**

Kp, *K. pneumoniae*; Aci, *Acinetobacter*; Sa, *S. aureus*
(n) number of laboratories that reported results for the antimicrobial; (N) total number of participating laboratories for the trial

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 tested by the AH laboratories on the *K. pneumoniae* trial, colistin, doripenem, levofloxacin, tigecycline and tobramycin were the least tested drugs (less than half of the participating laboratories). Similarly, colistin, tigecycline and tobramycin, as well as doxycycline were tested by only one or two laboratories in the *Acinetobacter* trial. Regarding the *S. aureus* trial, few laboratories (25.0%) reported results for fusidate, kanamycin and vancomycin (**Table 17**). In contrast, ciprofloxacin was tested by all laboratories for all trials. Ampicillin, gentamicin, meropenem, tetracycline and trimethoprim/sulfamethoxazole were also among the drugs tested by at least 80% of the laboratories (**Table 17**). Scattering of missing data or incomplete AST results entries were observed for all three trials (**Tables 18-20**). Two of the six laboratories participating in the *K. pneumoniae* trial revealed incomplete results (**Table 18**). Laboratory #18 reported cefoxitin results for strain Kp EQASIA 22.1 only, which does not allow for a proper assessment of the laboratory's capacity for testing this specific antimicrobial. Laboratory #41 missed to report the interpretation of the inhibition zone diameter results obtained for cefoxitin, ceftazidime and nalidixic acid when testing strain Kp EQASIA 22.4. As only the categorisation as R, I or S is evaluated, the results for these antimicrobials could not be scored.

For the *Acinetobacter* trial, two of the five participating laboratories did not submit complete results of their own available antimicrobial agents (**Table 19**). Laboratory #18 reported results for 12 different antimicrobials; however, the laboratory did not report doripenem result for strain Aci EQASIA 22.1, and only reported results for two antimicrobials for strain Aci EQASIA 22.4.

Lastly, laboratory #40 did not report results for strain Sa EQASIA 22.7 tested against vancomycin in the *S. aureus* trial (**Table 20**).

Table 18. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by AH laboratories (n=6) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Kp EQAsia 22.1	Kp EQAsia 22.2	Kp EQAsia 22.4	Kp EQAsia 22.5	Kp EQAsia 22.7
#18	-	FOX	FOX	FOX	FOX
#41	-	-	FOX; TAZ; NAL	-	-

Kp, *K. pneumoniae*

Table 19. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter* strains reported by AH laboratories (n=6) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Aci EQAsia 22.1	Aci EQAsia 22.3	Aci EQAsia 22.4	Aci EQAsia 22.5	Aci EQAsia 22.6
#18	DOR	-	FEP; CIP; COL; DOR; IMI; LEVO; MERO; MIN; TGC; SXT	-	-
#22	-	-	GEN	-	-

Aci, *Acinetobacter*

Table 20. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by AH laboratories (n=8) participating in the 4th EQA of the EQAsia project.

Lab ID No.	Sa EQAsia 22.1	Sa EQAsia 22.2	Sa EQAsia 22.3	Sa EQAsia 22.6	Sa EQAsia 22.7
#40	-	-	-	-	VAN

Sa, *S. aureus*

4.2 *Klebsiella pneumoniae* trial

Six laboratories from five countries uploaded results for the *K. pneumoniae* trial.

4.2.1 Bacterial identification

All six participating laboratories correctly identified the five *K. pneumoniae* strains among the seven test strains provided (**Table 21**).

Table 21. Bacterial identification of each of the seven test strains provided related to the *K. pneumoniae* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Kp EQASIA 22.1	*K. pneumoniae*	6/6
Kp EQASIA 22.2	*K. pneumoniae*	6/6
Kp EQASIA 22.3	Non-*K. pneumoniae* (*Escherichia coli*)	5/6
Kp EQASIA 22.4	*K. pneumoniae*	6/6
Kp EQASIA 22.5	*K. pneumoniae*	6/6
Kp EQASIA 22.6	Non-*K. pneumoniae* (*Shigella flexneri*)	5/6
Kp EQASIA 22.7	*K. pneumoniae*	6/6

Kp, *K. pneumoniae*

One laboratory, however, identified the two non-*K. pneumoniae* strains (Kp EQASIA 22.3 and Kp EQASIA 22.6) as *K. pneumoniae*, suggesting that laboratory #41 may not have performed bacterial identification and simply reported all seven strains as *K. pneumoniae* (**Table 21**).

4.2.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 87.8% (strain Kp EQASIA 22.7) to 99.5% (strain Kp EQASIA 22.5) for each strain, with three strains revealing a deviation above 10% (**Table 22**). The high deviation for strain Kp EQASIA 22.7 seems to have been mainly caused by some instances of results' misinterpretation of, for example, meropenem, tetracycline and trimethoprim, as well as trimethoprim/sulfamethoxazole being reported as resistant when expected to be susceptible.

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

Strain	AST in total	% Correct
Kp EQASIA 22.1	95	94.5
Kp EQASIA 22.2	94	89.6
Kp EQASIA 22.4	91	89.0
Kp EQASIA 22.5	94	99.5
Kp EQASIA 22.7	94	87.8

Kp, *K. pneumoniae*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were tigecycline (30.0%), chloramphenicol (22.5%), doripenem (22.5%), imipenem (22.0%) and azithromycin (21.3%), whereas ampicillin, cefepime, cefoxitin, gentamicin, levofloxacin, nalidixic acid, sulfamethoxazole and tobramycin revealed no deviation from the expected results (**Figure 13**).

Of the 24 tested and scored antimicrobial agents, nine revealed to exceed a 10% deviation.

Tigecycline and doripenem were tested by only one and two laboratories, respectively. Despite the low number of incorrect results, it caused the high deviation observed.

The deviation observed for chloramphenicol was mostly due to misinterpretation of results (strain Kp EQASIA 22.1).

Most of the incorrect results observed for imipenem were due to reported Inhibition Zone Diameters smaller than what would be expected.

In the case of azithromycin, the incorrect reported results were close to the threshold for categorising the strain as susceptible or resistant (intermediate option is not available), which resulted in a heavy score penalty (score of 0 or 1 out of the possible full score 4).

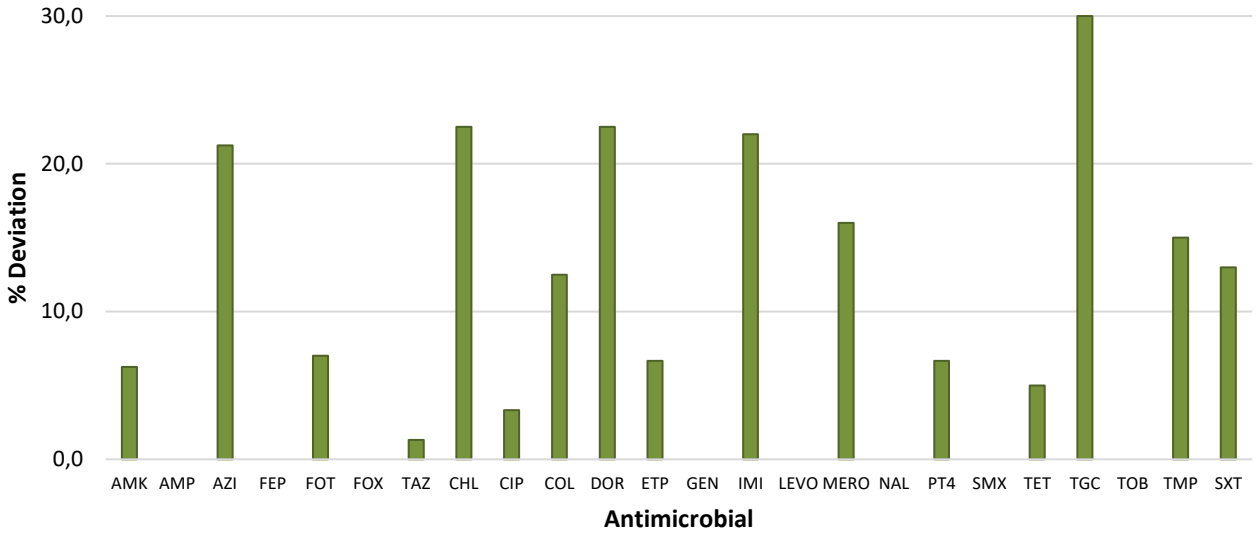

Figure 13. Percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by AH laboratories (n=6) participating in the 4th EQA in the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/I/S) was observed for none of the six participants (**Figure 14**). In average, the deviation was 8.0% (ranging from 6.6 to 8.7%). As the acceptance level was set to 5% deviation, all laboratories did not perform within the expected range for the *K. pneumoniae* trial. Yet, all deviations were below 10%.

