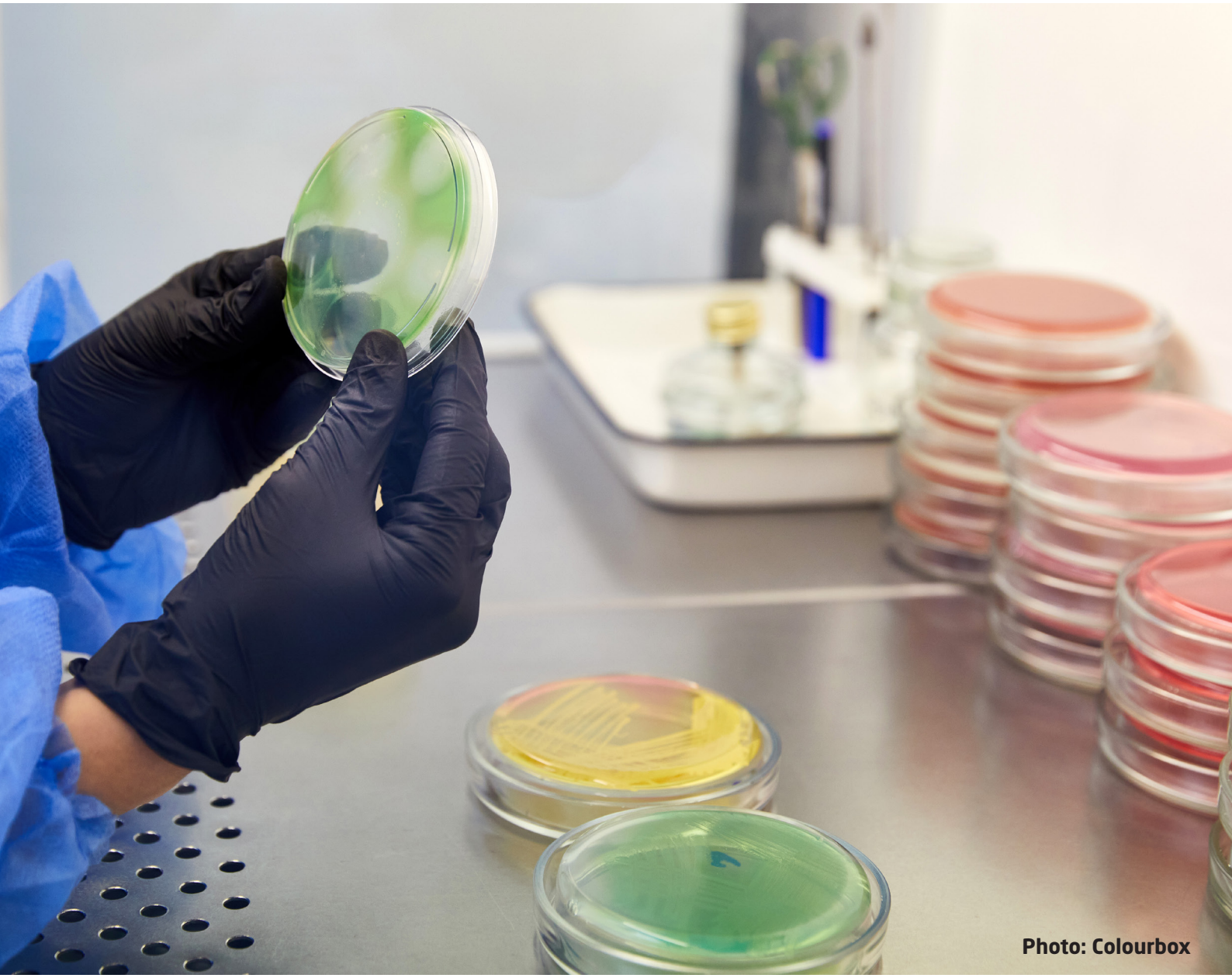
The 2nd EQAsia External Quality Assessment trial: Klebsiella pneumoniae, Shigella spp., Acinetobacter spp. and Staphylococcus aureus - 2021



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The 2nd EQAsia External Quality Assessment trial: *Klebsiella pneumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* – 2021

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The 2nd EQAsia External Quality Assessment trial: *Klebsiella pneumoniae, Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* – 2021

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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].

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 local 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. The EQA trials focus 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*), Enterococci (*E. faecium* and *E. faecalis*) and *Streptococcus pneumoniae*. In addition, two Matrix EQAs are offered, aligning with the scope of WHO Tricycle and suggested from FAO, aiming to assess the veterinary laboratories' ability to detect AmpC beta-lactamases (AmpC), extended-spectrum beta-lactamases (ESBL) and carbapenemase producing *E. coli* from animal caeca samples and 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.

Each EQA trial encompasses the testing of a total of 11 test strains of a given organism. Of these, eight of the test strains are of the organism in focus (target organism), whereas three test strains are different from the targeted species (reported as non-[organism], e.g. non-*Shigella*). For each of the 11 test strains, participants are requested to report which eight strains belong to the expected target organism. For the three organisms different from the expected, no further testing is required. For the remaining eight test strains of the target organism, results in relation to AST are requested.

This report contains results from the second EQA trial of the EQAsia project carried out in August-October 2021. This second EQA trial includes identification and AST of *Klebsiella pbeumoniae*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus 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 European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Interpretative criteria referring to both disk diffusion and MIC determination are listed in the EQA protocol (Appendix 1) and allow for the obtained results to be interpreted into categories as resistant or susceptible depending on the method used. Results in agreement with the expected interpretation are categorised as '1' (correct), while results deviating from the expected interpretation are categorised as '0' (incorrect). 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 route cause analysis procedure performed by individual participants (self-evaluation) when the EQA results are disclosed. 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 ±3mm, respectively. If the expected MIC/Zone Diameter is close to the threshold for categorising the strain as susceptible or resistant, a one-fold dilution/±3mm difference may result in different interpretations. Since this report evaluates the interpretations of MIC/Zone Diameter and not the values, some participants may find their results classified as incorrect even though the actual MIC/Zone Diameter measured is only one-fold dilution/±3mm appart 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 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), Navin Karan (Pacific Pathology Training Centre, New Zealand) and Monica Lahra (WHO Collaborating Center for STI and AMR, NSW Health Pathology Microbiology, New South Wales, Australia).

2. Materials and Methods

2.1 Participants in EQAsia EQA2

A total of 24 laboratories participated in the second EQA survey of the EQAsia project: 14 laboratories belonging to the HH Sector and 10 belonging to the AH Sector, originating from: Bangladesh, Bhutan, Indonesia, Lao's People Democratic Republic, Maldives, Nepal, Pakistan, 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 11 bacterial strains of which only eight 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 eight 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 EQA2, 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 azithromycin, cefotaxime, cefotaxime and clavulanic acid, cefoxitin, ceftazidime and clavulanic acid, chloramphenicol, nalidixic acid, sulfamethoxazole and trimethoprim (*K. pneumoniae*); amikacin, cefotaxime, cefotaxime and clavulanic acid, cefoxitin, ceftazidime, ceftazidime and clavulanic acid, colistin, ertapenem, imipenem, sulfamethoxazole and tigecycline (*Shigella*); cefotaxime, and levofloxacin (*Acinetobacter*); and cefoxitin, chloramphenicol, erythromycin, kanamycin, mupirocin, quinupristin/dalfopristin, streptomycin, sulfamethoxazole, tiamulin and trimethoprim (*S. aureus*). Expected MIC values (Appendix 2) of the selected strains for this EQA were further confirmed by NIH (*K. pneumoniae*, *Shigella*, *Acinetobacter* and *S. aureus*) and CUVET (*K. pneumoniae*, *Shigella* and *S. aureus*).

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 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 3) were retrieved from Clinical and Laboratory Standards Institute (CLSI) in document M100-31st Ed. [3], tables 4A-1 and 5A-1, and from EUCAST in document "Routine and extended internal quality control for MIC determination and disk diffusion" [4].

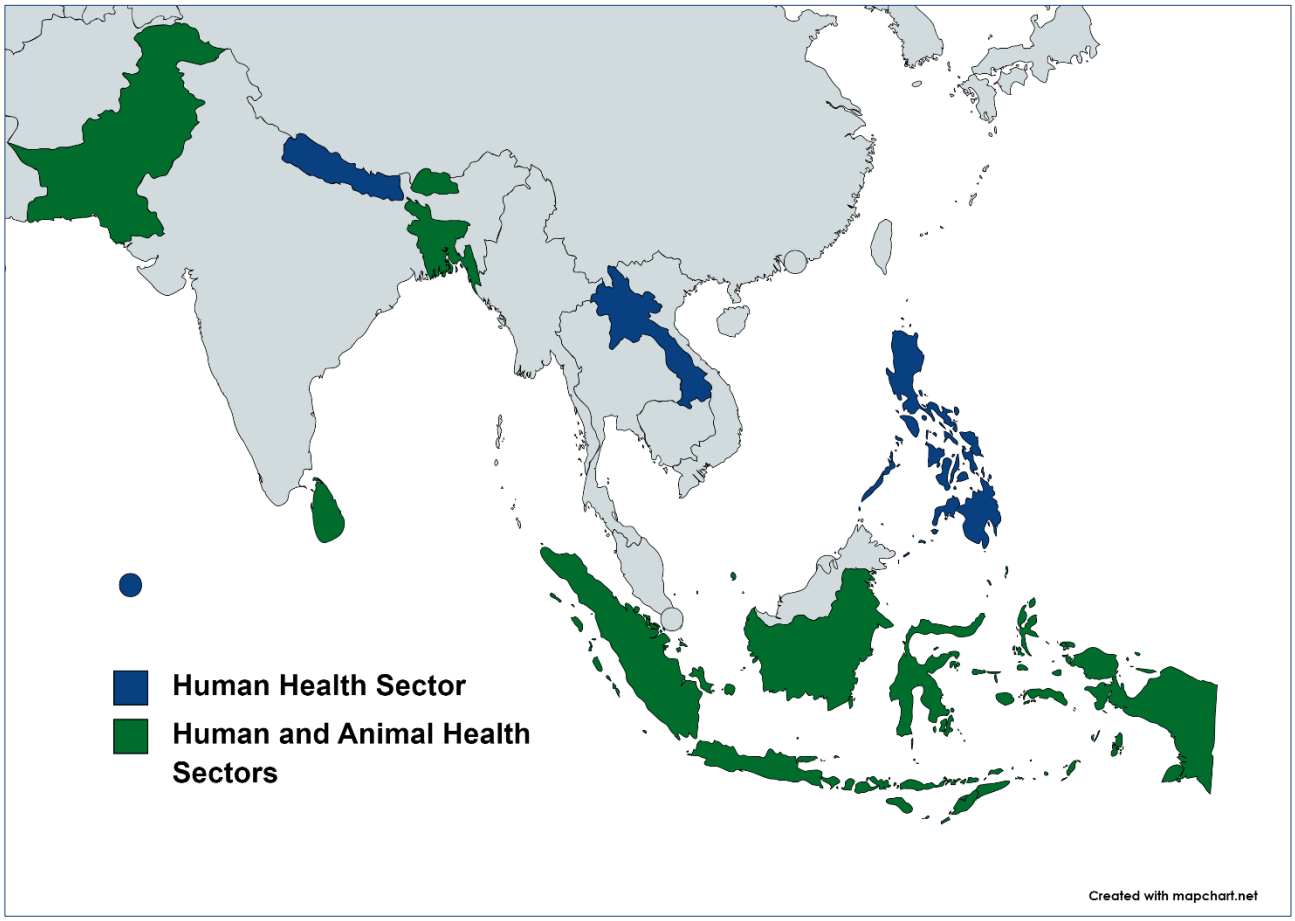

Figure 1: Countries participating in the second EQA of the EQAsia 2021 project on antimicrobial susceptibility testing. Color indicates sector affiliation of the participating laboratory as Human Health laboratory (blue) or both Human and Animal Health laboratories (green).

2.3 Antimicrobials

The antimicrobials recommended for AST in this trial for all four organisms are listed in the EQA 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.

Table 1. Panel of antimicrobials for antimicrobial susceptibility testing included in EQAsia EQA2 2021. For the antimicrobials in grey, no interpretative criteria were available and/or scored in the informatics module.

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

The reference values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current epidemiological cut-off values developed by EUCAST [5]. When not available, CLSI zone diameter and MIC breakpoint values were used instead [3]. Cefotaxime/ clavulanic acid and ceftazidime/ clavulanic acid results 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, also included in the EQA protocol (Appendix 1).

Participants were encouraged to test as many as possible of the antimicrobials listed.

2.4 Distribution

The bacterial strains were dispatched as lyophilized strains in August 2021 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, 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/susceptible (R/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

2.6.1 Adjusted data

Data analysis revealed several instances of misinterpretation of results. Participating laboratories were recommended to interpret the obtained results using the tables provided in the EQA protocol (Appendix 1). Due to misunderstanding or lack of clarification, several laboratories followed the guidelines routinely used in their work. This resulted in different categorisation as resistant or susceptible for each strain/antimicrobial combination, despite identical MIC/Inhibition Zone Diameter values. Such mistakes do not necessarily indicate a poor laboratory performance. The participating laboratories were then invited to re-enter the informatics module and re-interpret their submitted results.

To guarantee that all submitted data was interpreted according to the EQAsia guidelines, the data retrieved from the informatics module was again revised and, when necessary, adjusted: supplementary MIC/ Inhibition Zone Diameter values reported by the participants were used for adjusting the interpretation (R/S) in accordance to the EQAsia interpretation tables. Adjusting the data allowed for an analysis of the submitted results, which more accurately reflects the laboratories' analytical performance.

In adition, antimicrobial susceptibility testing of some of the reference strains revealed a number of incorrect results. These deviations (results outside the acceptance interval) were caused by the method used for MIC determination. This issue was also verified on EQA1 and reported. Briefly, MIC determination by broth microdilution often tests 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' reported an MIC ≤ 1. The informatics module scores such result as '0' (incorrect). We are aware, however, that this is a method limitation and the laboratories cannot test for lower antimicrobial concentrations. For these specific occurrences, the score was changed to '1', as the reported values are not necessarily incorrect. **Table 2** summarizes all the situations where this change was applied.

2.6.2 Omitted data

Data analysis exposed a high percentage of incorrect results for some strain/antimicrobial combinations, likely caused by a "breakpoint issue" and/or a "MIC not possible to interpret issue":

- A "breakpoint issue" is defined as a case where the expected MIC value is equal to the breakpoint (or close to), and participants that obtained a MIC value one dilution step above/below or a Zone Diameter 3mm above/below the breakpoint value obtained an interpretation different from the

expected. As this is a method variation, such results should not be considered incorrect;

- A "MIC not possible to interpret issue" is defined as a situation where the expected MIC value and breakpoint are below the antimicrobial range tested by a laboratory. In this case, the laboratory cannot interpret the result as resistant or susceptible, as both interpretations could be possible. For example, if the expected MIC for ertapenem is 0.12 and ECOFF ≥ 0.06, the strain is categorized as resistant. However, if the result reported by the laboratory is MIC ≤ 0.50, the result cannot be interpreted.

After examining each individual strain/antimicrobial combination with a great percentage of incorrect results, the EQAsia Consortium agreed to omit the following strain/antimicrobial combinations from the general analysis, as these test results were not considered representative of the laboratories' capacity for performing AST:

- *Klebsiella pneumoniae* trial – Kp EQAsia 21.2/ETP, Kp EQAsia 21.3/SMX, Kp EQAsia 21.11/IMI and Kp EQAsia 21.11/NAL;
- *Shigella* trial – Shi EQAsia 21.3/CIP, Shi EQAsia 21.6/CIP, Shi EQAsia 21.9/CIP, Shi EQAsia 21.11/FEP (only MIC results) and Shi EQAsia 21.11/TAZ;
- *Staphylococcus aureus* trial – Sa EQAsia 21.8/CIP.

In addition, the data reported by laboratories #02, #05, #07 and #35 regarding strain Aci EQAsia 21.4 did not seem to correspond to the expected phenotypic profile (very resistant strain compared to what would be expected). Such deviating results suggest that the strain tested by the laboratories was likely a different strain (perhaps one of the non-target strains). For that reason, the data submitted by laboratories #02, #05, #07 and #35 regarding strain Aci EQAsia 21.4 was not analysed, and thus not presented in the report.

Finally, strain Sa EQAsia 21.4, sent as a non-*S. aureus* strain was misidentified as *S. aureus* by most of the laboratories. This strain was in fact a *S. argenteus*, a species genetically closely related to *S. aureus*, which could not be distinguished by phenotypic testing and properly identified. For that reason, strain Sa EQAsia 21.4 was excluded from the analysis of results.

Upon omisson of the abovementioned results, the laboratories' performance and deviations were recalculated and presented in this report.

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 method applied.

***E. coli* ATCC 25922**			
Antimicrobial	**MIC Quality Control Range**	**MIC reported by the labs**	**Score**
Cefepime	0.016-0.12	≤ 1	Changed to '1'
Ceftazidime	0.06-0.5	≤ 1	Changed to '1'
Ciprofloxacin	0.004-0.016	≤ 0.25 or ≤ 0.125	Changed to '1'
Ertapenem	0.004-0.016	≤ 0.5 or ≤ 0.25	Changed to '1'
Meropenem	0.008-0.06	≤ 0.25 or ≤ 0.125	Changed to '1'
Tigecycline	0.03-0.25	≤ 0.5	Changed to '1'
***S. aureus* ATCC 29213**			
Antimicrobial	**MIC Quality Control Range**	**MIC reported by the labs**	**Score**
Rifampicin	0.004-0.016	≤ 0.5 or ≤ 0.25 or ≤ 0.03	Changed to '1'

3. Results – Human Health Laboratories

3.1 Overall participation

Among the Human Health laboratories, 14 laboratories submitted results for *K. pneumoniae, Acinetobacter* spp., and *S. aureus* trials, and 12 laboratories submitted results for the *Shigella* spp. trial. The methodologies applied by the laboratories varied greatly and are summarized in **Figure 2**. Some laboratories opted for only one method, whereas others performed AST using different methodologies and reported both Inhibition Zone Diameters and MIC depending on the antimicrobial drug tested.

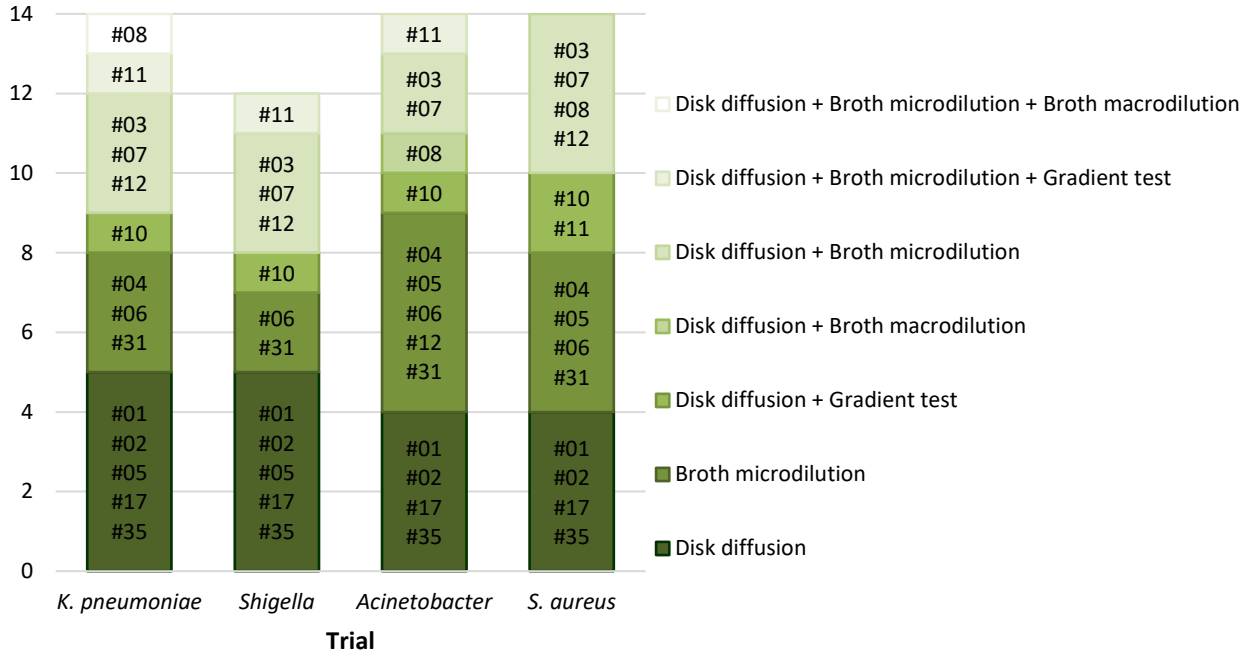

Figure 2. Methodologies applied by the laboratories in each of the trials.

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R') 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 antimicrobials (**Table 1**). The highest number of total ASTs was reported for *K. pneumoniae* in comparison to *Shigella, Acinetobacter* and *S. aureus* (**Table 3**). For the Gram negative bacteria, the last resort antibiotics such as colistin and tigecycline were tested only by few laboratories (**Table 3**). In contrast, amikacin, ampicillin, cefepime, ciprofloxacin, gentamicin and meropenem were tested by most laboratories for the *K. pneumoniae* trial, whereas only ampicillin and gentamicin were tested by most laboratories for the *Shigella* trial. Ceftazidime, ciprofloxacin, gentamicin and meropenem were tested by most laboratories for the *Acinetobacter* trial, as well as erythromycin and gentamicin were tested by most laboratories for the *S. aureus* trial (**Table 2**).

Table 3. Total of ASTs performed for each antimicrobial and in total for each of the trials by HH laboratories.

Antimi-crobial	ASTs in total			
	Kp	Shi	Aci	Sa
AMK	110 (7.2%)	87 (7.0%)	90 (8.7%)	--
AMP	112 (7.3%)	90 (7.3%)	--	--
AZI	66 (4.3%)	62 (5.0%)	--	--
FEP	106 (6.9%)	79 (6.4%)	--	--
FOT	70 (4.6%)	57 (4.6%)	59 (5.7%)	--
FOX	75 (4.9%)	71 (5.7%)	--	77 (7.0%)
TAZ	99 (6.5%)	67 (5.4%)	101 (9.7%)	--
CHL	69 (4.5%)	68 (5.5%)	--	82 (7.5%)
CIP	111 (7.2%)	56 (4.5%)	101 (9.7%)	86 (7.8%)
CLI	--	--	--	77 (7.0%)
COL	48 (3.1%)	28 (2.3%)	47 (4.5%)	--
DOR	--	--	37 (3.6%)	--
ETP	92 (6.0%)	78 (6.3%)	--	--
ERY	--	--	--	99 (9.0%)
FUS	--	--	--	15 (1.4%)
GEN	110 (7.2%)	91 (7.3%)	102 (9.8%)	99 (9.0%)
IMI	82 (5.3%)	80 (6.4%)	88 (8.5%)	--
KAN	--	--	--	25 (2.3%)
LEVO	--	--	74 (7.1%)	--
LZD	--	--	--	86 (7.8%)
MERO	109 (7.1%)	84 (6.8%)	101 (9.7%)	--
MIN	--	--	44 (4.2%)	--
MUP	--	--	--	8 (0.7%)
NAL	57 (3.7%)	58 (4.7%)	--	--
PEN	--	--	--	88 (8.0%)
P/T4	--	--	94 (9.1%)	--
SYN	--	--	--	53 (4.8%)
RIF	--	--	--	65 (5.9%)
STR	--	--	--	1 (0.1%)
SMX	22 (1.4%)	8 (0.6%)	--	20 (1.8%)
TET	79 (5.2%)	77 (6.2%)	--	90 (8.2%)
TIA	--	--	--	1 (0.1%)
TGC	60 (3.9%)	53 (4.3%)	40 (3.9%)	--
TOB	--	--	59 (5.7%)	--
TMP	56 (3.7%)	47 (3.8%)	--	53 (4.8%)
VAN	--	--	--	71 (6.5%)
Total	**1533**	**1241**	**1037**	**1096**

Kp, *K. pneumoniae*; Shi, *Shigella*; Aci, *Acinetobacter*; Sa, *S. aureus*

Scattering of missing data or incomplete AST results entries were observed in all four types of bacteria. Nine of the 14 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 #05, #06 and #12 (**Table 4**).

Similarly, more than half of the laboratories selecting *Shigella* (n=7) submitted incomplete results of their own available antimicrobial agents (**Table 5**). The highest number of incomplete results in the *Shigella* trial was seen for laboratory #05 (**Table 5**).

Seven out of 14 laboratories selecting *Acinetobacter* revealed incomplete results of their own available antimicrobial agents (**Table 6**). The highest number of incomplete results in the *Acinetobacter* trial was seen for laboratories #03, #04 and #07 (**Table 6**).

Four out of the 14 laboratories selecting *S. aureus* did not submit complete results of their own available antimicrobial agents (**Table 7**). The highest number of incomplete results in the *S. aureus* trial was seen for laboratory #05 (**Table 7**).

Table 4. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Kp EQASIA 21.2	Kp EQASIA 21.3	Kp EQASIA 21.4	Kp EQASIA 21.7	Kp EQASIA 21.8	Kp EQASIA 21.9	Kp EQASIA 21.10	Kp EQASIA 21.11
#02	--	--	--	--	TAZ	--	TET	--
#03	MERO	FOT	FOT	FOT	FOT	--	FEP; FOT; TAZ; CIP; ETP; MERO	FOT
#05	AMK; FOT	FOT; CHL; IMI; NAL; TMP	FOT; CHL; IMI; NAL; SMX; TMP	FOT; CHL; GEN; IMI; NAL; SMX; TET; TMP	FOT; CHL; MERO; IMI; NAL; SMX; TET; TMP	FEP; CHL; IMI; NAL; SMX; TET; TMP	AMK; FOT; ETP; SMX; TMP	FEP; FOT; CHL; SMX; TMP
#06	FEP; TAZ; TGC	TAZ; ETP; TGC	TAZ; ETP; TGC	FEP; TAZ; ETP; TGC	TAZ	TAZ; ETP; TGC	--	--
#07	AZI; IMI; TMP	--	--	--	TGC	--	--	--
#12	AZI; FOT; FOX; TAZ; CHL; TET	AZI; FOT; FOX; TAZ; CHL; TET	--	--	AZI; FOT; FOX; TAZ; CHL; TET	AZI; FOT; FOX; TAZ; CHL; TET	FEP	AZI; FOT; FOX; TAZ; CHL; TET
#17	--	--	TGC	IMI; TGC	IMI; TGC	IMI; TGC	TGC	--
#31	--	--	--	GEN	--	--	--	TGC
#35	--	NAL	--	--	--	--	--	--

Kp, *K. pneumoniae*

Table 5. Distribution of incomplete or missing data of antimicrobial agents among *Shigella* strains reported by HH laboratories (n=12) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Shi EQASIA 21.1	Shi EQASIA 21.2	Shi EQASIA 21.3	Shi EQASIA 21.5	Shi EQASIA 21.6	Shi EQASIA 21.7	Shi EQASIA 21.9	Shi EQASIA 21.11
#03	FOT	--	FOT	FOT; CIP	--	CIP	FOT; FOX	GEN
#05	AMK; ETP; TGC	AMK: ETP; TGC	AMK; ETP; TGC	AMK; ETP; TGC	CHL; IMI; NAL; TGC	FEP; TAZ; CHL; IMI; NAL; TET	AMK; ETP; TGC	FEP; CHL; IMI; NAL; TGC
#07	AZI	--	--	--	--	--	ETP	--
#10	--	COL; IMI	--	--	--	--		--
#12	TMP	TGC	TMP	TMP	TMP	TMP	TMP	TMP
#31	AMP		--	--	AMP	--	--	--
#35	FOX	FOT; FOX	FOX	FOX	FOX	FOX	FOT	FOX

Shi, *Shigella*; blue shade, strains not tested

Table 6. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter* strains reported by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Aci EQASIA 21.1	Aci EQASIA 21.2	Aci EQASIA 21.3	Aci EQASIA 21.4	Aci EQASIA 21.8	Aci EQASIA 21.9	Aci EQASIA 21.10	Aci EQASIA 21.11
#03	FOT	FOT	FOT	AMK; TAZ; CIP; GEN; MERO; P/T4; TGC	FOT	FOT; TGC	AMK; FOT; TAZ; CIP; GEN; MERO; P/T4; TGC	FOT
#04	--	--	--	COL; IMI; LEVO; P/T4; TGC	--	--	--	LEVO; MERO
#05	--	--	--		TAZ	--	--	CIP
#06	--	--	--		IMI	--	--	--
#07	--	--	--		--	--	TAZ; CIP; GEN; MERO; P/T4; TGC	--
#12	--	AMK	AMK	--	AMK	AMK	AMK	AMK
#17	COL	COL	COL	FOT	COL	COL	FOT; COL	COL

Aci, *Acinetobacter*; blue shade, strains not tested or ommited from analysis

Table 7. Distribution of incomplete or missing data of antimicrobial agents among *S. aureus* strains reported by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Sa EQASIA 21.1	Sa EQASIA 21.2	Sa EQASIA 21.3	Sa EQASIA 21.5	Sa EQASIA 21.6	Sa EQASIA 21.7	Sa EQASIA 21.8	Sa EQASIA 21.9
#03	--	--	--	--	CLI	--	--	--
#04	STR	STR	STR	--	STR	STR	STR	STR
#05	TIA	KAN; RIF; TIA	KAN; RIF; TIA	KAN; RIF; TIA	KAN; RIF; TET	KAN; RIF; TIA	KAN; RIF; TIA	KAN; RIF; TIA
#07	--	--	--	--	--	--	--	PEN

Sa, *S. aureus*

3.2 *Klebsiella pneumoniae* trial

Fourteen laboratories from 10 countries uploaded results for the *K. pneumoniae* trial.

3.2.1 Bacterial identification

All 14 participating laboratories submitted results for bacterial identification (**Table 8**). All of them correctly identified the eight *K. pneumoniae* strains among the 11 test strains provided. However, laboratory #05 did not identify properly the three non-*K. pneumoniae* strains Kp EQASIA 21.1, Kp EQASIA 21.5 and Kp EQASIA 21.6. This misidentification suggests that bacterial

identification was not performed by laboratory #05 and that all 11 strains were simply reported as *K. pneumoniae.*

Table 8. Bacterial identification of each of the 11 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 21.1	Non-*K. pneumoniae* (*Enterobacter sakazakii*)	13/14
Kp EQASIA 21.2	*K. pneumoniae*	14/14
Kp EQASIA 21.3	*K. pneumoniae*	14/14
Kp EQASIA 21.4	*K. pneumoniae*	14/14
Kp EQASIA 21.5	Non-*K. pneumoniae* (*Citrobacter freundii*)	13/14
Kp EQASIA 21.6	Non-*K. pneumoniae* (*Shigella boydii*)	13/14
Kp EQASIA 21.7	*K. pneumoniae*	14/14
Kp EQASIA 21.8	*K. pneumoniae*	14/14
Kp EQASIA 21.9	*K. pneumoniae*	14/14
Kp EQASIA 21.10	*K. pneumoniae*	14/14
Kp EQASIA 21.11	*K. pneumoniae*	14/14

Kp, *K. pneumoniae*

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/S) ranged from 93.3% (strain Kp EQASIA 21.9) to 98.3% (strain Kp EQASIA 21.11) for each strain (**Table 9**). All strains revealed deviations below 10%.

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were tigecycline (28.3%), gentamicin (18.2%), sulfamethoxazole (18.2%), azithromycin (7.6%), trimethoprim (7.1%) and colistin (6.3%), whereas ampicillin and ciprofloxacin revealed no deviation from the expected results (**Figure 3**). Of the 19 tested and scored antimicrobial agents, three revealed to exceed a 10% deviation. This is likely due to, for example, the uncommon testing of sulfamethoxazole and tigecycline, which are not recommended by CLSI, the guidelines followed by most of the HH laboratories. In addition, gentamicin is rarely used for treatment, and colistin testing requires standard broth microdilution, which may require more experience for some of the laboratories.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed for only seven participants (**Figure 4**). In average, the deviation was 4.7% (ranging from 0.0 to 11.2%). As the acceptance level was set to 5% deviation, seven laboratories (#01, #07, #04, #12, #02, #31 and #03) did not perform within the expected range for the *K. pneumoniae* trial.

Table 9. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 14 HH laboratories for the *K. pneumoniae* trial.