Laboratory #37 owes its deviation to a few misinterpreted results, as well as the incorrect

results obtained for colistin, as all strains were found to be resistant (MIC ≥ 16), opposite to what would be expected (MIC ≤ 0.25); laboratory #33 interpreted the obtained Zone Inhibition Diameters of some antimicrobials (imipenem, meropenem, tetracycline and trimethoprim) as resistant whereas intermediate would be expected, resulting in a slight score penalty; laboratory #18 presented occasional deviations when testing, for example, imipenem, meropenem and tigecycline; the remaining three laboratories also presented some occasional mistakes.

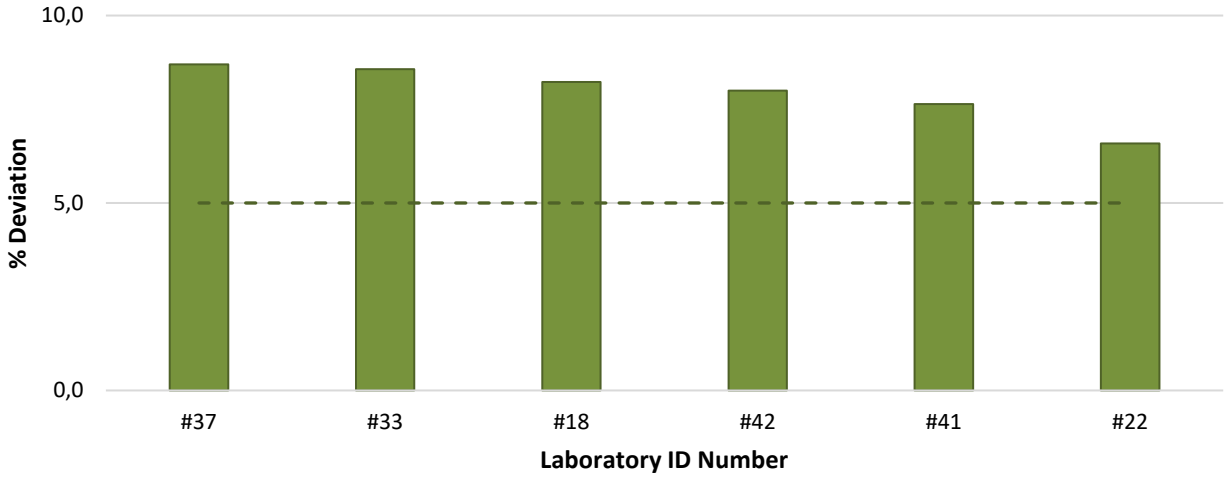

Figure 14. Percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by AH laboratories (n=6) participating in the 4th EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 β-lactamase producing *K. pneumoniae*

Four out of the six participating laboratories uploaded results for this component of the *K. pneumoniae* trial (laboratories #18, #22, #37 and #41). Laboratory #18, however, did not test strain Kp EQASIA 22.7 for ESBL-production. Discrepancies from the expected results are summarized in **Table 23**.

Table 23. 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 22.1	Kp EQASIA 22.2	Kp EQASIA 22.4	Kp EQASIA 22.5	Kp EQASIA 22.7
Expected results		Carbapenemase	Carbapenemase	Carbapenemase	Carbapenemase	Carbapenemase
Obtained results (n/N)	ESBL	1/4 (25.0%)	1/4 (25.0%)	--	--	--
	AmpC	1/4 (25.0%)	--	--	1/4 (25.0%)	1/3 (33.3%)
	ESBL + AmpC	--	--	2/4 (50.0%)	1/4 (25.0%)	1/3 (33.3%)
	Carbapenemase	**2/4 (50.0%)**	**1/4 (25.0%)**	**1/4 (25.0%)**	**2/4 (50.0%)**	**1/3 (33.3%)**
	Other	--	--	--	--	--
	Susceptible*	--	2/4 (50.0%)	1/4 (25.0%)	--	--

Kp, *K. pneumoniae*
*no AmpC, ESBL and carbapenemase. (n/N) number of responses (n) out of the total of reported results (N)

Firstly, laboratories identified the strains that produced ESBL/AmpC/carbapenemase, and then reported the specific phenotype. All five *K. pneumoniae* strains were expected to be carbapenemase-producers, and laboratory #41 correctly classified all of them. On the contrary, laboratories #18 and #22 wrongly identified the *K. pneumoniae* strains as either susceptible, ESBL-, AmpC- or ESBL+AmpC-producers, even though the MIC reported for meropenem was > 0.12 µg/mL (laboratory #18) and the Inhibition Zone Diameter < 25 mm (laboratory #22). Laboratory #37 incorrect results were similar for strains Kp EQASIA 22.4 and Kp EQASIA 22.7; however, for strain Kp EQASIA 22.4, the laboratory reported an Inhibition Zone Diameter of 26 mm and therefore reported the strain as susceptible.

4.2.4 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 distributed free of charge (in this trial or in previous trials) to all participating laboratories to be used as reference strains for the *K. pneumoniae* trial.

Among the six participating laboratories, four submitted results for the reference strain *E. coli* ATCC 25922 and none of the laboratories tested colistin and reported results for *E. coli* NCTC 13846. The laboratories used disk diffusion method for testing the reference strain *E. coli* ATCC 25922 (**Table 24**).

Test results outside of the expected range were only observed for sulfamethoxazole (1 out of 3) and tetracycline (1 out of 4) (**Table 24**). These two deviations were reported by laboratory #37, one found to be below the acceptance interval and the other above. Laboratories #22, #33 and #42 presented no deviation from the expected range (**Figure 15**).

Table 24. AST of the reference strains *E. coli* ATCC 25922 in the *K. pneumoniae* trial. Proportion of test results outside of expected range is presented by methodology used.

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

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

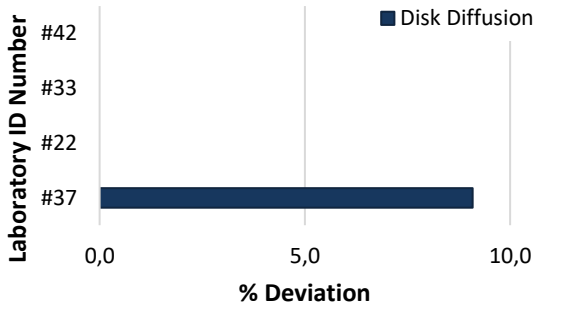

Figure 15. Percentage of deviation in the AST of *E. coli* ATCC 25922 in the *K. pneumoniae* trial by the AH laboratories.

4.3 *Acinetobacter* trial

A total of five laboratories from four countries uploaded results for the *Acinetobacter* trial.

4.3.1 Bacterial identification

All five participating laboratories correctly identified the five *Acinetobacter* strains among the seven test strains provided. However, one laboratory identified the two non-*Acinetobacter* strains as *Acinetobacter*, suggesting that laboratory #42 may not have performed bacterial identification and simply reported all seven strains as *Acinetobacter* (**Table 25**).

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

Strain	Bacterial ID	No. correct
Aci EQASIA 22.1	*A. baumannii*	5/5
Aci EQASIA 22.2	Non-*Acinetobacter* (*P. aeruginosa*)	4/5
Aci EQASIA 22.3	*A. baumannii*	5/5
Aci EQASIA 22.4	*A. lowffii*	5/5
Aci EQASIA 22.5	*A. baumannii*	5/5
Aci EQASIA 22.6	*A. pittii*	5/5
Aci EQASIA 22.7	Non-*Acinetobacter* (*P. aeruginosa*)	4/5

Aci, *Acinetobacter*

4.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 73.8% (strain Aci EQASIA 22.4) to 98.1% (strain Aci EQASIA 22.5) for each strain (**Table 26**). Strain Aci EQASIA 22.4 owes its high deviation to laboratory #37, which reported a very susceptible strain (**Appendix 2b**) as resistant to the majority of the tested antimicrobials.

Table 26. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/I/S). Results are from 5 AH laboratories for the *Acinetobacter* trial.

Strain	AST in total	% Correct
Aci EQASIA 22.1	52	89.4
Aci EQASIA 22.3	53	92.0
Aci EQASIA 22.4	42	73.8
Aci EQASIA 22.5	53	98.1
Aci EQASIA 22.6	53	91.5

Aci, *Acinetobacter*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were tigecycline (50.0%), followed by trimethoprim/sulfamethoxazole (28.9%), doripenem (23.1%) and doxycycline (20.0%) (**Figure 16**). In contrast, amikacin revealed no deviation from the expected results (**Figure 16**).