Strain	AST in total	% Correct
Kp EQASIA 21.2	183	94.5
Kp EQASIA 21.3	192	95.3
Kp EQASIA 21.4	201	97.5
Kp EQASIA 21.7	196	93.9
Kp EQASIA 21.8	192	93.8
Kp EQASIA 21.9	194	93.3
Kp EQASIA 21.10	198	95.5
Kp EQASIA 21.11	177	98.3

Kp, *K. pneumoniae*

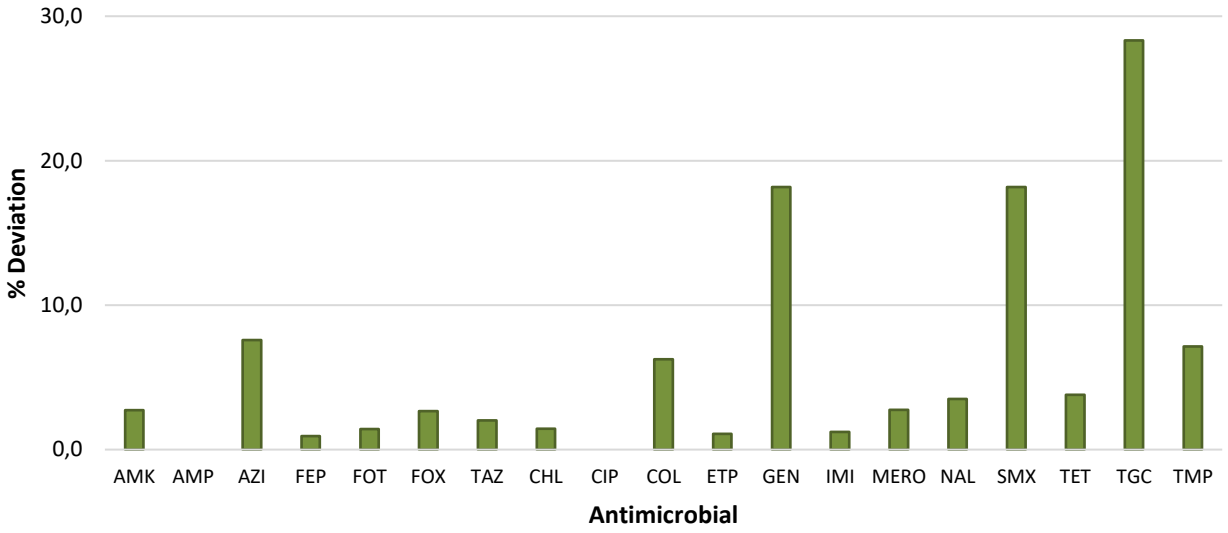

Figure 3. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=14) participating in the 2nd EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

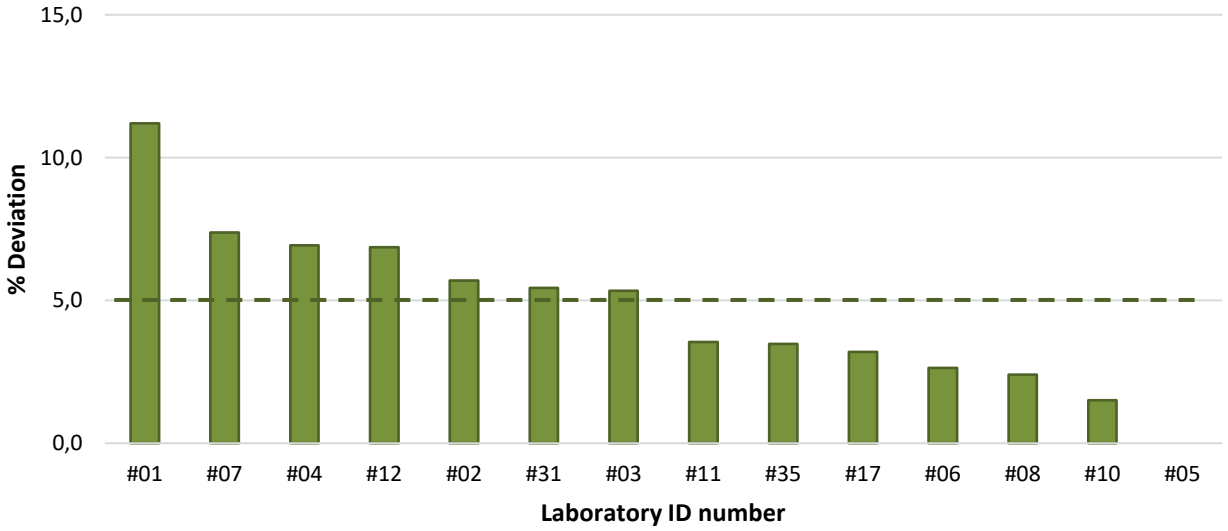

Figure 4. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by HH laboratories (n=14) participating in the 2nd EQA in the EQAsia project. Results are categorized by laboratory ID number.

3.2.3 β-lactamase producing *K. pneumoniae*

Eleven out of the 14 participating laboratories uploaded results for this component of the *K. pneumoniae* trial. Yet, for strains Kp EQASIA 21.2 and Kp EQASIA 21.10, only nine laboratories tested for ESBL-production, and for strains Kp EQASIA 21.3, Kp EQASIA 21.8, Kp EQASIA 21.9 and Kp EQASIA 21.11 only 10 laboratories reported results (**Table 10**). Of the 11 laboratories, only laboratories #04, #06, #10 and #11 correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the eight *K. pneumoniae* strains. The highest deviation from the expected results was obtained for strains Kp EQASIA 21.8 and EQASIA 21.9 (**Table 10**). Four out of 10 laboratories wrongly identified this carbapenemase-producing *K. pneumoniae* strain as an ESBL- or ESBL + AmpC-producer, even though three of the laboratories found both strains resistant to meropenem.

Table 10. 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 11 Human Health laboratories.

Strain code		Kp EQASIA 21.2	Kp EQASIA 21.3	Kp EQASIA 21.4	Kp EQASIA 21.7	Kp EQASIA 21.8	Kp EQASIA 21.9	Kp EQASIA 21.10	Kp EQASIA 21.11
Expected results		ESBL	Carbapene-mase	Carbapene-mase	Carbapene-mase	Carbapene-mase	Carbapene-mase	Susceptible	Carbapene-mase
Obtained results (n/N)	ESBL	**8/9 (88.9%)**	--	--	--	2/10 (20.0%)	2/10 (20.0%)	1/9 (11.1%)	1/10 (10.0%)
	ESBL + AmpC	--	--	--	--	2/10 (20.0%)	2/10 (20.0%)	--	--
	Carbapenemase	--	**9/10 (90.0%)**	**10/11 (90.9%)**	**10/11 (90.9%)**	**6/10 (60.0%)**	**6/10 (60.0%)**	--	**8/10 (80.0%)**
	Other	1/9 (11.1%)	--	--	--	--	--	--	1/10 (10.0%)
	Susceptible*	--	1/10 (10.0%)	1/11 (9.1%)	1/11 (9.1%)	--	--	**8/9 (88.9%)**	--

Kp, *Klebsiella 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 to all participating laboratories to be used as reference strains for both *K. pneumoniae* and *Shigella* trials. Antimicrobial susceptibility test results for the quality control strains were evaluated separately for each of the trials.

Among the 14 participating laboratories, 12 submitted results for the reference strain *E. coli* ATCC 25922 and only six performed colistin testing and reported results for *E. coli* NCTC 13846. The laboratories used different methodologies for testing the reference strain *E. coli* ATCC 25922: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, broth macro or microdilution (**Table 11**). For testing *E. coli* NCTC 13846, MIC was determined by standard method either broth macro or microdilution. One laboratory (#10) tested colistin by gradient test, which is not the recommended standard method due to colistin's large molecule. This result was therefore considered incorrect although the obtained interpretation value was the same as the expected interpretation value (**Table 11**, *).

Table 11. 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	0/5	--	0/4	**0/9**
AMP	2/5	0/1	1/5	**3/11**
FEP	2/5	0/1	0/4	**2/10**
FOT	2/5	0/1	--	**2/6**
FOX	1/5	0/1	--	**1/6**
TAZ	1/7	0/1	0/3	**1/11**
CHL	1/7	-	-	**1/7**
CIP	1/6	0/1	1/4	**2/11**
COL	--	1/1*	0/5	**1/6**
ETP	0/4	0/1	0/4	**0/9**
GEN	1/5	0/1	0/5	**1/11**
IMI	0/5	0/1	0/3	**0/9**
MERO	0/6	0/1	0/4	**0/11**
NAL	0/6	--	1/2	**1/8**
SMX	2/2	--	--	**2/2**
TET	0/6	0/1	--	**0/7**
TGC	0/1	--	1/5	**1/6**
TMP	0/3	--	0/1	**0/4**

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

The highest proportion of test results outside of the expected range were observed for sulfamethoxazole (2 out of 2), cefotaxime (2 out of 6), and ampicillin (3 out of 11) (**Table 11**). Moreover, the majority of the inaccurate results seemed to be caused by disk diffusion.

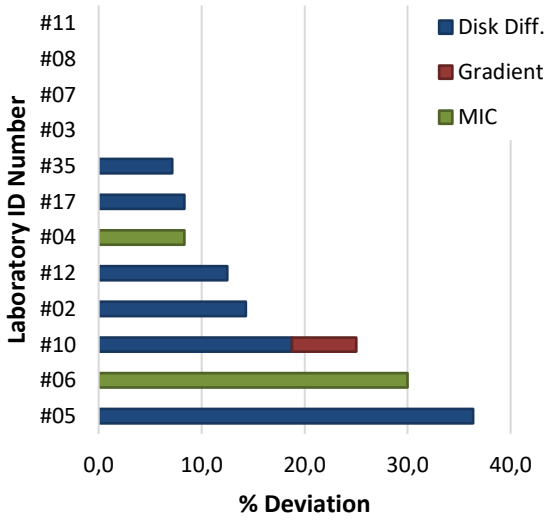

Figure 5. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *K. pneumoniae* trial by the HH laboratories.

Considering the deviations, the laboratories' performance seems to be independent of the methodology applied for AST of the quality control strains (**Figure 5).** Laboratories #03, #07, #08 and #11 presented no deviation. While laboratories #03 and #07 applied broth microdilution, laboratory #08 used mostly disk diffusion (exception for broth macrodilution for colistin testing) and laboratory #11 used a mixture of disk diffusion and gradient test (exception for broth microdilution for colistin testing). The remaining eight laboratories presented deviations that ranged from 7.1% to 36.4% (**Figure 5**). Laboratories #35, #17 and #04 had only one deviation each, whereas laboratories #12 and #02 presented two deviations. Laboratory #10 obtained 4 deviations, being one of them due to testing colistin by gradient test. Laboratories #06 and #05 presented 3 and 4 deviations, respectively. As mentioned above, most of the deviations were seen when disk diffusion methodology was applied. For those inaccurate results, the Inhibition Zone Diameters reported were usually below the expected range.

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

3.3 *Shigella* trial

Twelve laboratories from 10 countries uploaded results for the *Shigella* trial.

3.3.1 Bacterial identification

All 12 laboratories participating in the *Shigella* trial submitted results for bacterial identification. Among these, eight laboratories correctly identified the eight *Shigella* strains and the three non-*Shigella* (**Table 12**). The *Shigella* strains Shi EQASIA 21.2, Shi EQASIA 21.9 and Shi EQASIA 21.11 were misidentified as non-*Shigella* by laboratory #31, laboratories #06 and #10, and laboratory #06, respectively. Inversely, the non-*Shigella* strains Shi EQASIA 21.4 and Shi EQASIA 21.10 were reported by laboratories #05 and #10, respectively, as *Shigella*.

Table 12. Bacterial identification of each of the 11 test strains provided related to the *Shigella* trial. Number of correct results out of the total of HH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Shi EQASIA 21.1	*S. sonnei*	12/12
Shi EQASIA 21.2	*S. sonnei*	11/12
Shi EQASIA 21.3	*S. flexneri*	12/12
Shi EQASIA 21.4	Non-*Shigella* (*K. pneumoniae*)	11/12
Shi EQASIA 21.5	*S. flexneri*	12/12
Shi EQASIA 21.6	*S. sonnei*	12/12
Shi EQASIA 21.7	*S. flexneri*	12/12
Shi EQASIA 21.8	Non-*Shigella* (*Salmonella*)	12/12
Shi EQASIA 21.9	*S. flexneri*	10/12
Shi EQASIA 21.10	Non-*Shigella* (*E. coli*)	11/12
Shi EQASIA 21.11	*S. flexneri*	11/12

Shi, *Shigella*

3.3.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/S) ranged from 89.3% (strain Shi EQASIA 21.5) to 96.1% (strain Shi EQASIA 21.9) for each strain. Deviations among the *Shigella* strains were below 5%, except for Shi EQASIA 21.5, where the deviation was as high as 10.7% (**Table 13**).

Antimicrobial-based analysis

The antimicrobials that resulted in highest percentage of deviations were sulfamethoxazole (37.5%), colistin (21.4%) and trimethoprim (19.1%), followed by gentamicin (14.3%) and cefepime (12.7%) (**Figure 6**). The results of two antimicrobial agents (imipenem and meropenem) revealed no deviation from the expected results.

Table 13. Total number of AST performed and percentage of results in agreement with expected interpretive results (R/S). Results are from 12 HH laboratories for the *Shigella* trial.

Strain	AST in total	% Correct
Shi EQASIA 21.1	167	92.8
Shi EQASIA 21.2	155	94.8
Shi EQASIA 21.3	157	94.3
Shi EQASIA 21.5	168	89.3
Shi EQASIA 21.6	156	92.9
Shi EQASIA 21.7	166	94.6
Shi EQASIA 21.9	129	96.1
Shi EQASIA 21.11	143	93.0

Shi, *Shigella*

Laboratory-based analysis

For the *Shigella* trial, six out of the 12 HH laboratories presented a deviation above the acceptance level of 5% (laboratories #10, #01, #02, #31, #35 and #12). The average deviation was 6.2% (ranging from 1.9 to 17.5%) (**Figure 7**).

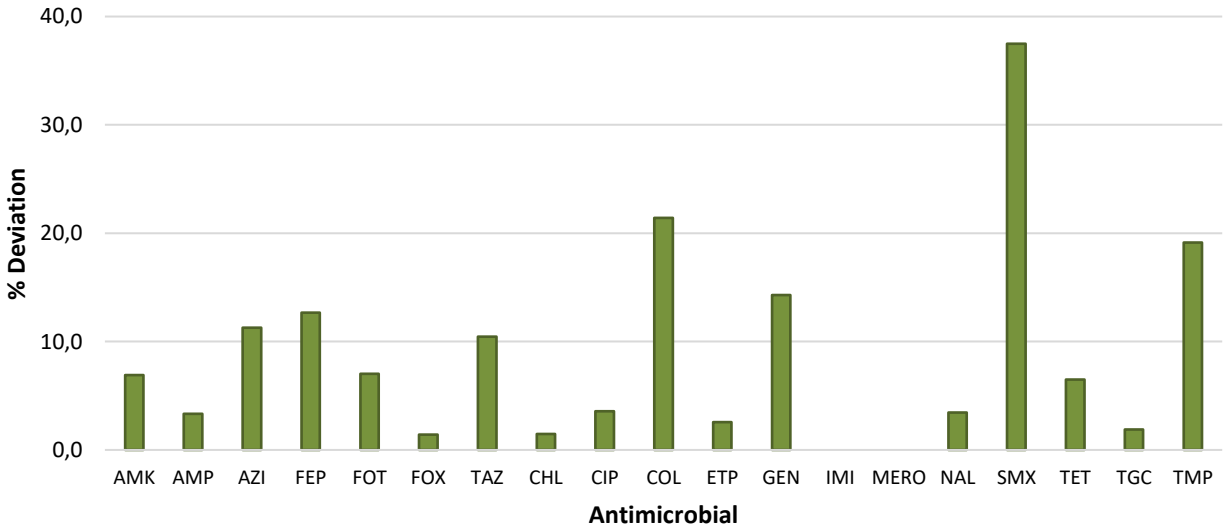

Figure 6. Percentage of deviation in the AST interpretation (R/S) among *Shigella* strains by HH laboratories (n=12) participating in the 2nd EQA of the EQAsia project. Results are categorized by antimicrobial agent.

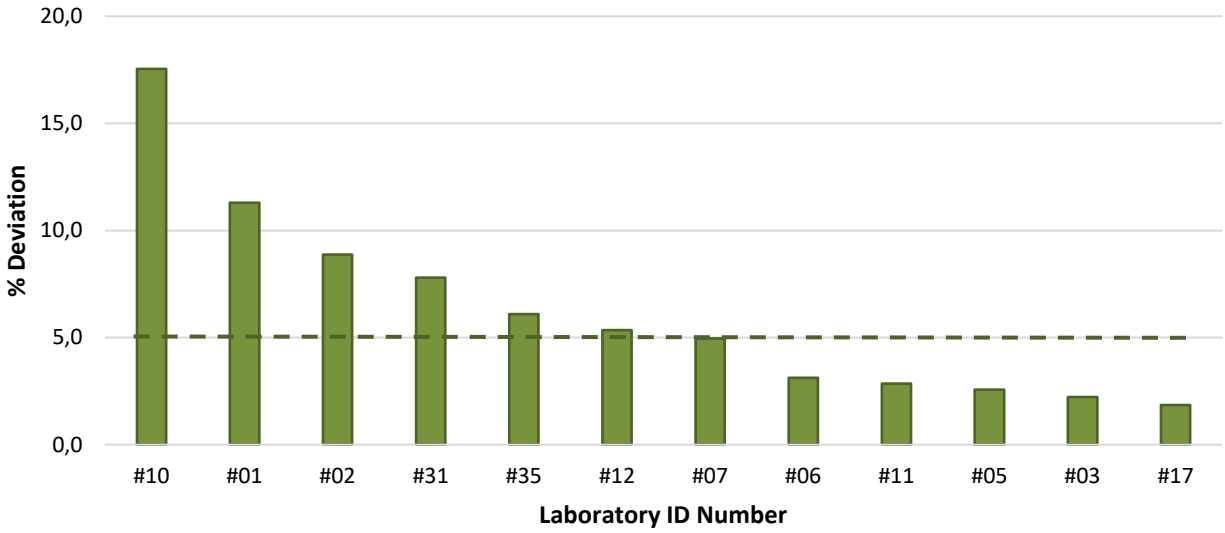

Figure 7. Percentage of deviation in the AST interpretation (R/S) among *Shigella* strains by HH laboratories (n=12) participating in the 2nd EQA of the EQAsia project. Results are categorized by laboratory ID number.