In the case of tigecycline, only laboratory #18 tested for it. Since the laboratory misinterpreted the results by reporting susceptible instead of resistant, it caused a score penalty that resulted in the high deviation observed in **Figure 16**. The deviation observed for trimethoprim/sulfamethoxazole was in part caused by laboratory #22, which reported all strains as resistant to the drug (Inhibition Zone Diameters of 6mm), even though only one out of the five strains was in fact resistant. The same laboratory also reported doripenem inhibition zones slightly larger than expected, contributing for the deviation of this antimicrobial.

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 five participants (**Figure 17**). In average, the deviation was 9.6% (ranging from 1.1 to 16.3%). As the acceptance level was set to 5% deviation, three laboratories (#37, #22 and #42) did not perform within the expected range for the *Acinetobacter* trial.

Laboratory #37 underperformance seems to be caused by the obtained results for strain Aci

EQASIA 22.4, as well as misinterpretation of results, including interpretation of colistin as susceptible instead of intermediate. Laboratory #22 obtained a few incorrect results for trimethoprim/sulfamethoxazole and doripenem, as already mentioned, as well as for meropenem (inhibition zones a bit larger than expected). Laboratory #42 only tested two antimicrobial agents (ciprofloxacin and trimethoprim/ sulfamethoxazole), meaning that even small score penalties result in a quite high overall deviation.

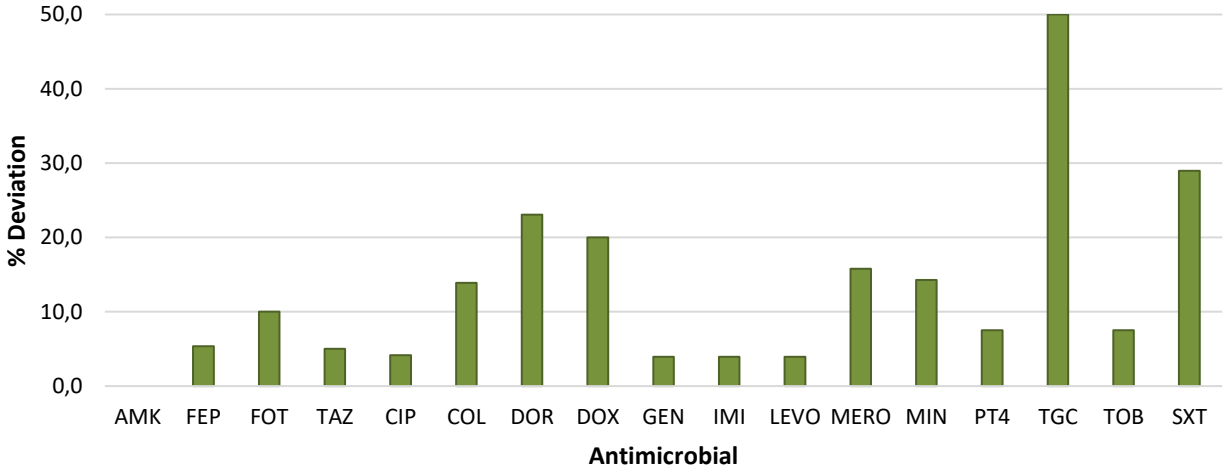

Figure 16. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* strains by AH laboratories (n=5) participating in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

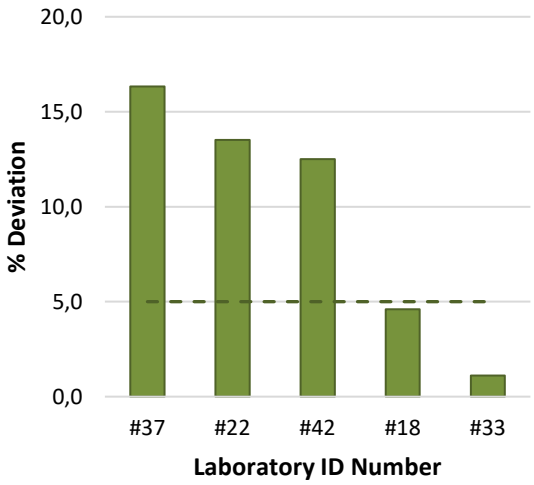

Figure 17. Percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* strains by AH laboratories (n=5) participating in the 4th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.3.3 Quality control strain *P. aeruginosa* ATCC 27853

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

Three laboratories (#22, #33 and #37) submitted AST results for *P. aeruginosa* ATCC 27853 reference strain in the *Acinetobacter* trial, and disk diffusion was the methodology applied by all three laboratories (**Table 27**). The highest proportion of test results outside of the expected range were observed for tigecycline (1 out of 1) levofloxacin (2 out of 3) and cefotaxime (1 out of 2) (**Table 27**).

In terms of performance, laboratory #33 presented no deviation for the nine antimicrobials tested. Inversely, laboratories #22 and #37 had five and three deviations each, respectively (**Figure 18**). Both laboratories presented deviations above the acceptance interval.

Table 27. AST of the reference strain *P. aeruginosa* ATCC 27853 in the *Acinetobacter* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range	
	Disk Diff.	Total
AMK	0/3	**0/3**
FEP	1/3	**1/3**
FOT	1/2	**1/2**
TAZ	1/3	**1/3**
CIP	0/3	**0/3**
COL	--	**--**
DOR	0/2	**0/2**
GEN	1/3	**1/3**
IMI	0/3	**0/3**
LEVO	2/3	**2/3**
MERO	1/3	**1/3**
P/T4	0/2	**0/2**
TGC	1/1	**1/1**
TOB	0/2	**0/2**
SXT	--	**--**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion

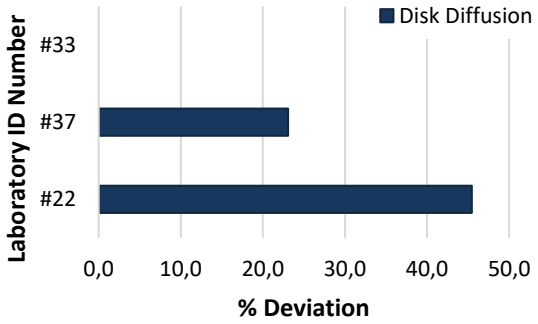

Figure 18. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *Acinetobacter* trial by the AH laboratories.

4.4 *Staphylococcus aureus* trial

Eight laboratories from five countries uploaded results for the *S. aureus* trial.

4.4.1 Bacterial identification

All eight participating laboratories submitted results for bacterial identification (**Table 28**). Six laboratories correctly identified the five *S. aureus* strains and two non-*S. aureus*. Regarding the *S. aureus* strains, Sa EQASIA 22.1 and Sa EQASIA 22.3 were misidentified as non-*S. aureus* by laboratory #33 (**Table 28**). For the non-*S. aureus* strains, *S. epidermidis* (Sa EQAsia 22.4) and *S. chromogenes* (Sa EQAsia 22.5) were wrongly identified as *S. aureus* by laboratories #33 and #41. These observations suggest that laboratory #41 may not have performed bacterial identification and simply reported all provided strains as *S. aureus*.

Table 28. Bacterial identification of each of the seven test strains provided related to the *S. aureus* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Sa EQASIA 22.1	*S. aureus*	7/8
Sa EQASIA 22.2	*S. aureus*	8/8
Sa EQASIA 22.3	*S. aureus*	7/8
Sa EQASIA 22.4	Non-*S. aureus* *(S. epidermis)*	6/8
Sa EQASIA 22.5	Non-*S. aureus* *(S. chromogenes)*	6/8
Sa EQASIA 22.6	*S. aureus*	8/8
Sa EQASIA 22.7	*S. aureus*	8/8

Sa, *S. aureus*

4.4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/I/S) ranged from 90.1% (strain Sa EQASIA 22.3) to 97.8% (strain Sa EQASIA 22.1) for each strain (**Table 29**). None of five strains revealed more than 10% deviation (**Table 29**). Yet, the deviation for strain Sa EQASIA 22.3 seems to have been caused in part by the results submitted by laboratory #38 that reported the strain as susceptible to all tested antimicrobials, even though that outcome was not expected (**Appendix 2c**). Similar results were observed for strain Sa EQASIA 22.2, which was reported by laboratory #33 as susceptible to the majority of the tested antimicrobials (quite large Inhibition Zone Diameters).

Table 29. 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* trial.

Strain	AST in total	% Correct
Sa EQASIA 22.1	78	97.8
Sa EQASIA 22.2	89	90.4
Sa EQASIA 22.3	78	90.1
Sa EQASIA 22.6	89	94.7
Sa EQASIA 22.7	88	96.9

Sa, *S. aureus*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were quinupristin/dalfopristin (13.0%) and cefoxitin (12.0%), whereas fusidate, kanamycin, linezolid and vancomycin revealed no deviation from the expected results (**Figure 19**).

The incorrect results reported for quinupristin/dalfopristin were due to misinterpretation of results: laboratory #44 obtained Inhibition Zone Diameters of 22mm for all five strains, but reported only one strain as susceptible (correct) and the remaining four as intermediate (incorrect); laboratory #38 reported three of the strains as resistant, even though the obtained Inhibition Zone Diameters were ≥19 mm, the threshold for classifying as susceptible.

For cefoxitin, the incorrect results varied amongst strains and laboratories.

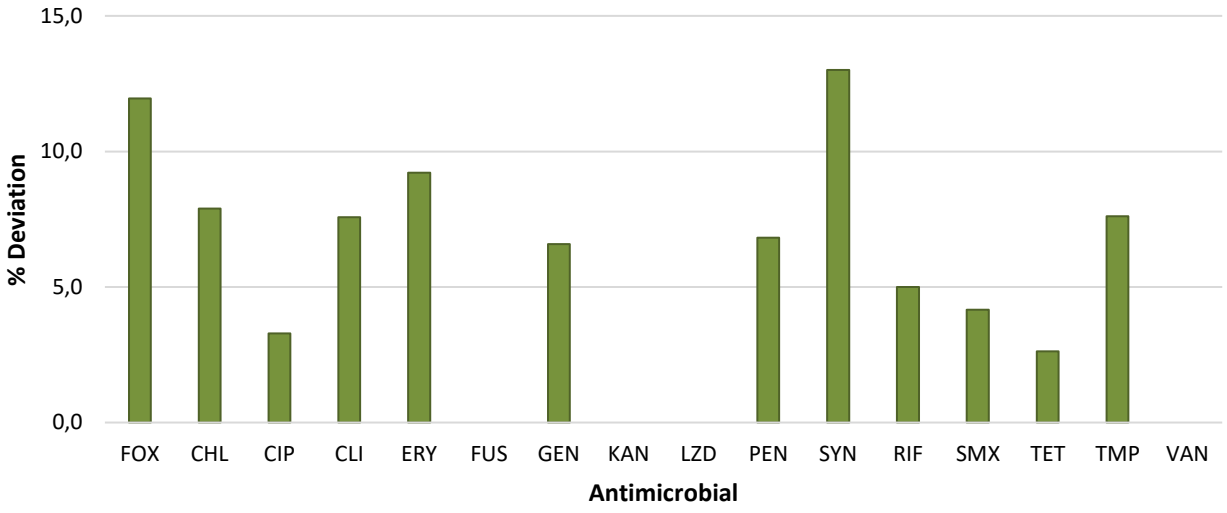

Figure 19. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent. Bars represent the average distribution of the deviation.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/I/S) was observed for five participants (**Figure 20**). In average, the deviation was 7.0% (ranging from 0.9 to 18.1%). As the acceptance level was set to 5% deviation, three laboratories (#38, #44 and #33) did not perform within the expected range for the *S. aureus* trial.

Laboratory #38 presented the highest deviation, which can be explained by the already mentioned incorrect results reported for strain Sa EQASIA 22.3 and the misinterpretations reported for quinupristin/dalfopristin. Similarly, laboratory #44 deviation is mostly due to the misinterpretation results reported for quinupristin/dalfopristin, as well as some other occasional mistakes. For laboratory #33, the deviation was mostly caused by the incorrect results reported for strain Sa EQASIA 22.2 as previously mentioned.

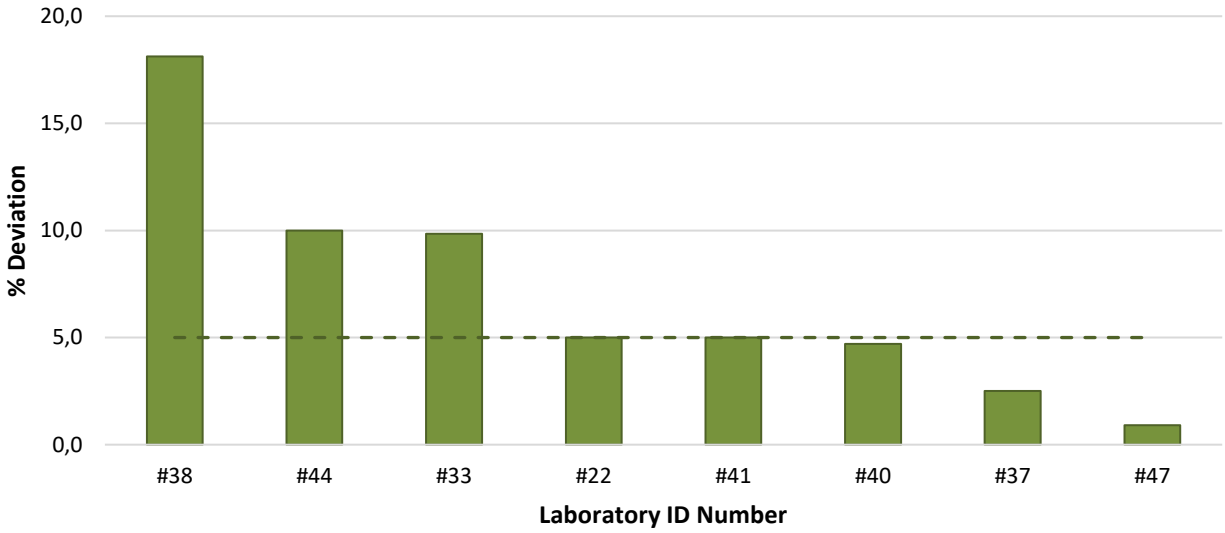

Figure 20. Percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 4th EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 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 this trial or in previous trials) to all participating laboratories to be used as reference strains for the *S. aureus* trial.

Six of the eight participating laboratories submitted AST results for the reference strains: five laboratories reported data for *S. aureus* ATCC 25923 reference strain as disk diffusion was the methodology applied (**Table 30**, *). Laboratory #40 selected *S. aureus* ATCC 29213 to test vancomycin by gradient test (**Table 30**, **). Laboratory #47 submitted AST results for *S. aureus* ATCC 29213 reference strain as broth microdilution was the methodology applied (**Table 30**, **).

The highest proportion of test results outside of the expected range was observed for fusidate (1 out of 2), and for vancomycin and sulfamethoxazole (2 out of 3) (**Table 30**).

Table 30. AST of the reference strain *S. aureus* ATCC 25923 in the *S. aureus* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimi-crobial	Proportion outside of range			
	Disk Diff. *	Gradient **	MIC **	**Total**
FOX	1/4	--	--	**1/4**
CHL	1/5	--	0/1	**1/6**
CIP	1/5	--	0/1	**1/6**
CLI	1/4	--	0/1	**1/5**
ERY	1/5	--	0/1	**1/6**
FUS	1/2	--	--	**1/2**
GEN	1/5	--	0/1	**1/6**
KAN	0/2	--	--	**0/2**
LZD	1/4	--	0/1	**1/5**
PEN	2/5	--	0/1	**2/6**
SYN	0/3	--	0/1	**0/4**
RIF	1/3	--	0/1	**1/4**
SMX	2/3	--	--	**2/3**
TET	2/5	--	0/1	**2/6**
TMP	1/3	--	--	**1/3**
VAN	1/1	1/1	0/1	**2/3**

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

A closer look at the laboratories' performance (**Figure 21**) shows that three laboratories had no deviation from the expected range (#22, #33 and #47). Inversely, laboratory #40 presented a 100% deviation, corresponding to incorrect results for all 13 tested antimicrobials. The MIC reported for vancomycin was below the expected range, whereas all Inhibition Zone Diameters were above the acceptance interval. These results could indicate, for example, too thin agar, too light inoculum, or inaccurate measurement of the Inhibition Zones.

The remaining two laboratories (#37 and #44) had one and three deviations each, respectively, all below the acceptance interval

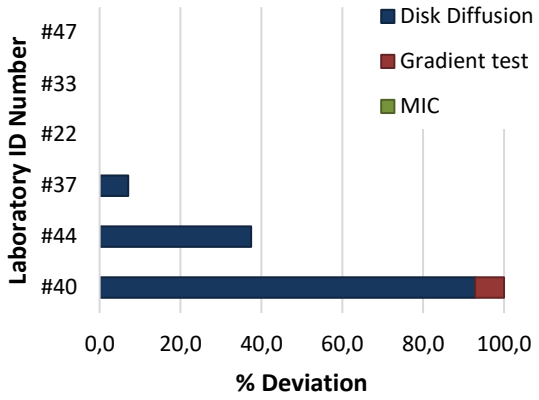

Figure 21. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial by the AH laboratories.