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

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

Among the 12 participating laboratories in the trial, 10 laboratories submitted results for the reference strain *E. coli* ATCC 25922 and only four performed colistin testing and reported results for *E. coli* NCTC 13846. Aligned with the *K. pneumoniae* trial, different methodologies were applied for testing the quality control strain *E. coli* ATCC 25922 (disk diffusion, gradient test and broth microdilution) (**Table 14**). For testing *E. coli* NCTC 13846, MIC was determined by either broth macro or microdilution. As for the *K. pneumoniae* trial, one laboratory (#10) tested colistin by gradient test, which is not the recommended standard method due to colistin's large molecule. This result was therefore considered incorrect although the obtained interpretation value was the same as the expected interpretation value (**Table 14**, *).

Table 14. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 1386 (blue shade) in the *Shigella* 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**
AMK	0/5	--	0/3	**0/8**
AMP	2/5	0/1	1/4	**3/10**
FEP	2/5	0/1	0/3	**2/9**
FOT	3/4	0/1	--	**3/5**
FOX	1/6	--	--	**1/6**
TAZ	1/6	0/1	0/2	**1/9**
CHL	1/5	0/1	--	**1/6**
CIP	1/5	0/1	1/3	**2/9**
COL	--	1/1*	0/3	**1/4**
ETP	0/3	0/1	0/2	**0/6**
GEN	1/6	--	0/4	**1/10**
IMI	0/4	0/1	0/2	**0/7**
MERO	0/5	0/1	0/3	**0/9**
NAL	0/4	--	1/2	**1/6**
SMX	--	--	--	**--**
TET	0/6	0/1	--	**0/7**
TGC	0/1	--	0/4	**0/5**
TMP	0/3	--	--	**0/3**

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

The highest proportion of test results outside of the expected range was observed for cefotaxime (3 out of 5) and ampicillin (3 out of 10) (**Table 14**). As seen for the *K. pneumoniae* trial, most of the deviations occurred when the disk diffusion methodology was applied.

Regarding the laboratories' performance, the observations are similar to the ones drawn for the *K. pneumoniae* trial (**Figure 8).** Once again, laboratories #03, #07 and #011 had no deviations, as well as laboratory #17. In reverse, the other six laboratories had deviations ranging from 12.5 to 37.5% (**Figure 8**). Laboratories #12, #02 and #35 presented two deviations each, and for all three, the deviations occurred when disk diffusion was applied. Laboratory #10 obtained 4 deviations, one of them caused by choosing gradient test for colistin testing. Laboratories #06 and #05 presented 3 deviations each. Laboratory #06 applied broth microdilution for testing the strains and all the deviations were due to MIC values way above the acceptance interval. All remaining deviations were seen when disk diffusion methodology was applied, where all the reported Inhibition Zone Diameters were below the expected range.

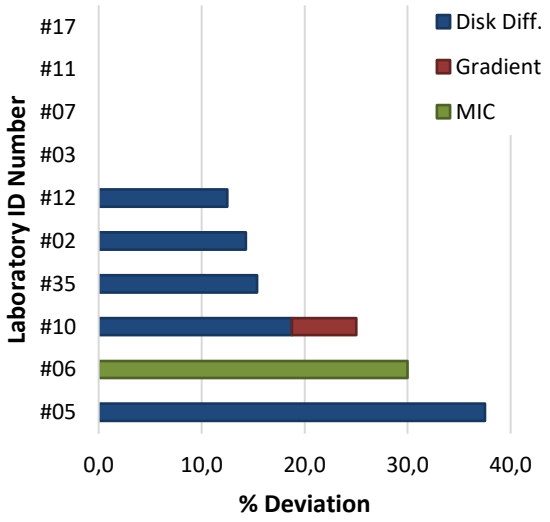

Figure 8. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *Shigella* trial by the HH laboratories.

3.4 *Acinetobacter* trial

A total of 14 laboratories from 10 countries uploaded results for the *Acinetobacter* trial.

3.4.1 Bacterial identification

All 14 participating laboratories submitted results for bacterial identification (**Table 15**). Eleven out of the 14 laboratories correctly identified the eight *Acinetobacter* strains and the three non-*Acinetobacter*, meaning that a few bacterial identification mistakes were committed: the non-*Acinetobacter* strain Aci EQASIA 21.7 was identified as *Acinetobacter* by laboratory #31, whereas strain Aci EQASIA 21.3 was misidentified as a non-*Acinetobacter* by laboratory #01. Two laboratories have also misidentified *A. lowffii* (Aci EQASIA 21.4), which is a species with high variation of phenotypic characteristics.

Table 15. Bacterial identification of each of the 11 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 21.1	*A. baumannii*	14/14
Aci EQASIA 21.2	*A. baumannii*	14/14
Aci EQASIA 21.3	*A. baumannii*	13/14
Aci EQASIA 21.4	*A. lowffii*	12/14
Aci EQASIA 21.5	Non-*Acinetobacter* (*P. aeruginosa*)	14/14
Aci EQASIA 21.6	Non-*Acinetobacter* (*P. aeruginosa*)	14/14
Aci EQASIA 21.7	Non-*Acinetobacter* (*P. aeruginosa*)	13/14
Aci EQASIA 21.8	*A. pittii*	14/14
Aci EQASIA 21.9	*A. baumannii*	14/14
Aci EQASIA 21.10	*A. radioresistens*	14/14
Aci EQASIA 21.11	*A.baumannii*	14/14

Aci, *Acinetobacter*

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/S) ranged from 88.7% (strain Aci EQASIA 21.2) to 100.0% (strain Aci EQASIA 21.10) for each strain (**Table 16**). The results from only one strain revealed more than 10% deviation (Aci EQASIA 21.2) and half of the strains had a deviation below or equal to 5% (**Table 16**).

Table 16. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 14 HH laboratories for the *Acinetobacter* trial.

Strain	AST in total	% Correct
Aci EQASIA 21.1	143	92.3
Aci EQASIA 21.2	142	88.7
Aci EQASIA 21.3	130	93.1
Aci EQASIA 21.4	74	98.6
Aci EQASIA 21.8	140	91.4
Aci EQASIA 21.9	141	95.0
Aci EQASIA 21.10	128	100.0
Aci EQASIA 21.11	139	98.6

Aci, *Acinetobacter*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were cefotaxime (18.6%), amikacin (13.3%), colistin (10.6%) and tigecycline (10.0%), whereas ciprofloxacin, doripenem and levofloxacin revealed no deviation from the expected results (**Figure 9**). Of the 14 tested and scored antimicrobial agents, three revealed to exceed a 10% deviation. In the case of tigecyline and colistin, this may be caused by the same reasons described in the *K. pneumoniae* trial.

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for half of the participants (#10, #05, #02, #03, #01, #35 and #04) (**Figure 10**), meaning that these seven laboratories did not perform within the expected range for the *Acinetobacter* trial. In average, the deviation was 6.0% (ranging from 1.2 to 15.0%).

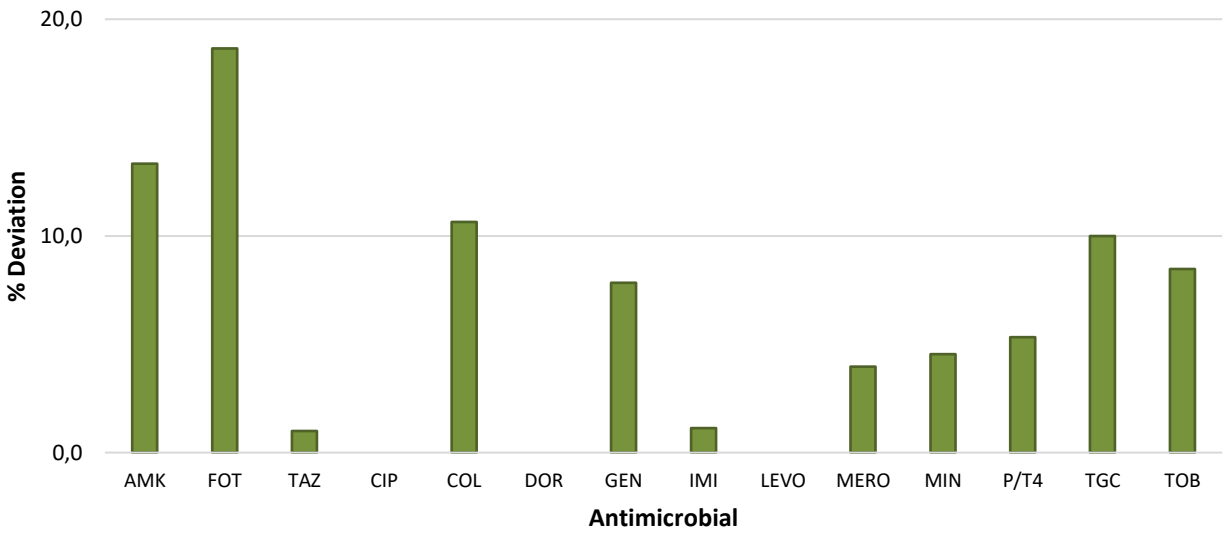

Figure 9. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter* strains by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

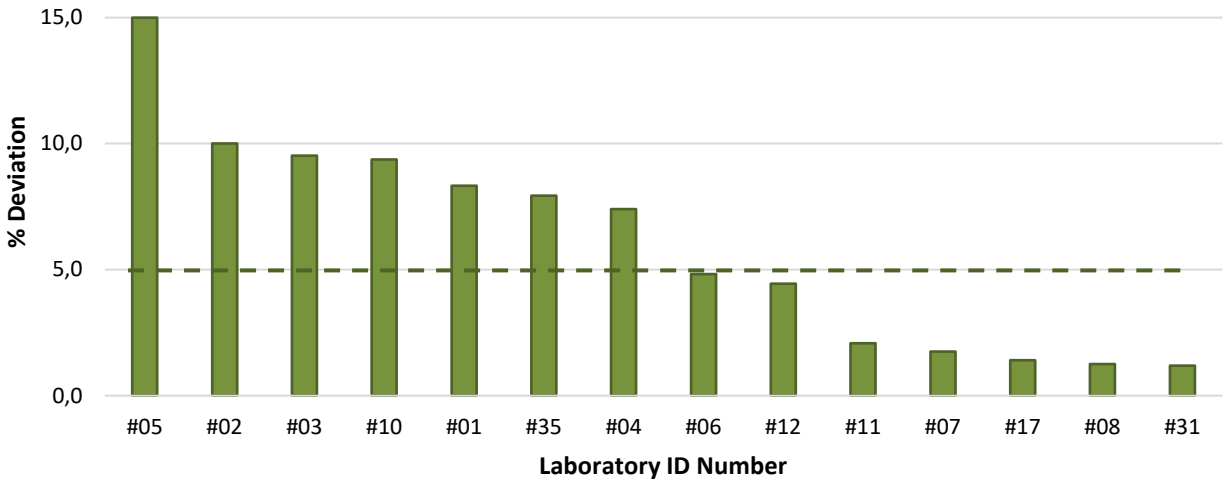

Figure 10. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter* strains by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project. Results are categorized by laboratory ID number.

3.4.3 Quality control strain *P. aeruginosa* ATCC 27853

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

Among the 14 particopating laboratories, 13 submitted results of reference strain. The participants used different methodologies for testing the reference strain: Inhibition Zone Diameter was determined by disk diffusion, and MIC was determined by either gradient test, broth macro or microdilution (**Table 17**). One laboratory (#10) tested colistin by gradient test, which is not the recommended standard method due to its large molecule. This result was therefore considered incorrect although the obtained interpretation value was the same as the expected interpretation value (**Table 17**, *).

The highest proportion of test results outside of the expected range were observed for cefotaxime (2 out of 7) and ceftazidime (2 out of 13) (**Table 17**). The majority of the inaccurate results seemed to be caused by disk diffusion, whereas no deviations were seen when MIC was determined by broth dilution.

Table 17. 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	0/7	--	0/5	**0/12**
FOT	2/6	0/1	--	**2/7**
TAZ	2/6	0/1	0/6	**2/13**
CIP	0/6	0/1	0/6	**0/13**
COL	--	1/1*	0/5	**1/6**
DOR	0/2	--	0/2	**0/4**
GEN	0/7	--	0/6	**0/13**
IMI	1/7	0/1	0/3	**1/11**
LEVO	0/5	0/1	0/3	**0/9**
MERO	1/5	0/1	0/6	**1/12**
P/T4	0/7	--	0/4	**0/11**
TGC	--	--	--	**--**
TOB	0/4	0/1	0/2	**0/7**

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

Regarding the laboratories' performance (**Figure 11**), eight laboratories (#03, #04, #05, #06, #08, #11, #12 and #35) presented no deviation. Most of these laboratories (#03, #04, #05, #06 and #12) tested the antimicrobials by using solely

broth microdilution. Laboratory #35 opted for disk diffusion (did not test for colistin), laboratory #08 did the same except for colistin testing (broth macrodilution was used) and laboratory #11 applied disk diffusion, gradient test and broth microdilution (for colistin). Except for the deviation caused by choosing gradient test for colistin testing (laboratory #10), all the deviations were caused by the disk diffusion methodology (**Figure 11**): laboratories #02, #17 and #07 had one deviation each, while laboratories #01 and #10 presented two deviations. In this trial, the reported deviations were both above and below the acceptance interval.

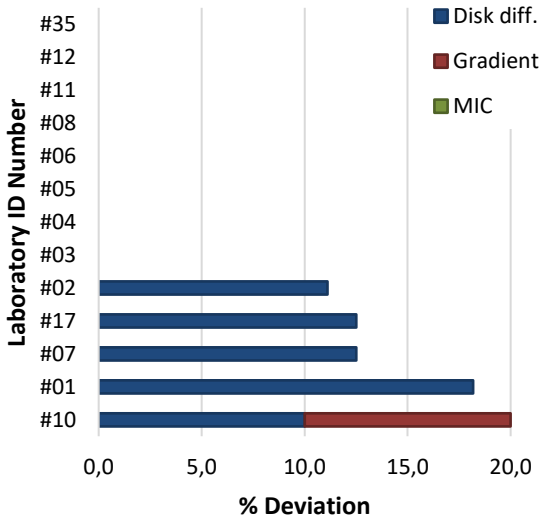

Figure 11. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *Acinetobacter* spp. trial by the HH laboratories.

3.5 *Staphylococcus aureus* trial

Fourteen laboratories from 10 countries uploaded results for the *S. aureus* trial.

3.5.1 Bacterial identification

All 14 participating laboratories submitted results for bacterial identification (**Table 18**). Only eight laboratories correctly identified the eight *S. aureus* strains and two non-*S. aureus*. Strain Sa EQASIA 21.1 was the most problematic as it was identified as non-*S. aureus* by five laboratories (#03, #06, #08, #17 and #31), followed by Sa EQASIA 21.2 misidentified by three laboratories (#02, #06 and #35). Besides these two *S. aureus* strains, laboratory #06 wrongly identified Sa EQASIA 21.5, Sa EQASIA 21.7, Sa EQASIA 21.8 and Sa EQASIA 21.9 as well. The latter was also misidentified by laboratory #35. Regarding the non-*S.aureus* strains, *Micrococcus leteus* (Sa EQASIA 21.10) was identified as *S. aureus* by laboratory #05, and *S. pseudintermedius* (Sa EQASIA 21.11) by laboratories #05, #10 and #35.

Table 18. Bacterial identification of each of the 10 test strains provided related to the *S. aureus* trial. Number of correct results out of the total of HH participating laboratories is presented. Sa EQASIA 21.4 is omitted from data analysis (see section 2.6.2 for details).

Strain	Bacterial ID	No. correct
Sa EQASIA 21.1	*S. aureus*	9/14
Sa EQASIA 21.2	*S. aureus*	11/14
Sa EQASIA 21.3	*S. aureus*	14/14
Sa EQASIA 21.5	*S. aureus*	13/14
Sa EQASIA 21.6	*S. aureus*	14/14
Sa EQASIA 21.7	*S. aureus*	13/14
Sa EQASIA 21.8	*S. aureus*	13/14
Sa EQASIA 21.9	*S. aureus*	12/14
Sa EQASIA 21.10	Non-*S. aureus* *(Micrococcus leteus)*	13/14
Sa EQASIA 21.11	Non-*S. aureus* *(S. pseudintermedius)*	11/14

Sa, *S. aureus*

3.5.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/S) ranged from 94.7% (strain Sa EQASIA 21.6) to 100% (strain Sa EQASIA 21.9) for each strain (**Table 19**). None of eight strains revealed more than 10% deviation and only one (Sa EQASIA 21.6) presented more than 5% deviation (**Table 19**).

Table 19. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 14 HH laboratories for the *S. aureus* trial.

Strain	AST in total	% Correct
Sa EQASIA 21.1	101	99.0
Sa EQASIA 21.2	130	97.7
Sa EQASIA 21.3	153	97.4
Sa EQASIA 21.5	146	96.6
Sa EQASIA 21.6	152	94.7
Sa EQASIA 21.7	145	98.6
Sa EQASIA 21.8	132	97.0
Sa EQASIA 21.9	137	100.0

Sa, *S. aureus*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were clindamycin (11.7%), trimethoprim (7.5%) vancomycin (7.0%) and cefoxitin (6.5%), whereas ciprofloxacin, fusidate, gentamicin, kanamycin, linezolid, mupirocin, quinupristin and dalfopristin, rifampin, streptomycin, sulfamethoxazole and tiamulin revealed no deviation from the expected results (**Figure 12**). Of the 19 tested and scored antimicrobial agents, only one revealed to exceed a 10% deviation (clindamycin). It is worth noting that the deviation observed for vancomycin seems to be a highly problematic as this drug is used to treat patients infected by methicillin-resistant *S. aureus* (MRSA).

Laboratory-based analysis

A deviation below or equal to 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for 12 participants (**Figure 13**). In average, the deviation was 2.3% (ranging from 0.0 to 14.9%). As the acceptance level was set to 5% deviation, two laboratories (#12 and #02) did not perform within the expected range for the *S. aureus* trial.

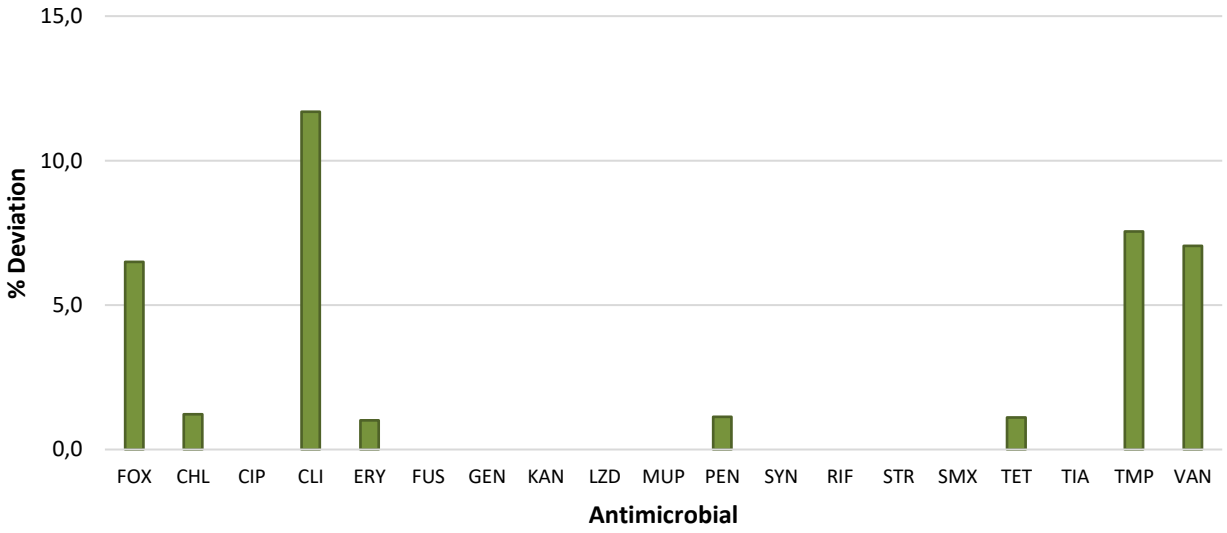

Figure 12. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

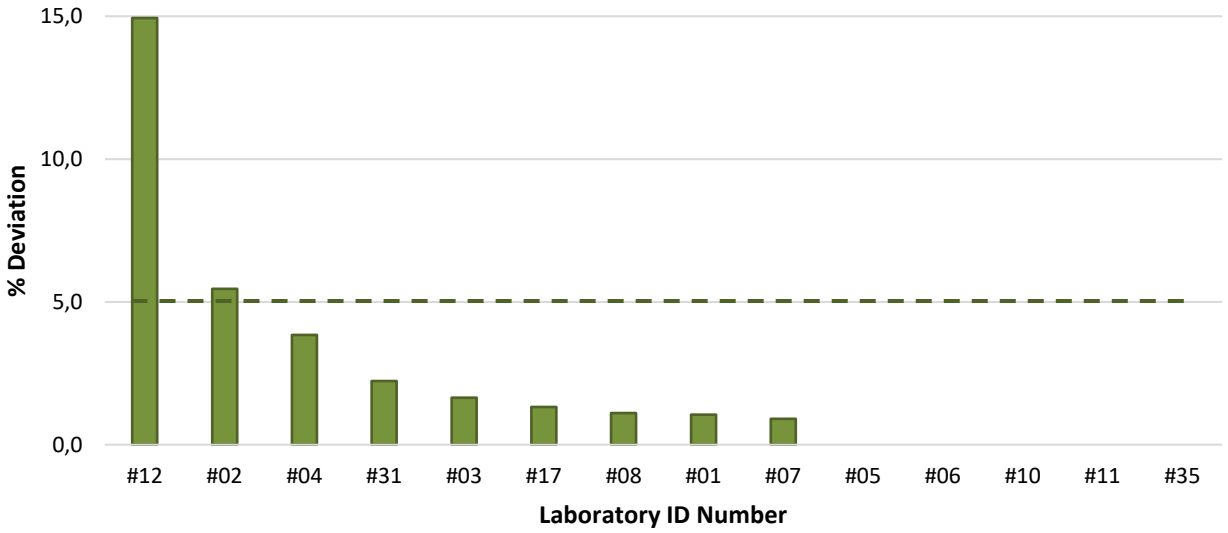

Figure 13. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by HH laboratories (n=14) participating in the 2nd EQA of the EQAsia project. Results are categorized by laboratory ID number.

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

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

Among the 14 participating laboratories, 12 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, broth macro or microdilution (**Table 20**, **). Inversely, the reference strain *S. aureus* ATCC 25923 could only be used to determine Inhibition Zone Diameters by disk diffusion (**Table 20**, *).

The highest proportion of test results outside of the expected range were observed for cefoxitin (3 out of 9), chloramphenicol (3 out of 10), penicillin (2 out of 9) and vancomycin (2 out of 10) (**Table 20**). Similar to the reference strains of previous trials, the majority of the inaccurate results seem to be caused by disk diffusion.

Table 20. 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	3/8	--	0/1	**3/9**
CHL	3/9	--	0/1	**3/10**
CIP	0/6	0/1	0/5	**0/12**
CLI	0/2	0/1	1/5	**1/8**
ERY	0/5	0/1	0/4	**0/10**
FUS	0/2	--	--	**0/2**
GEN	1/5	0/1	0/5	**1/11**
KAN	1/3	--	--	**1/3**
LZD	0/3	0/1	0/5	**0/9**
PEN	1/5	0/1	1/3	**2/9**
SYN	0/1	--	0/3	**0/4**
RIF	1/3	--	0/4	**1/7**
STR	--	--	--	**--**
SMX	--	--	--	**--**
TET	0/5	0/1	0/4	**0/10**
TMP	0/5	--	0/1	**0/6**
VAN	1/2	0/2	1/6	**2/10**

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

A closer look at the laboratories' performance (**Figure 14**) shows that seven laboratories had no deviation. Of those, laboratories #03, #04 and #06 opted for broth microdilution as the sole methodology; in reverse, laboratories #08, #11, #17 and #35 applied disk diffusion as the only or main methodology. The remaining five laboratories had deviations ranging from 8.3 to 36.4% (**Figure 14**). Laboratory #12, not only presented the highest number of deviations (n=4), but also had deviations on both methodologies applied. Laboratories #02, #07 and #10 accounted for three deviations each, and laboratory #01 for only one. While the Inhibition Zone Diameters reported by laboratories #01, #02 and #07 were below the acceptance interval, the results from laboratories #10 and #12 were above the expected range.

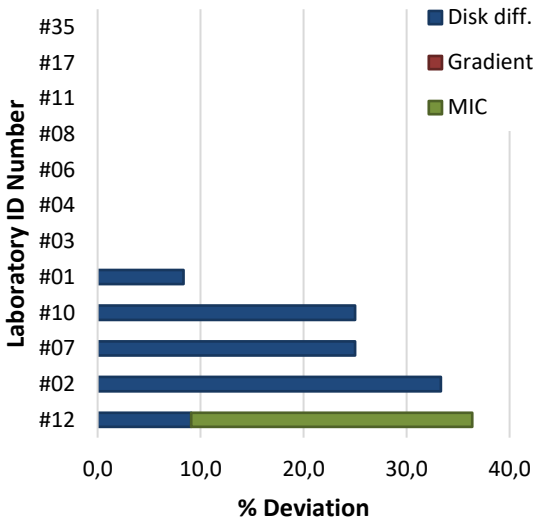

Figure 14. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial by the HH laboratories.

4. Results – Animal Health laboratories

4.1 Overall participation

Among the Animal Health laboratories, five submitted results for the *K. pneumoniae* trial, three for each of the *Shigella* and *Acinetobacter* trials, and nine for the *S. aureus* trial (**Figure 15**). Applied AST methodologies for the four trials are presented in **Figure 15**. Disk diffusion as the sole method was the preferred choice for the *K. pneumoniae* and *S. aureus* trials. Some laboratories, depending on the trial, used a mixture of disk diffusion and broth microdilution, or disk diffusion and gradient test. Laboratory #19 was the only participant reporting MIC values obtained by agar dilution. It is also worth noticing that laboratories #42 and #19 performed bacterial identification but did not submit AST results for the *Acinetobacter* and *S. aureus* trials, respectively (**Figure 15**).

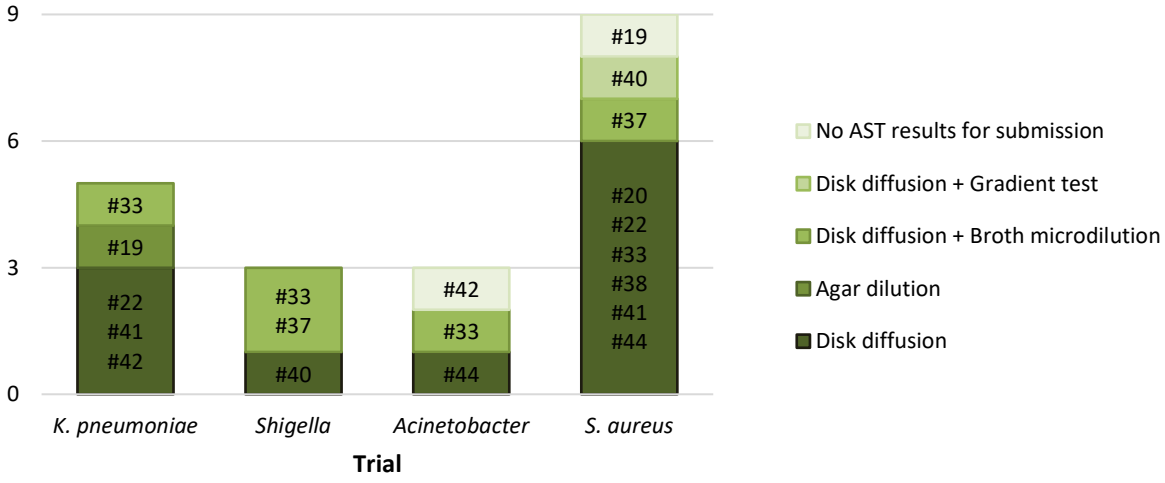

Figure 15. Methodologies applied by the laboratories in each of the trials

The participants were invited to report Inhibition Zone Diameters/MIC values and categorisation as resistant ('R') 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 antimicrobials (**Table 1**). The higher number of laboratories participating in the *S. aureus* trial resulted in a larger number of total ASTs reported for this pathogen in comparison to *K. pneumoniae*, *Shigella* and *Acinetobacter* trials (**Table 21**). Regarding the number of tests performed for each individual antimicrobial agent, azithromycin, cefepime, colistin, ertapenem and sulfamethoxazole were the least tested drugs amongst the AH laboratories on the *K. pneumoniae* trial, as well as ciprofloxacin, colistin, ertapenem, sulfamethoxazole, tigecycline and trimethoprim in the *Shigella* trial. For the *Acinetobacter* trial, no AST results on doripenem, minocycline and tobramycin were reported. Few or no results were reported for fusidate, mupirocin, streptomycin, tiamulin and vancomycin in the *S. aureus* trial. On the contrary, gentamicin and tetracycline were tested by most laboratories for this trial (**Table 21**).

Scattering of missing data or incomplete AST results entries were observed in two of the trials (**Tables 22** and **23**). One of the five laboratories participating in the *K. pneumoniae* trial (laboratory #42) revealed incomplete results (**Table 22**). A closer looks suggests that this laboratory may have wrongly selected tigecycline instead of tetracycline for strain Kp EQASIA 21.11 when submitting results. Participants need to be careful when entering results in the informatics system, as these mistakes will lead to a wrong assessment of their performance. When it comes to the *Acinetobacter* trial, laboratory #33 did not report results for strain Aci EQASIA 21.4 tested against colistin (**Table 23**).

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

Antimicrobial	ASTs in total			
	Kp	Shi	Aci	Sa
AMK	24 (5.3%)	22 (6.0%)	8 (6.8%)	--
AMP	40 (8.8%)	22 (6.0%)	--	--
AZI	16 (3.5%)	22 (6.0%)	--	--
FEP	16 (3.5%)	22 (6.0%)	--	--
FOT	24 (5.3%)	22 (6.0%)	14 (12.0%)	--
FOX	24 (5.3%)	22 (6.0%)	--	37 (6.6%)
TAZ	24 (5.3%)	20 (5.4%)	14 (12.0%)	--
CHL	32 (7.0%)	22 (6.0%)	--	58 (10.3%)
CIP	32 (7.0%)	13 (3.5%)	14 (12.0%)	45 (8.0%)
CLI	--	--	--	29 (5.2%)
COL	16 (3.5%)	15 (4.1%)	7 (6.0%)	--
DOR	--	--	0 (0.0%)	--
ETP	7 (1.5%)	14 (3.8%)	--	--
ERY	--	--	--	44 (7.8%)
FUS	--	--	--	14 (2.5%)
GEN	32 (7.0%)	22 (6.0%)	14 (12.0%)	50 (8.9%)
IMI	21 (4.6%)	22 (6.0%)	8 (6.8%)	--
KAN	--	--	--	22 (3.9%)
LEVO	--	--	8 (6.8%)	--
LZD	--	--	--	22 (3.9%)
MERO	24 (5.3%)	22 (6.0%)	14 (12.0%)	--
MIN	--	--	0 (0.0%)	--
MUP	--	--	--	7 (1.2%)
NAL	28 (6.2%)	22 (6.0%)	--	--
PEN	--	--	--	36 (6.4%)
P/T4	--	--	8 (6.8%)	--
SYN	--	--	--	28 (5.0%)
RIF	--	--	--	29 (5.2%)
STR	--	--	--	8 (1.4%)
SMX	14 (3.1%)	14 (3.8%)	--	30 (5.3%)
TET	39 (8.6%)	22 (6.0%)	--	58 (10.3%)
TIA	--	--	--	0 (0.0%)
TGC	17 (3.7%)	15 (4.1%)	8 (6.8%)	--
TOB	--	--	0 (0.0%)	--
TMP	24 (5.3%)	14 (3.8%)	--	30 (5.3%)
VAN	--	--	--	15 (2.7%)
Total	**454**	**369**	**117**	**562**

Kp, *K. pneumoniae*; Shi, *Shigella*; Aci, *Acinetobacter*; Sa, *S. aureus*

Table 22. Distribution of incomplete or missing data of antimicrobial agents among *K. pneumoniae* strains reported by AH laboratories (n=5) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Kp EQASIA 21.2	Kp EQASIA 21.3	Kp EQASIA 21.4	Kp EQASIA 21.7	Kp EQASIA 21.8	Kp EQASIA 21.9	Kp EQASIA 21.10	Kp EQASIA 21.11
#42	TGC	TGC	TGC	TGC	TGC	TGC	TGC	TET

Kp, *K. pneumoniae*

Table 23. Distribution of incomplete or missing data of antimicrobial agents among *Acinetobacter* strains reported by AH laboratories (n=3) participating in the 2nd EQA of the EQAsia project.

Lab ID No.	Aci EQASIA 21.1	Aci EQASIA 21.2	Aci EQASIA 21.3	Aci EQASIA 21.4	Aci EQASIA 21.8	Aci EQASIA 21.9	Aci EQASIA 21.10	Aci EQASIA 21.11
#33	--	--	--	COL	--	--	--	--

Aci, *Acinetobacter*

4.2 *Klebsiella pneumoniae* trial

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

4.2.1 Bacterial identification

All five participating laboratories submitted results for bacterial identification (**Table 24**). All of them correctly identified the eight *K. pneumoniae* strains among the 11 test strains provided. However, one laboratory identified the three non-*K. pneumoniae* strains as *K. pneumoniae*, suggesting that laboratory #41 may have not performed bacterial identification and simply reported all 11 strains as *K. pneumoniae* (**Table 24**).