5. Results – Overall

5.1 Bacterial identification

In this fourth EQA round, a total of 19 laboratories participated and submitted results for both the *K. pneumoniae* and *S. aureus* trials, and 16 for the *Acinetobacter* trial. 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 22** shows the distribution of the deviation for each of the trials. As seen, there is no deviation for the majority of the laboratories, except a few outliers. These outliers mainly correspond to the laboratories that did not perform bacterial identification and simply reported all tested isolates as being the target organism.

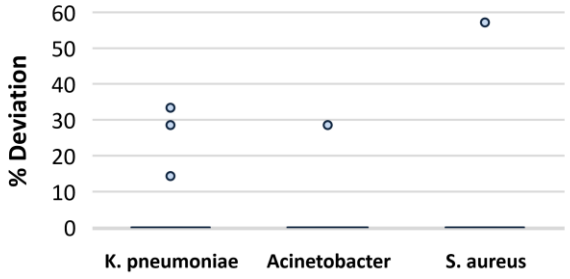

Figure 22. Percentage of deviation in the bacterial identification of *K. pneumoniae*, *Acinetobacter* and *S. aureus* isolates by the participating laboratories.

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 trials, the antimicrobials were tested by a varying number of laboratories. **Figures 23-25** 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).

In the *K. pneumoniae* trial (**Figure 23**), the median deviation for half of the antimicrobials included in the trial (amikacin, ampicillin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, levofloxacin, nalidixic acid, piperacillin/tazobactam, sulfamethoxazole and tobramycin) was 0%, where few outliers can be observed; such distribution suggests that the majority of the laboratories testing these antimicrobials may be proficient at testing them; the distribution of deviations for tetracycline shows that all 12 laboratories submitting results

presented a deviation of 5%. On the contrary, antimicrobials such as azithromycin, chloramphenicol, doripenem, imipenem, meropenem, tigecycline, trimethoprim and trimethoprim/sulfamethoxazole showed more dispersed deviations and quite high median deviation (10% or above), demonstrating that these antimicrobials originated more incorrect results and may have been found as more difficult to test by the participants. Distributions are difficult to visualize for antimicrobials tested by few laboratories (n<5), which is the case of tigecycline; this antimicrobial was tested by only three laboratories, which presented varying deviations (30.0, 35.0 and 75.0 %).

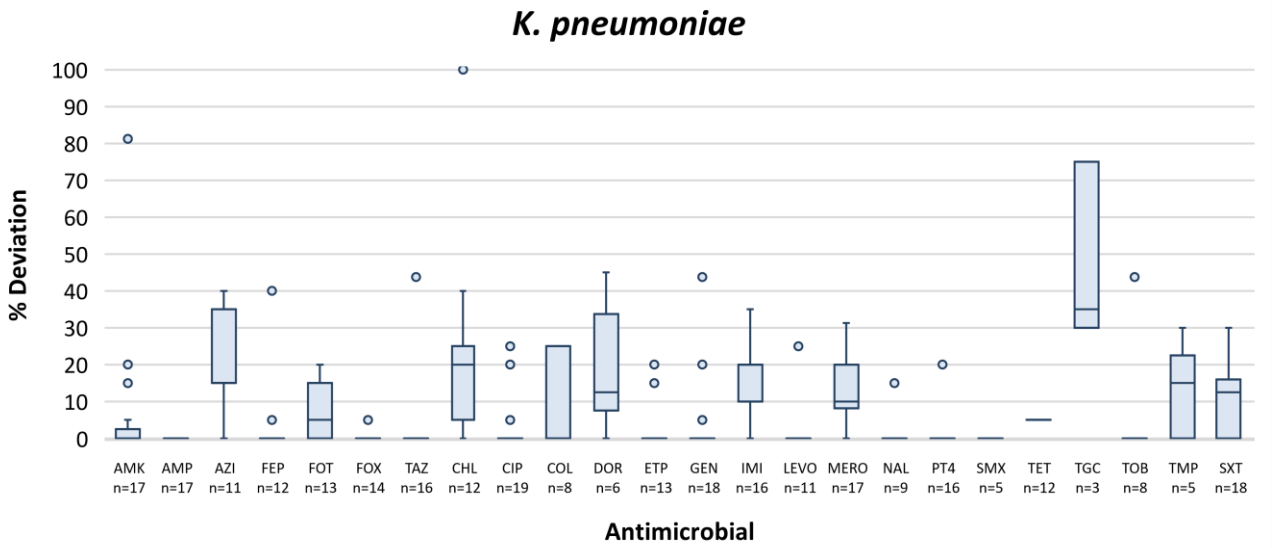

Figure 23. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *K. pneumoniae* strains by the participating laboratories (n=19) in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

In the *Acinetobacter* trial (**Figure 24**), amikacin, ciprofloxacin, gentamicin and imipenem deviations are around 0%, with the exception of a few outliers. Some other antimicrobials have also a median deviation of 0%, but the results are a bit more dispersed, meaning that some of the laboratories presented higher deviations. For instance, the majority of laboratories that submitted results for cefepime (n=8) had a deviation of 0%, but the remaining (n=3) had all a deviation of 15%; similar results are observed for levofloxacin, meropenem and tobramycin.

Antimicrobials such as cefotaxime, ceftazidime, colistin, doripenem, minocycline, piperacillin/ tazobactam and trimethoprim/sulfamethoxazole generated higher and more dispersed deviations, suggesting that not all laboratories participating in the program may be comfortable with testing *Acinetobacter* against these antimicrobials.

As mentioned in the *K. pneumoniae* trial, antimicrobials tested by less than five laboratories are more difficult to be analysed for their deviations' distribution. Doxycycline was tested by three participants, which presented deviations of 0% (n=1) or 20% (n=2); tigecycline was tested by only two laboratories that had deviations of 15 and 50%.

In the *S. aureus* trial (**Figure 25**), fusidate, kanamycin and linezolid presented no deviations, whereas ciprofloxacin, erythromycin, gentamicin, rifampicin, tetracycline and vancomycin had median deviations close to 0% with a few outliers; the remaining antimicrobials presented higher median deviations and/or more dispersed deviations suggesting that these

antimicrobials may have been found more difficult to test by the participating laboratories and therefore generated more incorrect results.

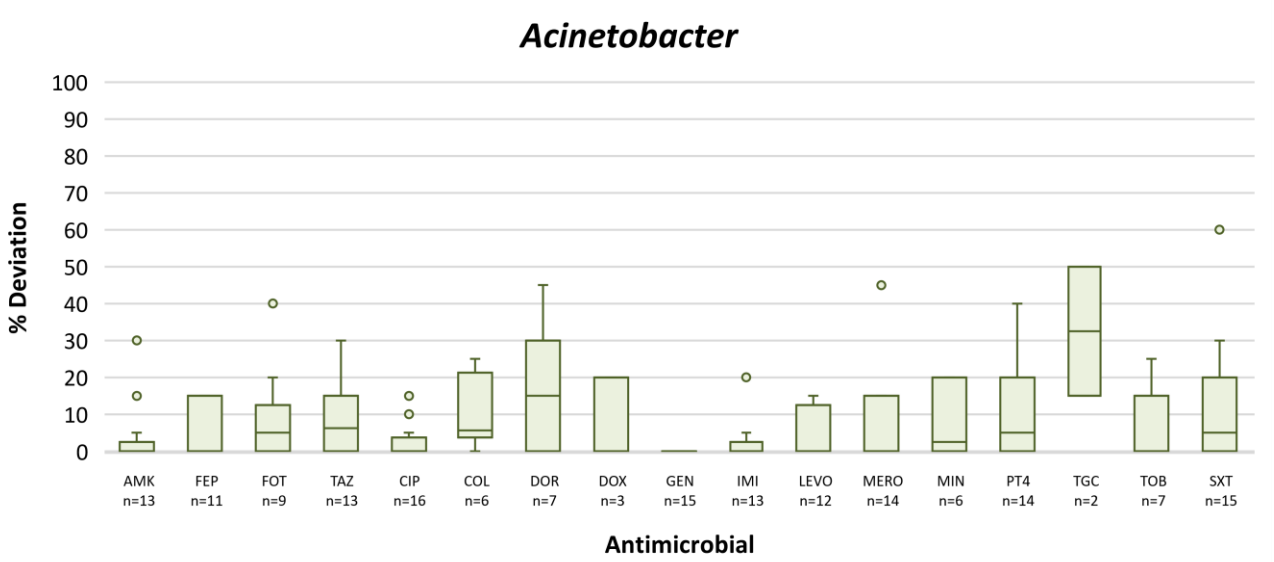

Figure 24. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *Acinetobacter* strains by the participating laboratories (n=16) in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

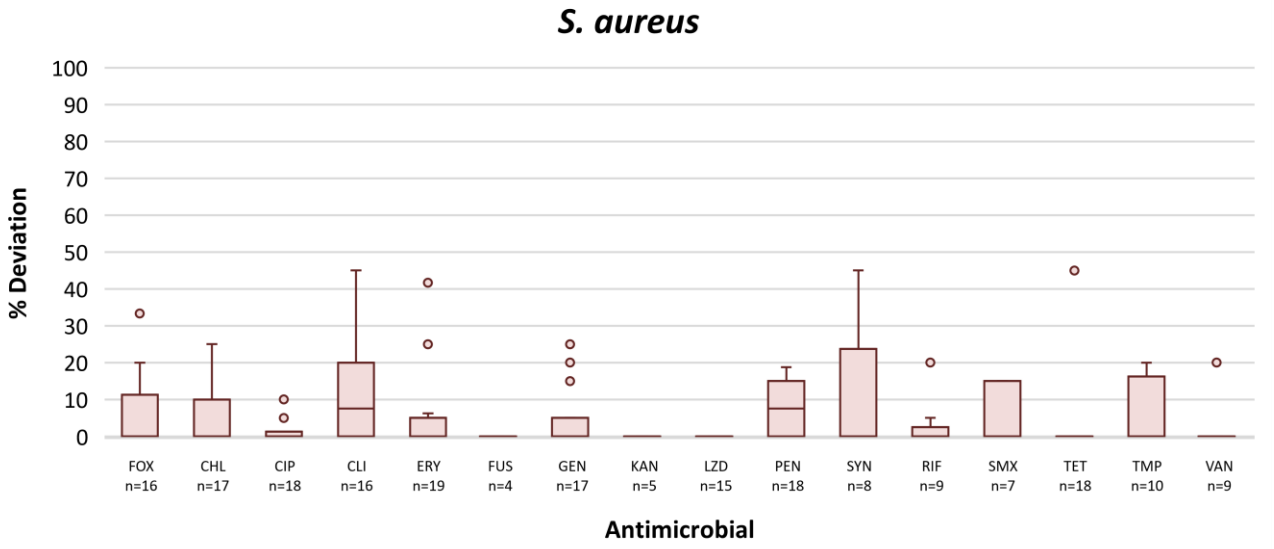

Figure 25. Distribution of the percentage of deviation in the AST interpretation (R/I/S) among *S. aureus* strains by the participating laboratories (n=19) in the 4th EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

5.2.2 Laboratories performance

In each of the trials, the laboratories performance varied. **Figure 26** presents the distribution of the deviations obtained for the laboratories participating in each of the trials (number of laboratories is indicated under each trial name). It can be observed that the deviations observed for the *Acinetobacter* trial are more dispersed than in the *K. pneumoniae* and *S. aureus* trials, suggesting that the level of proficiency testing *Acinetobacter* varies among the participating laboratories.

In the *K. pneumoniae* trial, it can be observed that the median deviation is above the

acceptance level of 5% deviation from expected results; with the exception of the outlier (deviation above 35%), the laboratories deviations were below 10%.

Regarding the *Acinetobacter* trial, it can be noticed that the median deviation is a bit lower than the one observed for the *K. pneumoniae* trial (slightly above 5%), but more laboratories presented deviations above 10%.

Lastly, the *S. aureus* trial was the only one with a median deviation below the acceptance level of 5% deviation from expected results, meaning that more than half of the participating laboratories performed within the expected.

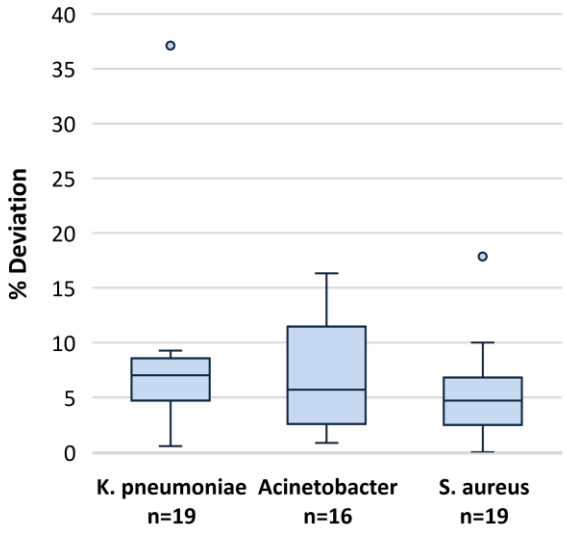

Figure 26. Distribution of the percentage of deviation in the AST interpretation (R/I/S) of obtained results by laboratories participating in the 4th EQA of the EQAsia project. Results are categorized by trial.

5.3 Quality control strains

Relevant quality control strains were tested for each of the trials: *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (for colistin) were used as reference strains for the *K. pneumoniae* trial, *P. aeruginosa* ATCC 27853 for the *Acinetobacter* trial, 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* trial. A total of 16 laboratories submitted results concerning the reference strains for the *K. pneumoniae* and *S. aureus* trials, and 13 laboratories for the *Acinetobacter* trial.

Figure 27 presents the distribution of the deviations obtained by the participating laboratories for the reference strains included in each of the trials. For both *K. pneumoniae* and *Acinetobacter* trials the median deviation is around 10%, whereas the median deviation for the *S. aureus* trial is 0%. The deviations observed are quite disperse in all of the trials, suggesting that the laboratories proficiency when testing the reference strains seems to vary greatly among the participants.

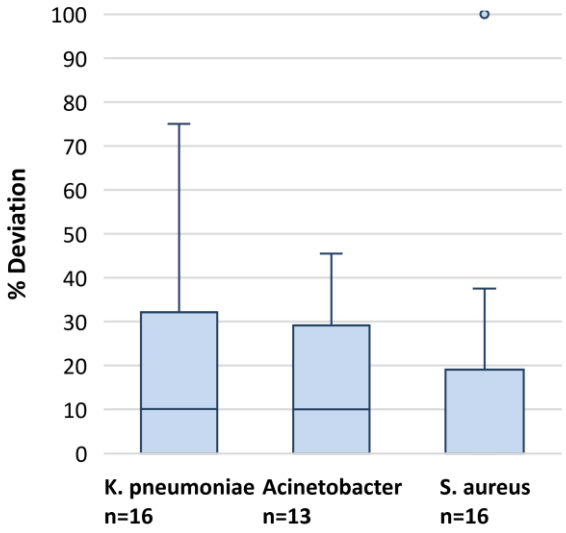

Figure 27. Distribution of the percentage of deviation in the AST of obtained results for the reference strains by laboratories participating in the 4th EQA of the EQAsia project. Results are categorized by trial.

6. Discussion

6.1 Human Health Laboratories

A total of 14 Human Health laboratories participated in the 4th EQA of the EQAsia programme. Disk diffusion and broth microdilution as solo methodologies were chosen by half of the participants for testing the recommended antimicrobials in each of the trials. The remaining laboratories opted for disk diffusion along with the other methods, such as gradient test, broth microdilution, broth macrodilution and/or agar dilution.

All laboratories that performed bacterial identification have also submitted AST results. Incomplete AST results' entries were, however, observed in all three trials, meaning that the participating laboratories did not submit complete results of their own available antimicrobial agents. It would be expected that the isolates of each trial would be tested against the same panel of antimicrobials, allowing for a solid assessment of the laboratories' performance and capacity.

Regarding the bacterial identification component, the participants showed high proficiency in correctly identifying the *S. aureus* species among the provided test strains. In the other two trials, the laboratories demonstrated limited capacity to properly identify the target species (*K. pneumoniae* or *Acinetobacter*), 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 trials (*K. pneumoniae* and *Acinetobacter*), some common antimicrobials presented a high deviation from the expected results, such as tigecycline (41.7% and 15.0% in the *K. pneumoniae* and *Acinetobacter* trials, respectively) and doripenem (17.1% and 11.8%, respectively). In general, the deviations observed were higher for antimicrobials that were tested by fewer laboratories. For the Gram-positive bacteria *S. aureus* trial, clindamycin revealed a rather high deviation (16.5%).

Regarding the HH laboratories' AST performance, on average, the deviation was 7.9% in the *K. pneumoniae* trial, 5.9% in the *Acinetobacter* trial and 4.9% in the *S. aureus* trial. Despite the average being close to acceptable (below the acceptance level of 5% or a bit above), there were some laboratories that had deviations above 5% in multiple trials. Laboratory #32, for example, presented deviations above 10% for all three trials.

Detection and confirmation of presumptive beta-lactamase producing *K. pneumoniae* was an optional component of this EQA, but highly encouraged due to its importance. Nine of the 13 participating laboratories submitted results but only two laboratories correctly identified all the phenotypes among the five *K. pneumoniae* strains, which were all carbapenemase-producers. Correct classification of the carbapenemase phenotypes seems to be an issue, even though the obtained values for meropenem were a lot of times > 0.12 µg/mL or < 25 mm. According to Figure 1 of the EQA4 protocol (**Appendix 1**), a strain presenting these values for meropenem should be classified as a carbapenem-resistant *K. pneumoniae* strain. This observation suggests a need for further clarification and support on capacity building.