Table 24. Bacterial identification of each of the 11 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 21.1	Non-*K. pneumoniae* (*Enterobacter sakazakii*)	4/5
Kp EQAsia 21.2	*K. pneumoniae*	5/5
Kp EQAsia 21.3	*K. pneumoniae*	5/5
Kp EQAsia 21.4	*K. pneumoniae*	5/5
Kp EQAsia 21.5	Non-*K. pneumoniae* (*Citrobacter freundii*)	4/5
Kp EQAsia 21.6	Non-*K. pneumoniae* *(Shigella boydii)*	4/5
Kp EQAsia 21.7	*K. pneumoniae*	5/5
Kp EQAsia 21.8	*K. pneumoniae*	5/5
Kp EQAsia 21.9	*K. pneumoniae*	5/5
Kp EQAsia 21.10	*K. pneumoniae*	5/5
Kp EQAsia 21.11	*K. pneumoniae*	5/5

Kp, *K. pneumoniae*

4.2.2 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/S) ranged from 91.4% (strain Kp EQASIA 21.8) to 100% (strains Kp EQASIA 21.7 and Kp EQASIA 21.11) for each strain. Five out of eight strains had a deviation below 5% (**Table 25**).

Table 25. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 5 AH laboratories for the *K. pneumoniae* trial.

Strain	AST in total	% Correct
Kp EQAsia 21.2	57	94.7
Kp EQAsia 21.3	56	98.2
Kp EQAsia 21.4	58	96.6
Kp EQAsia 21.7	58	100.0
Kp EQAsia 21.8	58	91.4
Kp EQAsia 21.9	58	98.3
Kp EQAsia 21.10	58	94.8
Kp EQAsia 21.11	51	100.0

Kp, *K. pneumoniae*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected results were meropenem (16.7%), imipenem (14.3%) and tigecycline (11.8%), whereas amikacin, azithromycin, cefepime, cefotaxime, cefoxitin, ceftazidime, chloramphenicol, ciprofloxacin colistin, ertapenem and sulfamethoxazole revealed no deviation from the expected results (**Figure 16**). Of the 19 tested and scored antimicrobial agents, three revealed to exceed a 10% deviation.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the results (R/S) was

observed for four out of the five participants (**Figure 17**). In average, the deviation was 4.7% (ranging from 0.0 to 12.5%). As the acceptance level was set to 5% deviation, one laboratory (#42) did not perform within the expected range for the *K. pneumoniae* trial.

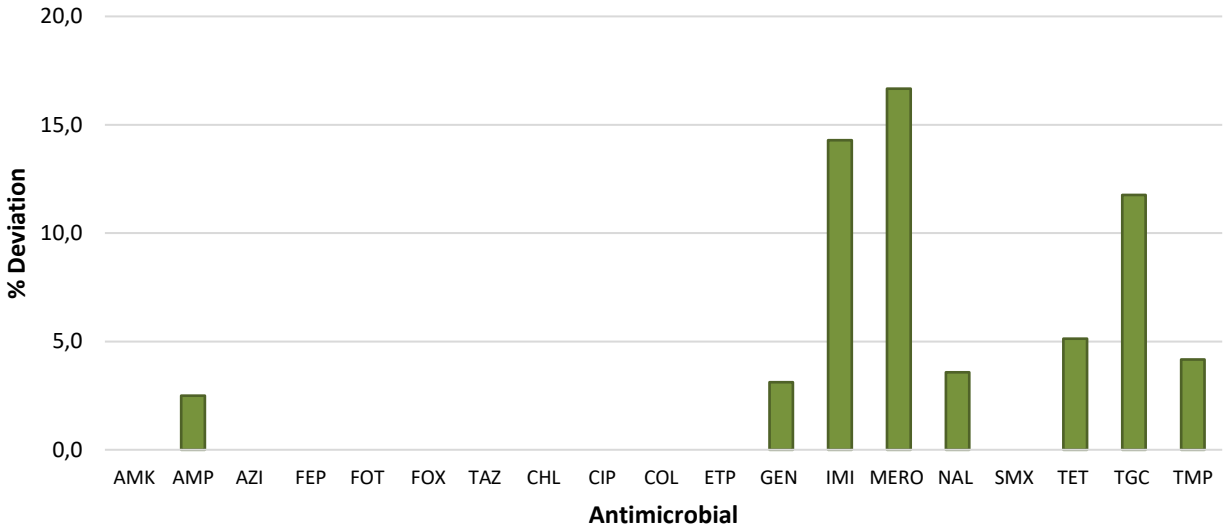

Figure 16. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=5) participating in the 2nd EQA of the EQAsia project. Results are categorized according to antimicrobial agent.

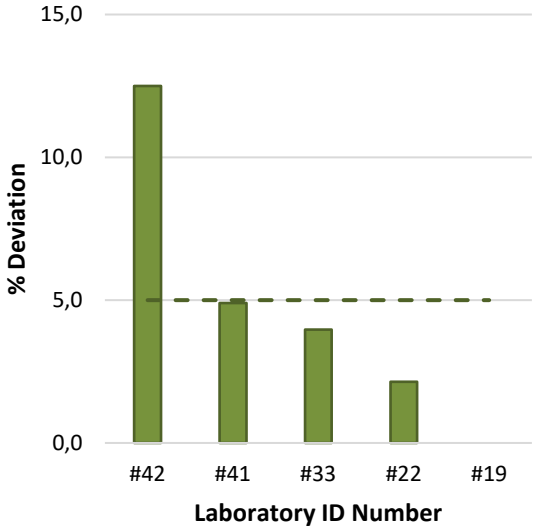

Figure 17. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=5) participating in the 2nd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.2.3 β-lactamase-producing *K. penumoniae*

Three out of the 5 participating laboratories uploaded results for this component of the *K. pneumoniae* trial (laboratories #22, #33 and #41). Discrepancies from the expected results are summarized in **Table 26**.

Firstly, laboratories identified the strains that produced ESBL/AmpC/carbapenemase, and then reported the specific phenotype. Strains Kp EQASIA 21.3, Kp EQASIA 21.4, Kp EQASIA 21.7, Kp EQASIA 21.8, Kp EQASIA 21.9 and Kp EQASIA 21.11 were expected to be carbapenemase-producers; however, laboratory #22 reported these strains (except Kp EQASIA 21.8) as resistant to cefotaxime, ceftazidime, cefoxitin, meropenem and negative synergy testing and wrongly classified the strains as 'other phenotypes'. It is worth mentioning that according to the EQA protocol (Figure 1 from Appendix 1), a strain resistant towards meropenem (MIC ≥ 0.25 or inhibition zone diameter < 25) should be classified as a carbapenemase-producer. Strain Kp EQASIA

21.8 was classified as ESBL+AmpC-phenotype by laboratories #22 and #33; this discrepancy can be explained by the wrong meropenem result (which should be resistant). Strain Kp EQASIA 21.2 was wrongly classified as a carbapenemase-producer by laboratory #33, as it was found by the laboratory to be resistant to meropenem. Lastly, strain Kp EQASIA 21.10 was expected to be a susceptible one, but laboratory #22 classified it as carbapenemase-producer, even though they reported the strain as susceptible to cefotaxime, ceftazidime, cefoxitin and meropenem.

Table 26. Expected and obtained classification of ESBL-, AmpC- and carbapenemase-producing *K. pneumoniae* test strains. Number of obtained results (n) out of the total of reported results (N) is presented for each phenotype and for each strain. Obtained results in accordance with the expected result are shown in bold. Results are from a total of 3 AH laboratories.

Strain code		Kp EQASIA 21.2	Kp EQASIA 21.3	Kp EQASIA 21.4	Kp EQASIA 21.7	Kp EQASIA 21.8	Kp EQASIA 21.9	Kp EQASIA 21.10	Kp EQASIA 21.11
Expected results		ESBL	Carbapene-mase	Carbapene-mase	Carbapene-mase	Carbapene-mase	Carbapene-mase	Susceptible	Carbapene-mase
Obtained results (n/N)	ESBL	**2/3 (66.7%)**	--	--	--	--	--	--	--
	ESBL + AmpC	--	--	--	--	2/3 (66.7%)	--	--	--
	Carbapenemase	1/3 (33.3%)	**2/3 (66.7%)**	**2/3 (66.7%)**	**2/3 (66.7%)**	**1/3 (33.3%)**	**2/3 (66.7%)**	1/3 (33.3%)	**2/3 (66.7%)**
	Other	--	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	--	1/3 (33.3%)	--	1/3 (33.3%)
	Susceptible*	--	--	--	--	--	--	**2/3 (66.7%)**	--

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

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

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

Among the five participating laboratories, four submitted results for the reference strain *E. coli* ATCC 25922 and only two 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 method (laboratories #22, #33 and #41), and MIC was determined by agar dilution method (laboratory #19). For testing *E. coli* NCTC 13846, MIC was determined by either broth microdilution (laboratory #33) or agar dilution (laboratory #19).

The highest proportion of test results outside of the expected range were observed for cefotaxime (2 out of 3) and ceftazidime (2 out of 3) (**Table 27**). Moreover, the majority of the inaccurate results seemed to be caused by Inhibition Zone Diameter determination methodologies. The sole deviation cause by broth microdilution was reported by laboratory #33 for the susceptibility testing of colistin (**Table 27** and **Figure 18**).

Considering the laboratories' performance, laboratories #19 and #22 presented no deviation from the expected range (**Figure 18**). All the deviations were observed for laboratories #41 (2 deviations) and #33 (9 deviations), which solely or mainly, repectively, used disk diffusion method and reported Inhibition Zone Diameters slightly above the expected range.

Table 27. 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.	MIC	Total
AMK	0/3	--	0/3
AMP	0/2	0/1	0/3
FEP	0/2	--	0/2
FOT	2/3	--	2/3
FOX	1/3	--	1/3
TAZ	2/3	--	2/3
CHL	1/3	0/1	1/4
CIP	1/3	0/1	1/4
COL	--	1/2	1/2
ETP	--	--	--
GEN	0/3	0/1	0/4
IMI	1/3	--	1/3
MERO	1/3	--	1/3
NAL	0/3	0/1	0/4
SMX	0/1	0/1	0/2
TET	1/3	0/1	1/4
TGC	0/2	--	0/2
TMP	0/2	0/1	0/3

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

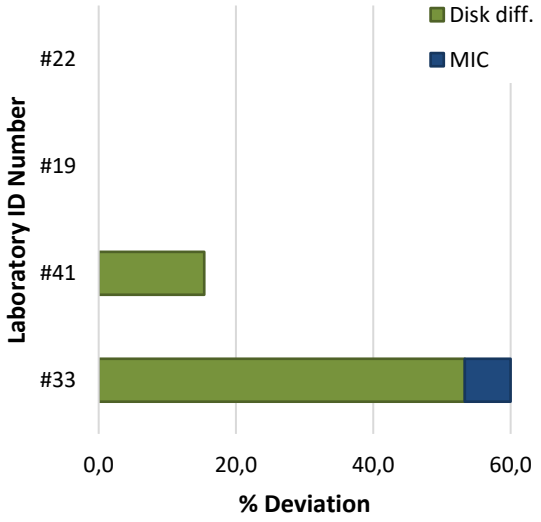

Figure 18. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 1386 in the *K. pneumoniae* trial by the AH laboratories.

4.3 *Shigella* trial

Three laboratories from two countries uploaded results for the *Shigella* trial.

4.3.1 Bacterial identification

The three laboratories participating in the *Shigella* trial submitted results for bacterial identification. Laboratory #37 misidentified Shi EQASIA 21.11 as non-*Shigella*, and laboratory #40 did not test Shi EQASIA 21.1 (**Table 28**).

Table 28. Bacterial identification of each of the 11 test strains provided related to the *Shigella* trial. Number of correct results out of the total of AH participating laboratories is presented.

Strain	Bacterial ID	No. correct
Shi EQAsia 21.1	*S. sonnei*	2/2
Shi EQAsia 21.2	*S. sonnei*	3/3
Shi EQAsia 21.3	*S. flexneri*	3/3
Shi EQAsia 21.4	Non-*Shigella* (*K. pneumoniae*)	3/3
Shi EQAsia 21.5	*S. flexneri*	3/3
Shi EQAsia 21.6	*S. sonnei*	3/3
Shi EQAsia 21.7	*S. flexneri*	3/3
Shi EQAsia 21.8	Non-*Shigella* (*Salmonella*)	3/3
Shi EQAsia 21.9	*S. flexneri*	3/3
Shi EQAsia 21.10	Non-*Shigella* (*E. coli*)	3/3
Shi EQAsia 21.11	*S. flexneri*	2/3

Shi, *Shigella*

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/S) ranged from 77.4% (strain Shi EQASIA 21.11) to 100.0% (strains Shi EQASIA 21.1, Shi EQASIA 21.3, Shi EQASIA 21.7 and Shi EQASIA 21.9) for each strain (**Table 29**). The results from two out of eight strains revealed more than 10% deviation (Shi EQASIA 21.2 and Shi EQASIA 21.11).

Table 29. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 3 AH laboratories for the *Shigella* trial.

Strain	AST in total	% correct
Shi EQAsia 21.1	35	100.0
Shi EQAsia 21.2	52	88.5
Shi EQAsia 21.3	49	100.0
Shi EQAsia 21.5	52	96.2
Shi EQAsia 21.6	49	98.0
Shi EQAsia 21.7	52	100.0
Shi EQAsia 21.9	49	100.0
Shi EQAsia 21.11	31	77.4

Shi, *Shigella*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected result were cefepime and cefotaxime, both with a deviation as high as 18.2%; inversely, amikacin, cefoxitin, colistin, ertapenem, gentamicin, imipenem, meropenem, sulfamethoxazole, tigecycline and trimethoprim revealed no deviation from the expected results (**Figure 19**).

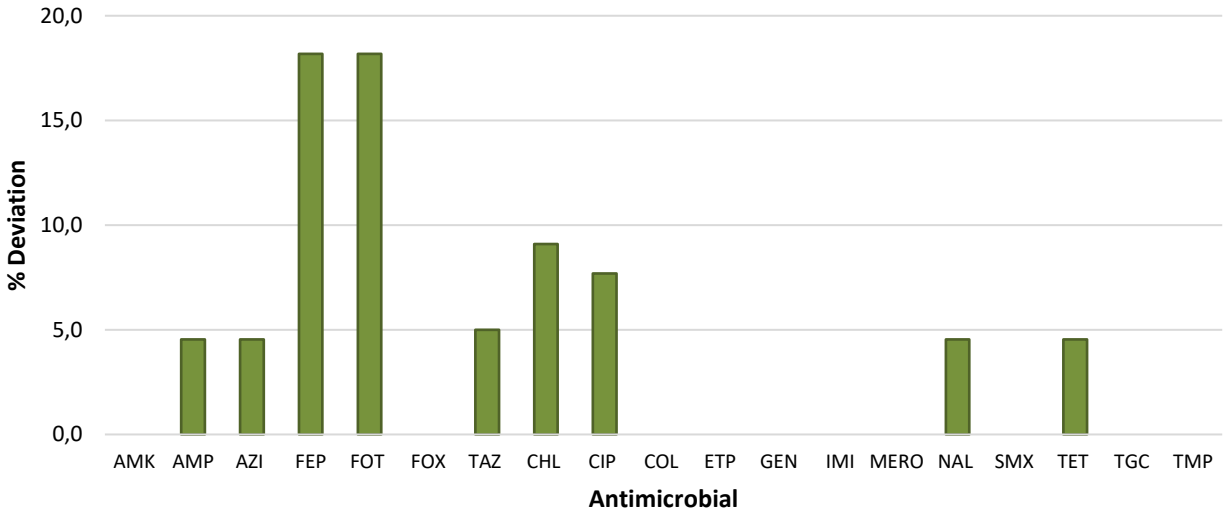

Figure 19. Percentage of deviation in the AST interpretation (R/S) among *E. coli* strains by AH laboratories (n=8) participating in the 1st EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below to 5% of laboratory performance in terms of interpretation of the results (R/S) was observed for 2 participants (**Figure 5**). In average, the deviation was 2.0% (ranging from 1.7 to 2.3%). As the acceptance level was set to 5% deviation, 2 laboratories (#37 and #40) performed within the expected range for the *Shigella* trial.

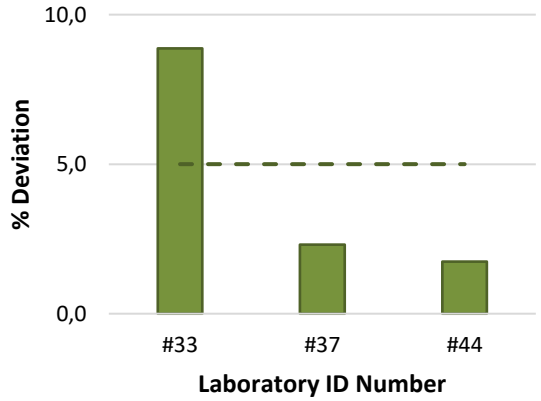

Figure 20. Percentage of deviation in the AST interpretation (R/S) among *K. pneumoniae* strains by AH laboratories (n=3) participating in the 2nd EQA in the EQAsia project. Results are categorized by laboratory ID number.