Among all laboratories, there was one laboratory (#32) that did not submit antimicrobial susceptibility testing results for the quality control strains. It was also noticed that some laboratories tested the test strains and the reference strain by different methodologies. For quality control purposes, the participating

laboratories should apply the same methodology for both the reference strains and the test strains, as well as test the same antimicrobials in both situations. According to the CLSI recommendation, 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. A systemic performance of internal quality control including testing of reference strains must be implemented to warrant the improvement of laboratory capacity.

6.2 Animal Health Laboratories

For the Animal Health sector, 10 laboratories participated in the 4th EQA of the EQAsia programme. The participating laboratories mostly applied disk diffusion for determining Inhibition Zone Diameters, though two participants opted for broth microdilution and one laboratory used a combination of disk diffusion and gradient test.

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 three trials. One laboratory, for example, reported results for a certain antimicrobial for one strain only, which does not allow for a proper assessment of the laboratory's capacity for testing this specific antimicrobial. Another laboratory missed to report the interpretation of the inhibition zone diameter results obtained for a number of antimicrobials; as only the categorisation as R, I or S is evaluated, the results for these antimicrobials could not be scored.

As mentioned above, bacterial identification was the first component in each of the trials. There were no major issues with bacterial identification of the five target strains among the seven isolates provided (except for one laboratory in the *S. aureus* trial). Yet, the two non-target strains included in each of the trials were misidentified by one participant in each of the trials, suggesting that these laboratories may not have performed bacterial identification and simply reported all seven strains as the target strain.

For the antimicrobial susceptibility testing performance, and as seen for the HH laboratories, tigecycline and doripenem presented quite high deviations in the *K. pneumoniae* and *Acinetobacter* trials (for TGC 30.0 and 50.0%, respectively and for DOR 22.5 and 23.1%, respectively), which can be explained by the fact that these antimicrobials were tested by very few laboratories (**Table 17**).

Regarding laboratories performance, the laboratories were ranked according to the percentage of deviating results in the antimicrobial susceptibility tests. The average deviation was, in fact, above the acceptance level of 5% for all three trials: 8.0% in the *K. pneumoniae* trial, 9.6% in the *Acinetobacter* trial, and 7.0% in the *S. aureus* trial. In addition, almost all AH laboratories (eight out of 10) had a deviation above 5% in at least one of the trials that they have participated in.

Four out of the six participating laboratories in the *K. pneumoniae* trial submitted results for the detection and confirmation of presumptive beta-lactamase producing bacteria. Only one laboratory (#41) correctly identified all the phenotypes among the five *K. pneumoniae* strains, which were all carbapenemase-producers. As seen for the HH laboratories, classification of the carbapenemase phenotypes seems to be problematic as well. Even though laboratories obtained Inhibition Zone Diameter values for meropenem < 25 mm and MIC > 0.12 µg/mL, they struggled to identify the correct classification. 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 trials. Four laboratories (#18, #38, #41 and #42) did not submit results for the reference strains. Testing the recommended reference strains is required in

terms of quality control and reliability of AST results and performance. 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 assure the laboratories' good performance.

7. Conclusions

This report presented the results of the EQAsia 4th EQA trial, which included *K. pneumoniae*, *Acinetobacter* and *S. aureus*. This EQA assessed the performance in 1) bacterial identification, 2) AST determination and interpretation, and 3) detection of beta-lactam resistance phenotypes mediated by ESBL/ AmpC/ carbapenemase.

The goal of EQAsia EQAs is to have all participating Human 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 need for supporting with training and capacity building the reference laboratories in the South and Southeast Asian region.

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.

As observed in the previous EQAsia EQAs, the laboratories still report incorrect interpretation of the MIC/Inhibition Zone Diameter values. Once again, it is recommended to solely use the interpretative criteria available in the EQA protocol, as it is a requirement that all participating laboratories follow the same interpretation criteria to allow for comparison of results. Several other incorrect results were also detected upon submission of the results, such as selection of the wrong antimicrobial or strains that seem to have been switched around. To avoid all these issues in future EQAs, it is recommended to implement quality control procedures such as having two different persons reading the results and the respective interpretations, both in the laboratory and when the data is entered in the informatics system.

Antimicrobial susceptibility testing of the reference strains is also highly important and, therefore, recommended. Relevant reference strains have been sent to the participating laboratories free of charge to be used not only in the EQAsia EQAs, but also in routine work. Thus, 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.

This EQAsia survey provides further valuable information for assessors, funders and participating laboratories. It enables identification of aspects of testing needing improvement and supports accurate testing for clinical and surveillance purposes, critical for detecting and monitoring AMR in the human and animal health sectors.

8. References

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

9. Appendices

Appendix 1: EQA4 Protocol

Protocol for EQAsia EQA4 2022

ID and antimicrobial susceptibility testing of *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Staphylococcus aureus* test strains

***Version 2** of the protocol includes changes in Table 1 reference values (marked in **bold**).*

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 during 2021. The EQA is organized by the consortium of EQAsia and supported by the Fleming Fund.

The EQAsia EQA4 2022 includes the antimicrobial susceptibility testing of five *Klebsiella pneumoniae*, five *Acinetobacter* spp. and five *Staphylococcus aureus* strains **identified** among a total of **seven** test strains for each microorganism, which include two non-target species strains.

Additionally, antimicrobial susceptibility testing of the relevant reference strains for quality control (QC) in relation to antimicrobial susceptibility testing is included. The QC reference strains supplied (or that have been supplied in previous EQAS) 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. Handle and maintain them as suggested in the manual 'Subculture and maintenance of quality control strains' available on the EQAsia website.

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

3 OUTLINE OF THE EQASIA EQA

3.1 Shipping, receipt and storage of strains

In April 2022, it is expected that around 25 laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- Seven test strains of which five are *Klebsiella pneumoniae*, in addition to two non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains (if not already received in previous EQAs).

- Seven test strains of which five are *Acinetobacter* spp., in addition to two non-target species strains. The *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 will be provided as reference strain (if not already received in previous EQAs).

- Seven test strains of which five are *Staphylococcus aureus*, in addition to two non-target species strains. The *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC) will be provided as reference strains (if not already received in previous EQAs).

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

All strains are shipped lyophilized. 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). This set of cultures 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 function as reference material available for reference at a later stage, when needed.

For reconstitution of the test strains, please see the document 'Instructions for opening and reviving lyophilised cultures of test strains on the EQAsia website.

For reconstitution of the QC reference strains, please see the document 'Subculture and maintenance of quality control strains' on the EQAsia website.

All provided strains belong to 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.

3.2 Identification of *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Staphylococcus aureus* test strains

For each test species, two out of the seven test strains related to each bacterial species does not belong to the target species of the EQA trial. To identify the five cultures of the correct target species among the seven test strains, you should use the method routinely used in your own laboratory for **identification** of the organism.

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

The strains identified as *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Staphylococcus aureus*, as well as the appropriate reference strains, should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form and in **Tables 1-3**. 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 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, 31st Ed.). When not available, EUCAST clinical breakpoints (Tables v. 11.0, 2021) or epidemiological cut off values (https://mic.eucast.org/) are used instead. The breakpoint values for *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Staphylococcus aureus* can be found in **Tables 1-3**, respectively. **Make sure to use the correct table for the interpretation**.

Interpretation of MIC or disk diffusion results will lead to categorization of the result into one of the categories: **resistant** (R), **intermediate** (I) or **susceptible** (S). In the evaluation report you receive upon the submission deadline, the obtained interpretation in comparison with the expected interpretation will be evaluated/scored as follows:

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

Table 1. Interpretive criteria for *Klebsiella pneumoniae* antimicrobial susceptibility testing

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 for ***K. pneumoniae*** are recommended:

- Reduced susceptibility to cefotaxime (FOT) and/or ceftazidime (TAZ): it indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype. 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). Resistance to MERO indicates that the bacterial strain is a carbapenemase-producer.

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.

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but it is not requested as part of this EQA.

Table 2. Interpretive criteria for *Acinetobacter* spp. antimicrobial susceptibility testing

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

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

Reference values are based on *Acinetobacter* spp. breakpoints from CLSI M100, **32nd** Ed.
*Reference values are based on *Acinetobacter* spp. clinical breakpoints from www.eucast.org (Tables v. 12.0, 2022).

Table 3. Interpretive criteria for *Staphylococcus aureus* antimicrobial susceptibility testing

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 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the Informatics Module. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The Informatics Module will allow you to view and print a report with your reported results. The scores for the results will be released after the result submission deadline where 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).