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

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

All three laboratories submitted results regarding AST of *E. coli* ATCC 25922 reference strain in the *Shigella* trial, but only two of them (laboratories #33 and #37) tested colistin and reported results for *E. coli* NCTC 13846. Disk diffusion was applied for testing the quality control strain *E. coli* ATCC 25922 by all three laboratories, whereas broth microdilution was used to test *E. coli* NCTC 13846 (for colistin) (**Table 30**).

Table 30. AST of the reference strains *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (blue shade) in the *Shigella* trial. Proportion of test results outside of expected range is presented by methodology used.

Antimicrobial	Proportion outside of range		
	Disk Diff.	MIC	Total
AMK	0/3	--	**0/3**
AMP	1/3	--	**1/3**
FEP	0/3	--	**0/3**
FOT	1/3	--	**1/3**
FOX	1/3	--	**1/3**
TAZ	2/3	--	**2/3**
CHL	2/3	--	**2/3**
CIP	1/3	--	**1/3**
COL	--	1/2	**1/2**
ETP	0/2	--	**0/2**
GEN	0/3	--	**0/3**
IMI	1/3	--	**1/3**
MERO	1/3	--	**1/3**
NAL	0/3	--	**0/3**
TET	3/3	--	**3/3**
TGC	0/2	--	**0/2**
TMP	0/2	--	**0/2**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; MIC – MIC determination by broth microdilution

The highest proportion of test results outside of the expected range were observed for ceftazidime (2 out of 3), chloramphenicol (2 out of 3) and tetracycline (3 out of 3) (**Table 30**). The only deviation caused by broth microdilution was reported by laboratory #33 (**Figure 21**). The remaining deviations came from disk diffusion testing and consisted in Inhibition Zone Diameters mostly above the expected range. While laboratories #40 and #37 had two and three deviations each, respectively, laboratory #33 presented a total of nine deviations (1 from MIC and 8 from disk diffusion) (**Figure 21**).

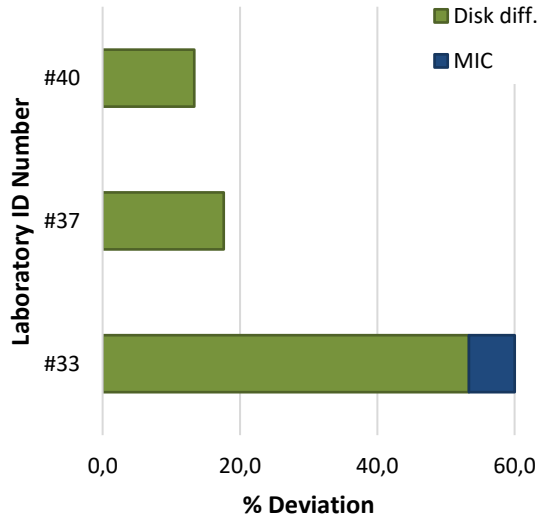

Figure 21. Percentage of deviation in the AST of *E. coli* ATCC 25922 and *E. coli* NCTC 13846 in the *Shigella* trial by the AH laboratories.

4.4 *Acinetobacter* trial

Three laboratories from three countries uploaded results for the *Acinetobacter* trial.

4.4.1 Bacterial identification

The three laboratories participating in the *Acinetobacter* trial submitted results for bacterial identification. Only laboratory #33 correctly identified the eight *Acinetobacter* strains and the three non-*Acinetobacter* (**Table 31**). Laboratories #42 and #44 misidentified the *Acinetobacter* strains Aci EQASIA 21.4 (*A. lowffii*) and Aci EQASIA 21.10 (*A. radioresistens*) as non-*Acinetobacter*, and the *P. aeruginosa* strains Aci EQASIA 21.5 and Aci EQASIA 21.7 as *Acinetobacter* (**Table 31**).

Table 31. Bacterial identification of each of the 11 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 21.1	*A. baumannii*	3/3
Aci EQAsia 21.2	*A. baumannii*	3/3
Aci EQAsia 21.3	*A. baumannii*	3/3
Aci EQAsia 21.4	*A. lowffii*	1/3
Aci EQAsia 21.5	Non-*Acinetobacter* (*P. aeruginosa*)	1/3
Aci EQAsia 21.6	Non-*Acinetobacter* (*P. aeruginosa*)	3/3
Aci EQAsia 21.7	Non-*Acinetobacter* (*P. aeruginosa*)	1/3
Aci EQAsia 21.8	*A. pittii*	3/3
Aci EQAsia 21.9	*A. baumannii*	3/3
Aci EQAsia 21.10	*A. radioresistens*	1/3
Aci EQAsia 21.11	*A.baumannii*	3/3

Aci, *Acinetobacter*

4.4.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/S) ranged from 87.5% (strains Aci EQASIA 21.2 and Aci EQASIA 21.8) to 100.0% (strains Aci EQASIA 21.1, Aci EQASIA 21.3, Aci EQASIA 21.4, Aci EQASIA 21.10, and Aci EQASIA 21.11) for each strain (**Table 32**).

Table 32. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 2 AH laboratories for the *Acinetobacter* trial.

Strain	AST in total	% correct
Aci EQAsia 21.1	16	100.0
Aci EQAsia 21.2	16	87.5
Aci EQAsia 21.3	16	100.0
Aci EQAsia 21.4	10	100.0
Aci EQAsia 21.8	16	87.5
Aci EQAsia 21.9	16	93.8
Aci EQAsia 21.10	11	100.0
Aci EQAsia 21.11	16	100.0

Aci, *Acinetobacter*

Antimicrobial-based analysis

Antimicrobials with the highest deviation from the expected result were colistin (14.3%), followed by amikacin, piperacillin/tazobactam and tigecycline, all with a deviation of 12.5% (**Figure 22**). In reverse, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin and meropenem revealed no deviation from the expected results (**Figure 22**).

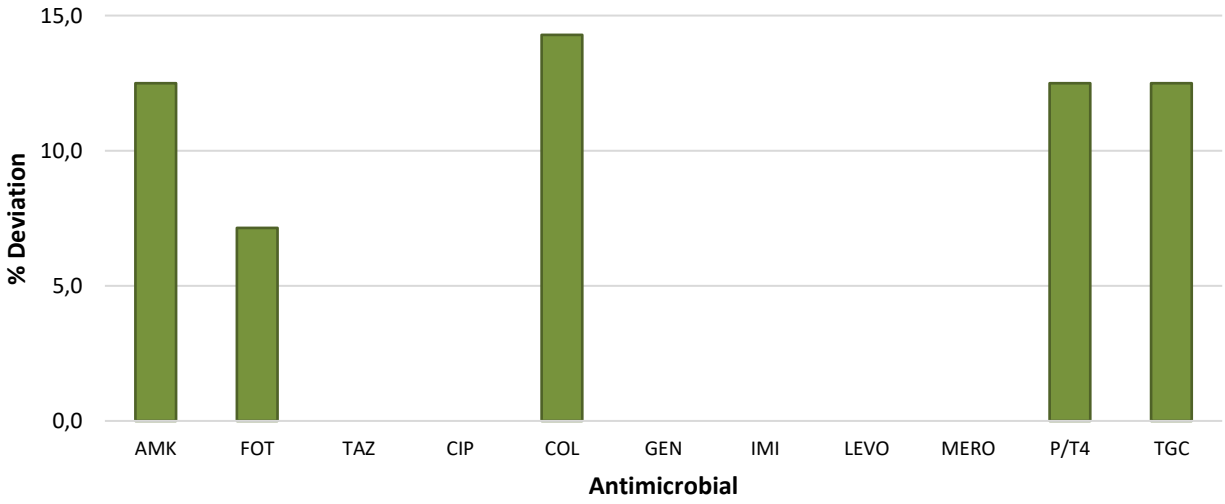

Figure 22. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter* strains by AH laboratories (n=2) participating in the 2nd EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for the two participating laboratories (**Figure 23**), meaning that both laboratories performed within the expected range for the *Acinetobacter* trial.

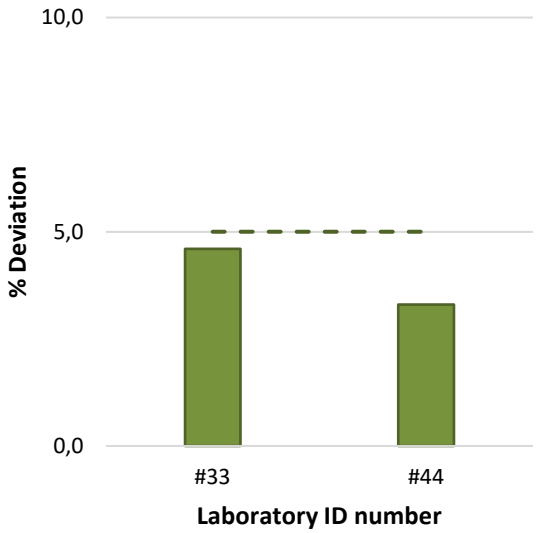

Figure 23. Percentage of deviation in the AST interpretation (R/S) among *Acinetobacter* strains by AH laboratories (n=2) participating in the 2nd EQA of the EQAsia project. Results are categorized by laboratory ID number.

4.4.3 Quality control strain *P. aeruginosa* ATCC 27853

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

Two laboratories (#33 and #44) submitted results regarding AST of *P. aeruginosa* ATCC 27853 reference strain in the *Acinetobacter* trial. While laboratory #44 opted for disk diffusion, laboratory #33 used both disk diffusion and broth microdilution (**Table 33**).

In terms of performance, laboratory #44 presented no deviation for the five antimicrobials tested (**Figure 24**). Laboratory #33 tested a few more antimicrobials, 10 in total, but presented deviating results for half of them (**Figure 24**).

Table 33. 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.	MIC	Total
AMK	0/1	--	**0/1**
FOT	1/1	--	**1/1**
TAZ	1/2	--	**1/2**
CIP	1/2	--	**1/2**
COL	--	0/1	**0/1**
DOR	--	--	**--**
GEN	0/2	--	**0/2**
IMI	0/1	--	**0/1**
LEVO	1/1	--	**1/1**
MERO	1/2	--	**1/2**
P/T4	--	0/1	**0/1**
TGC	0/1	--	**0/1**
TOB	--	--	**--**

Disk Diff. – Inhibition Zone Diameter determination by Disk Diffusion; MIC – MIC determination by broth microdilution

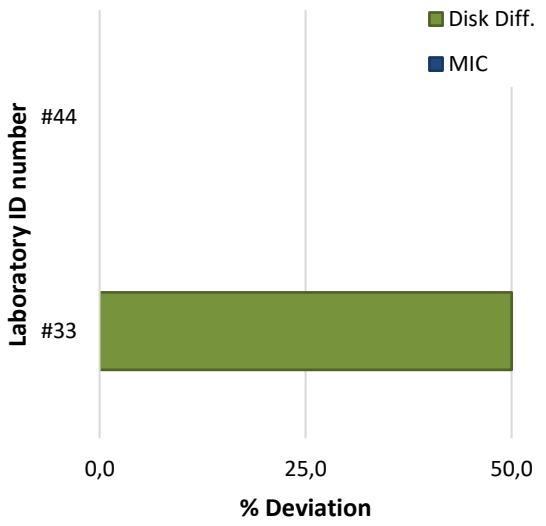

Figure 24. Percentage of deviation in the AST of *P. aeruginosa* ATCC 27853 in the *Acinetobacter* trial by the AH laboratories.

4.5 *Staphylococcus aureus* trial

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

4.5.1 Bacterial identification

All 9 participating laboratories submitted results for bacterial identification, but only three correctly identified all 11 strains (**Table 34**). Regarding the *S. aureus* strains, Sa EQASIA 21.1 was misidentified as non-*S. aureus* by three laboratories (#22, #33 and #44), Sa EQASIA 21.7 by one laboratory (#38), and Sa EQASIA 21.8 by two laboratories (#38 and #40) (**Table 34**). When it comes to the non-*S. aureus* strains, *Micrococcus leteus* (Sa EQAsia 21.10) was wrongly identified as *S. aureus* by laboratory #41, and *S. pseudintermedius* (Sa EQAsia 21.11) was misidentified by laboratories #38 and #41. These observations suggests that laboratory #41 may have not performed bacterial identification and simply reported all provided strains as *S. aureus*.

Table 34. Bacterial identification of each of the 11 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 21.1	*S. aureus*	6/9
Sa EQAsia 21.2	*S. aureus*	9/9
Sa EQAsia 21.3	*S. aureus*	9/9
Sa EQAsia 21.5	*S. aureus*	9/9
Sa EQAsia 21.6	*S. aureus*	9/9
Sa EQAsia 21.7	*S. aureus*	8/9
Sa EQAsia 21.8	*S. aureus*	7/9
Sa EQAsia 21.9	*S. aureus*	9/9
Sa EQAsia 21.10	Non-*S. aureus* (*Micrococcus leteus*)	8/9
Sa EQAsia 21.11	Non-*S. aureus* (*S. pseudintermedius*)	7/9

Sa, *S. aureus*

4.5.2 AST performance

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

Strain-based analysis

The percentage of results in agreement with expected interpretative results (R/S) ranged from 88.5% (strain Sa EQASIA 21.2) to 98.7% (strains Sa EQASIA 21.3 and Sa EQASIA 21.9) for each strain (**Table 35**). The results from three out of eight strains revealed more than 5% deviation.

Table 35. Total number of AST performed and percentage of correct results in agreement with expected interpretive results (R/S). Results are from 8 AH laboratories for the *S. aureus* trial.

Strain	AST in total	% correct
Sa EQAsia 21.1	45	97.8
Sa EQAsia 21.2	78	88.5
Sa EQAsia 21.3	78	98.7
Sa EQAsia 21.5	78	97.4
Sa EQAsia 21.6	78	96.2
Sa EQAsia 21.7	73	94.5
Sa EQAsia 21.8	54	94.4
Sa EQAsia 21.9	78	98.7

Sa, *S. aureus*

Antimicrobial-based analysis

Antimicrobials with highest deviations from the expected results were streptomycin (62.5%) and kanamycin (13.6%), whereas fusidate, linezolid, mupirocin, penicillin, rifampin, trimethoprim and vancomycin revealed no deviation from the expected results (**Figure 25**). Of the 18 tested and scored antimicrobial agents, only three had a deviation equal or higher than 10%.

Laboratory-based analysis

A deviation below 5% of laboratory performance in terms of interpretation of the result (R/S) was observed for five of the participants (**Figure 26**). In average, the deviation was 3.8% (ranging from 0.0 to 7.7%). As the acceptance level was set to 5% deviation, three laboratories (#22, #37 and #44) did not perform within the expected range for the *S. aureus* trial.

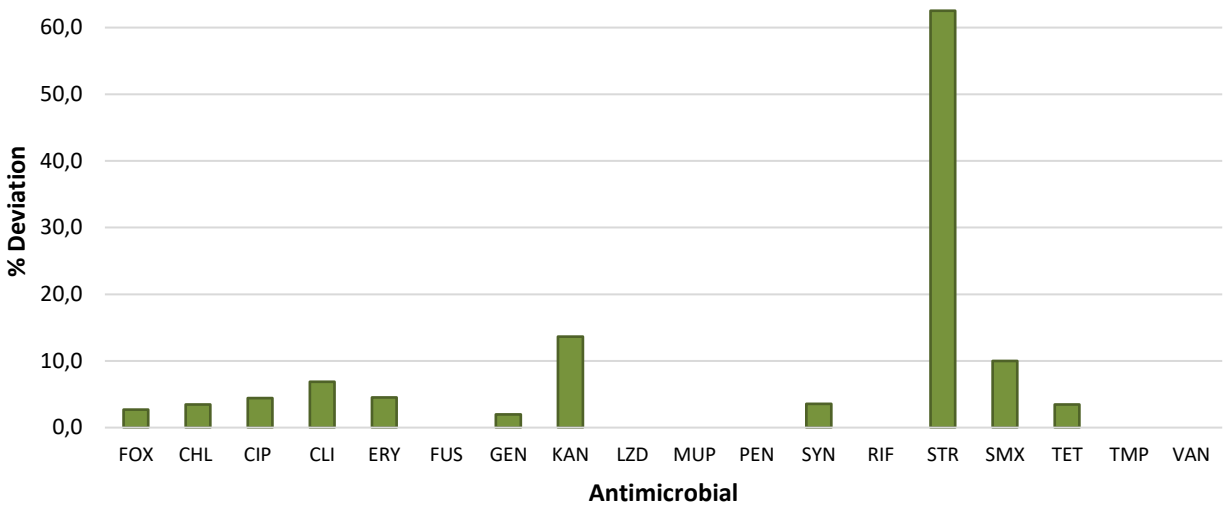

Figure 25. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 2nd EQA in the EQAsia project. Results are categorized according to antimicrobial agent.