Results must be submitted no later than June 10th 2022.

If you have trouble in entering your results, please contact the EQA Coordinator directly, explaining the issues that you encountered:

Patrícia T. dos Santos
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK
E-mail: pado@food.dtu.dk

Direct communication with the EQA Coordinator must be in English.

5 HOW TO SUBMIT RESULTS VIA THE INFORMATICS MODULE

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

Access the Informatics Module (incognito window) using this address. See below how to login to the Informatics Module.

When you submit your results, remember to have by your side the completed test forms (template available for download from the EQAsia website).

Do not hesitate to contact us if you have trouble with the Informatics Module.

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 data entry.

Login to the Informatics Module:

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 pado@food.dtu.dk

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

Appendix 2a: Reference values (MIC values and interpretation) – *K. pneumoniae*

	Amikacin AMK		Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		FOT+Cl F/C	Cefoxitin FOX		Ceftazidime TAZ		TAZ+Cl T/C
Kp EQASIA 22.1	> 128	R	> 32	R	64	R	> 32	R	> 64	R	> 64/4	> 64	R	128	R	128/4
Kp EQASIA 22.2	≤ 4	S	> 32	R	32	R	0.5	S	1	S	1/4	8	S	0.5	S	0.5/4
Kp EQASIA 22.4	≤ 4	S	> 32	R	16	S	16	R	> 64	R	4/4	> 64	R	128	R	16/4
Kp EQASIA 22.5	≤ 4	S	> 32	R	> 64	R	> 32	R	> 64	R	> 64/4	> 64	R	> 128	R	> 128/4
Kp EQASIA 22.7	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	2/4	> 64	R	> 128	R	2/4

R, Resistant; I, Intermediate; S, Susceptible

	Chloramphenicol CHL		Ciprofloxacin CIP		Colistin COL		Doripenem DOR		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Levofloxacin LEVO		Meropenem MERO	
Kp EQASIA 22.1	32	R	> 8	R	≤ 0.25	I	> 2	R	> 4	R	> 16	R	> 16	R	8	R	> 16	R
Kp EQASIA 22.2	≤ 8	S	0.06	S	≤ 0.25	I	0.5	S	2	R	≤ 0.5	S	2	I	≤ 0.5	S	1	S
Kp EQASIA 22.4	≤ 8	S	> 8	R	≤ 0.25	I	0.5	S	> 4	R	> 16	R	≤ 1	S	> 8	R	2	I
Kp EQASIA 22.5	> 64	R	> 8	R	≤ 0.25	I	> 2	R	> 4	R	> 16	R	16	R	> 8	R	> 16	R
Kp EQASIA 22.7	> 64	R	> 8	R	≤ 0.25	I	1	S	> 4	R	> 16	R	0.5	S	> 8	R	2	I

R, Resistant; I, Intermediate; S, Susceptible

	Nalidixic acid NAL		Piperacillin/ tazobactam P/T4		Sulfamethoxazole SMX		Tetracycline TET		Tigecycline TGC		Tobramycin TOB		Trimethoprim TMP		Trimethoprim/ sulfamethoxazole SXT	
Kp EQASIA 22.1	> 64	R	> 64/4	R	> 512	R	4	S	0.5	S	> 8	R	1	S	1/19	S
Kp EQASIA 22.2	≤ 4	S	> 64/4	R	≤ 8	S	≤ 2	S	0.5	S	≤ 1	S	0.5	S	≤ 0.5/9.5	S
Kp EQASIA 22.4	> 64	R	> 64/4	R	> 512	R	> 32	R	1	S	> 8	R	> 16	R	> 4/76	R
Kp EQASIA 22.5	> 64	R	> 64/4	R	> 512	R	> 32	R	8	R	> 8	R	> 16	R	> 4/76	R
Kp EQASIA 22.7	> 64	R	> 64/4	R	> 512	R	8	I	1	S	> 8	R	2	S	1/19	S

R, Resistant; I, Intermediate; S, Susceptible

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

	Amikacin AMK		Cefepime FEP		Cefotaxime FOT		Ceftazidime TAZ		Ciprofloxacin CIP		Colistin COL		Doripenem DOR		Doxycycline DOX		Gentamicin GEN	
Aci EQASIA 22.1	> 32	R	> 16	R	> 32	R	> 16	R	> 2	R	> 4	R	> 4	R	> 16	R	> 8	R
Aci EQASIA 22.3	8	S	> 16	R	> 32	R	> 16	R	> 2	R	≤ 0.25	I	> 4	R	> 16	R	> 8	R
Aci EQASIA 22.4	≤ 4	S	≤ 2	S	≤ 1	S	≤ 1	S	≤ 0.25	S	≤ 0.25	I	≤ 0.12	S	≤ 2	S	≤ 1	S
Aci EQASIA 22.5	≤ 4	S	> 16	R	> 32	R	> 16	R	> 2	R	≤ 0.25	I	1	S	≤ 2	S	> 8	R
Aci EQASIA 22.6	≤ 4	S	4	S	16	I	4	S	≤ 0.25	S	0.5	I	> 4	R	≤ 2	S	≤ 1	S

R, Resistant; I, Intermediate; S, Susceptible

	Imipenem IMI		Levofloxacin LEVO		Meropenem MERO		Minocycline MIN		Piperacillin/tazobactam P/T4		Tigecycline TGC		Tobramycin TOB		Trimethoprim/ sulfamethoxazole SXT	
Aci EQASIA 22.1	> 8	R	> 8	R	> 8	R	16	R	> 64/4	R	1	R	> 8	R	1/19	S
Aci EQASIA 22.3	> 8	R	> 8	R	> 8	R	≤ 2	S	> 64/4	R	1	R	> 8	R	> 4/76	R
Aci EQASIA 22.4	≤ 1	S	≤ 1	S	≤ 1	S	≤ 2	S	≤ 8/4	S	≤ 0.25	S	≤ 1	S	≤ 0.5/9.5	S
Aci EQASIA 22.5	≤ 1	S	8	R	≤ 1	S	≤ 2	S	> 64/4	R	≤ 0.25	S	> 8	R	≤ 0.5/9.5	S
Aci EQASIA 22.6	> 8	R	≤ 1	S	> 8	R	≤ 2	S	32/4	I	≤ 0.25	S	≤ 1	S	≤ 0.5/9.5	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 2c: Reference values (MIC values and interpretation) – *S. aureus*

	Cefoxitin FOX		Chloramphenicol CHL		Ciprofloxacin CIP		Clindamycin CLI		Erythromycin ERY		Fusidate FUS		Gentamicin GEN		Kanamycin KAN	
Sa EQASIA 22.1	16	R	≤ 4	S	≤ 0.25	S	≤ 0.12	S	> 8	R	> 4	R	≤ 0.5	S	> 32	R
Sa EQASIA 22.2	8	R	64	R	≤ 0.25	S	> 4	R	> 8	R	≤ 0.25	S	≤ 0.5	S	> 32	R
Sa EQASIA 22.3	8	R	8	S	≤ 0.25	S	> 4	R	> 8	R	≤ 0.25	S	16	R	> 32	R
Sa EQASIA 22.6	16	R	8	S	8	R	≤ 0.12	S	4	I	≤ 0.25	S	> 16	R	> 32	R
Sa EQASIA 22.7	4	S	8	S	0.5	S	≤ 0.12	S	> 8	R	≤ 0.25	S	≤ 0.5	S	≤ 4	S

R, Resistant; I, Intermediate; S, Susceptible

	Linezolid LZD		Penicillin PEN		Quinupristin/dalfopristin SYN		Rifampin RIF		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP		Vancomycin VAN	
Sa EQASIA 22.1	≤ 1	S	> 1	R	≤ 0.5	S	≤ 0.015	S	512	R	≤ 0.5	S	> 16	R	≤ 1	S
Sa EQASIA 22.2	≤ 1	S	≤ 0.06	S	≤ 0.5	S	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQASIA 22.3	≤ 1	S	> 1	R	≤ 0.5	S	4	R	≤ 64	S	> 16	R	> 16	R	≤ 1	S
Sa EQASIA 22.6	2	S	> 1	R	≤ 0.5	S	≤ 0.015	S	≤ 64	S	≤ 0.5	S	> 16	R	≤ 1	S
Sa EQASIA 22.7	2	S	> 1	R	≤ 0.5	S	≤ 0.015	S	≤ 64	S	≤ 0.5	S	≤ 1	S	≤ 1	S

R, Resistant; I, Intermediate; S, Susceptible

Appendix 3: Quality control ranges for the reference strains

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

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

MIC ranges and disk diffusion ranges are according to CLSI M100 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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