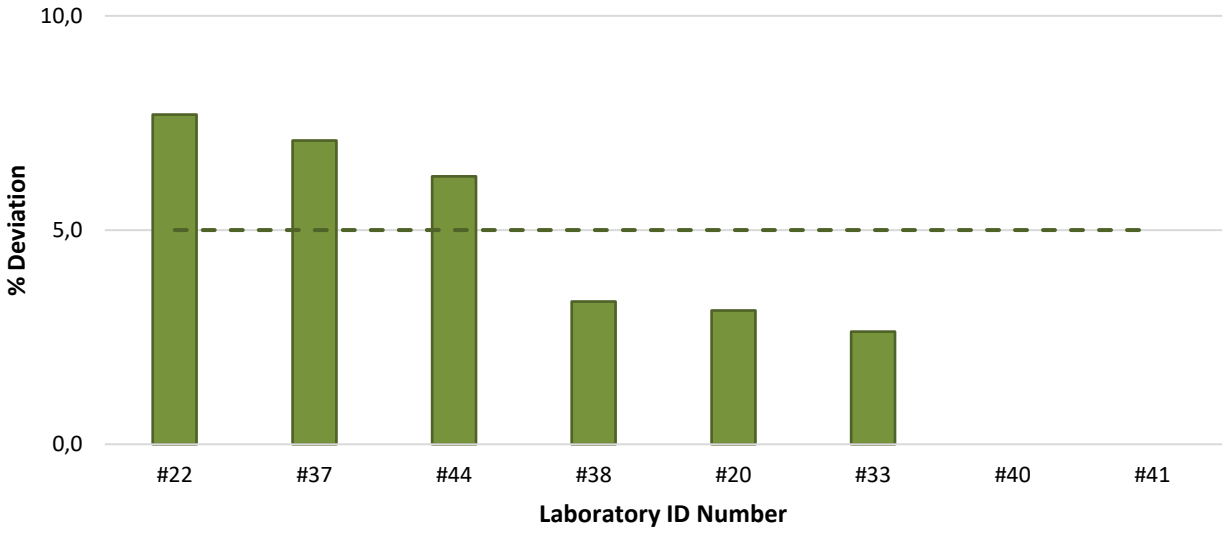

Figure 26. Percentage of deviation in the AST interpretation (R/S) among *S. aureus* strains by AH laboratories (n=8) participating in the 2nd EQA in the EQAsia project. Results are categorized by laboratory ID number.

4.5.3 Quality control strain *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 to all participating laboratories to be used as reference strains for the *S. aureus* trial.

Eight laboratories submitted AST results for *S. aureus* ATCC 25923 reference strain as disk diffusion was the methodology applied (**Table 36**, *). Only 2 laboratories (#37 and #40) selected *S. aureus* ATCC 29213 to test vancomycin by either broth microdilution or gradient test (**Table 36**, **).

The highest proportion of test results outside of the expected range was observed for cefoxitin (3 out of 5), ciprofloxacin (3 out of 7), erythromycin

(2 out of 6) and tetracycline (3 out of 8) (**Table 36**).

Table 36. 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	3/5	--	--	**3/5**
CHL	1/8	--	--	**1/8**
CIP	3/7	--	--	**3/7**
CLI	1/4	--	--	**1/4**
ERY	2/6	--	--	**2/6**
FUS	0/2	--	--	**0/2**
GEN	0/7	--	--	**0/7**
KAN	0/3	--	--	**0/3**
LZD	1/3	--	--	**1/3**
PEN	2/5	--	--	**2/5**
SYN	0/4	--	--	**0/4**
RIF	1/4	--	--	**1/4**
STR	0/3	--	--	**0/3**
SMX	--	--	--	**--**
TET	3/8	--	--	**3/8**
TMP	0/4	--	--	**0/4**
VAN	--	0/1	0/1	**0/2**

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

Laboratories #22, #37 and #38 presented no deviation from the expected range (**Figure 27**). The highest percentage of deviation was observed for laboratory #33, which presented a total of nine deviations. The remaining four laboratories (#20, #41, #44 and #40) had two deviations each. These deviations imply a poor performance of individual laboratories on applying the disk diffusion methodology.

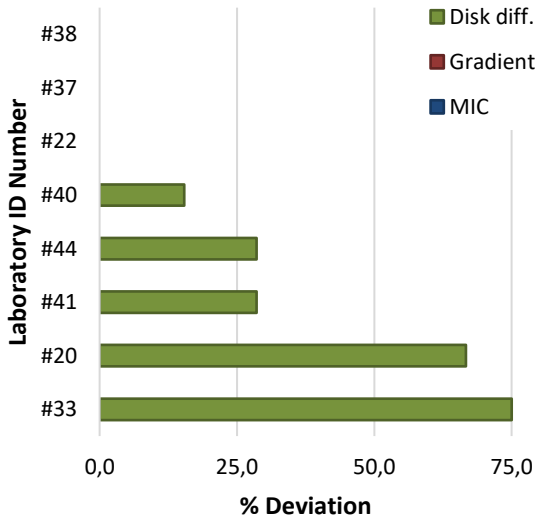

Figure 27. Percentage of deviation in the AST of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 in the *S. aureus* trial by the AH laboratories.

5. Discussion

5.1 Human Health Laboratories

A total of 14 Human Health laboratories participated in the 2nd EQA of the EQAsia programme. Disk diffusion and broth microdilution as solo methodologies were chosen by the majority of the participants for testing the recommended antimicrobials in each of the trials. The remaining laboratories opted for disk diffusion along with another method, such as gradient test, broth microdilution and/or broth macrodilution.

All laboratories that performed bacterial identification have also submitted AST results. Incomplete AST results' entries were, however, observed in all four 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 *K. pneumoniae* species among the provided test strains, even though one laboratory apparently

did not perform bacterial identification and simply reported all 11 strains as *K. pneumoniae*. In the other three trials, the laboratories demonstrated limited capacity to properly identify the target species (*Shigella*, *Acinetobacter* or *S. aureus*), as some misidentifications were observed. In the *Acinetobacter* trial, for example, two laboratories misidentified the *Acinetobacter* strain *A. lowffii* as a non-*Acinetobacter* strain, which can be explained by the high variation of phenotypic characteristics of this species. Neverthless, 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. Therefore, the strain-based analysis revealed no major issues in testing any of the *K. pneumoniae* and *S. aureus* test strains, as none of them revealed more than 10% deviation. Only two strains (Shi EQASIA 21.5 and Aci EQASIA 21.2) shown a deviation higher than 10%; in both cases, a notable proportion of deviations was caused by expected MIC/ Inhibition Zone Diameter values being close to the cut-off epidemiological values. In this situation, a one-fold dilution/±3mm difference from an expected value resulted in a different interpretation and was scored as incorrect.

For the Gram-negative bacteria trials (*K. pneumoniae*, *Shigella* and *Acinetobacter*), some common antimicrobials presented a high deviation from the expected results, such as: sulfamethozaxole (18.5% and 37.5% in the *K. pneumoniae* and *Shigella* trials, respectively), trimethoprim (7.1% and 19.1% deviation) and tigecycline (28.3% in the *K. pneumoniae* trial and 10.0% in the *Acinetobacter* trial). These high deviations can be explained by the uncommon testing of sulfamethoxazole, trimethoprim and tigecycline by the HH laboratories, as these drugs are not recommended by CLSI, the guidelines followed by most of them. Gentamicin also revealed high deviations in the *K. pneumoniae* (18.2%) and *Shigella* (14.3%) trials most likely because this drug is rarely used for treatment. Lastly, testing of colistin seemed to be a bit problematic for all three trials, which might be due to the need of using a standard broth microdilution method rather than disk diffusion or gradient test methodologies for testing colistin. Broth microdilution is a method that requires proper experience for a good performance.

In the *S. aureus* trial, vancomycin revealed a rather high deviation (7.0%), which can be problematic since this drug is used to treat patients infected by methicillin-resistant *S. aureus* (MRSA). In such situation, it is expected that no mistakes are made when treating a patient and, therefore, vancomycin susceptibility testing should always be accurate.

Regarding the HH laboratories' AST performance, on average, the deviation was 4.7% in the *K. pneumoniae* trial, 6.2% in the *Shigella* trial, 6.0% in the *Acinetobacter* trial and 2.3% in the *S. aureus* trial. Despite the average being close to acceptable (below the acceptance level of 5% or just slightly above), there were some laboratories with deviations of more than 10%. Also, some laboratories had deviations only slightly above 5% in multiple trials, whereas others seemed to only struggle in a specific trial. In all situations, it is clear that the laboratories' performance can still be strengthened.

Detection and confirmation of presumptive beta-lactamase producing *K. pneumoniae* was an optional component of this EQA, but highly encouraged due to its importance. Eleven out of the 14 participating laboratories submitted results and, in most of the cases, were able to differentiate the susceptible (no ESBL, AmpC or carbapenemase) from the ESBL/AmpC/ carbapenemase-producers. However, only four laboratories correctly identified all the different ESBL / AmpC / carbapenemase phenotypes among the eight *K. pneumoniae* strains. The main mistake observed was the incorrect classification of the carbapenemase phenotypes, even though the strains were reported as resistant to meropenem. The observations suggest a need for further

carification and support on capacity building.

Among all laboratories, there were three laboratories that did not submit antimicrobial susceptibility testing results for the quality control strains: laboratory #05 did not submit results for the reference strain in the *S. aureus* trial, laboratory #01 for the reference strains in the *K. pneumoniae* and *Shigella* trials, and laboratory #31 did not submit results for the reference strains in any of the four trials. 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.

5.2 Animal Health Laboratories

For the Animal Health sector, 10 laboratories participated in the 2nd EQA of the EQAsia programme. The participating laboratories mostly applied disk diffusion for determining Inhibition Zone Diameters, or disk diffusion together with gradient test or broth microdilution. One laboratory (#19) opted for agar dilution to test the *K. penumoniae* strains.

The participants were asked to firstly perform bacterial identification and then proceed with AST of the target strains. Although laboratories #42 and #19 performed bacterial identification, they did not submit AST results for the *Acinetobacter* and *S. aureus* trials, respectively. Besides these missing data, incomplete AST results' entries were observed in two of the trials. In one of the cases, it seems that the laboratory may have wrongly selected the antimicrobial for one of the strains, which can lead to a wrong assessment of the laboratory's performance.

As mentioned above, bacterial identification was the first component in each of the trials. The reported results from laboratory #41, for example, indicate that bacterial identification was not performed and that all strains were reported as target strains (no non-target strains reported). The results from the other laboratories additionally suggest limited capacity for performing bacterial identification, at least in one of the trials, since several occasions of bacterial misidentification were observed. In the *Acinetobacter* trial, for instance, the laboratories demonstrated difficulties in identifying *Acinetobacter* strains other than *A. baumannii*. The identification of *S. aureus* and non-*S. aureus* strains seems to be difficult for the majority of the laboratories, suggesting that advice and training on the subject may be required among the AH laboratories.

The antimicrobial susceptibility testing performance revealed that testing of the *K. pneumoniae* test strains generated the lowest deviation from the expected results, whereas the *Acinetobacter* strains, two in particular, presented deviations as high as 12.5%. Regarding the antimicrobials, the ones with the highest deviation from the expected results varied from trial to trial. In the *K. pneumoniae* trial, the carbapenems meropenem and imipenem, together with tigecycline, presented the highest deviations, whereas the cephalosporins cefepime and cefotaxime were problematic in the *Shigella* trial. Colistin presented the highest deviation in the *Acinetobacter* trial. For the gram-positive bacteria *S. aureus*, the highest deviations from the expected results were seen for streptomycin and kanamycin.

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, below the acceptance level of 5% for all four trials: 4.7% in the *K. pneumoniae* trial, 2.0% in the *Shigella* trial, 4.0% in the *Acinetobacter* trial, and 3.8% in the *S. aureus* trial. Still, some AH laboratories did not perform within the expected range (deviations above the acceptance level of 5%), which is the case of laboratories #22 (*S. aureus* trial), #33 (*Shigella* trial), #37 (*S. aureus* trial), #42 (*K. pneumoniae* trial) and #44 (*S. aureus* trial).

Only three out of the 5 participating laboratories in the *K. pneumoniae* trial submitted results for

the detection and confirmation of presumptive beta-lactamase producing bacteria. One of the laboratories (#22) was not able to correctly classify the susceptible isolate Kp EQASIA 21.10 (no ESBL, AmpC or carbapenemase), even though the isolate was reported as susceptible to cefotaxime, ceftazidime, cefoxitin and meropenem. The remaining inaccurate results were mostly due to the incorrect classification of the carbapenemase phenotypes. This issue was also observed for the HH laboratories and suggests that further clarification on the classification of the different phenotypes is required.

Lastly, laboratories performed antimicrobial susceptibility testing of the quality control strains relevant for each of the trials. One laboratory (#42) did not submit results for the reference strains in the *K. pneumoniae* trial, which is required in terms of quality control and reliability of AST results and performance. 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, and often very different from the expected interval of values, demonstrating technical problems in performing AST.

6. Conclusions

This report presented the results of the second EQAsia EQA trial 2021, which included *K. pneumoniae*, *Shigella*, *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 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.

In this report, the data was adjusted due to erroneous interpretation of MIC/Inhibition Zone Diameter values, which were otherwise obtained within the acceptable range. This was caused by laboratories using guidelines different from those indicated in the EQA protocol. In future EQAs, the interpretation criteria will be adjusted to meet the needs and routine work of the participating laboratories. Still, 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 icorrections were also detected upon submission of the results, such as selection of the wrong antimicrobial or wrong interpretation. 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 their routine work. Thus, it is recommended proper storage and maintenance of these reference strains. 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.

7. References

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

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

[3] Clinical and Laboratory Standards Institute; M100. Performance standards for Antimicrobial Susceptibility Testing 31st edit, January 2021.

[4] The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 12.0, 2022. http://www.eucast.org.

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

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

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

8. Appendices

Appendix 1: EQA2 Protocol

Appendix 1: EQA2 protocol

Protocol for EQAsia EQAS – 2nd round

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

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1 INTRODUCTION

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

The 2nd iteration of EQAsia EQAS includes the antimicrobial susceptibility testing of eight *Klebsiella pneumoniae*, eight *Shigella* spp., eight *Acinetobacter* spp. and eight *Staphylococcus aureus* strains **identified** among a total of 11 test strains for each microorganism, which include three non-target species strains.

Additionally, antimicrobial susceptibility testing of five reference strains for quality control (QC) in relation to antimicrobial susceptibility testing is included. The QC reference strains supplied 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 stored for future internal quality control

for antimicrobial susceptibility testing in your laboratory. The QC reference strains included in the 2nd EQA will not be included in the parcel related to future EQAS-iterations. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual 'Subculture and maintenance of quality strain' available on the EQAsia website.

2 OBJECTIVES

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

3 OUTLINE OF THE EQAS 2021

3.1 Shipping, receipt and storage of strains

In July/August 2021, it is expected that approximately 31 laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- 11 test strains of which eight are *Klebsiella pneumoniae*, in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 (if not already received for EQA1) and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains.

- 11 test strains of which eight are *Shigella* spp., in addition to three non-target species strains. The *Escherichia coli* ATCC 25922/CCM 3954 (if not already received for EQA1) and *E. coli* NCTC 13846/CCM 8874 (for colistin) will be provided as reference strains.

- 11 test strains of which eight are *Acinetobacter* spp., in addition to three non-target species strains. The *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 will be provided as reference strain.

- 11 test strains of which eight are *Staphylococcus aureus*, in addition to three 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.

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 (Human health laboratories)' OR 'Instructions for opening and reviving lyophilised cultures of test strains (Animal health laboratories)' on the EQAsia website.

For reconstitution of the QC reference strains, please see the document 'Subculture and maintenance of quality strain' 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*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* test strains

For each test species, three out of the 11 test strains related to each bacterial species does not belong to the target species of the EQAS. To identify the eight cultures of the correct target species among the 11 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*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* test strains, and of the reference strains

The strains identified as *Klebsiella pneumoniae*, *Shigella* spp., *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 forms and in **Tables 1-4**. Should it however not be possible to test all antimicrobials, the optional antimicrobials are marked with [a]. Please use the methods routinely used in your own laboratory.

The reference values used in this EQAS for interpreting MIC and disk diffusion results are in accordance with current epidemiological cut-off values or clinical breakpoint values developed by EUCAST. When not available, CLSI zone diameter and MIC breakpoint values are used instead. The epidemiological cut-off values or clinical breakpoint values for *Klebsiella pneumonia*, *Shigella* spp., *Acinetobacter* spp. and *Staphylococcus aureus* can be found in **Tables 1-4**, 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 two categories: **resistant** (R) or **susceptible** (S). In the evaluation report you receive upon the submission deadline, you can find that obtained interpretations in accordance with the expected interpretation will be evaluated as '1' (correct), whereas obtained interpretations not in accordance with the expected interpretation will be evaluated as '0' (incorrect).

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

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK[a]	≥ 16	< 18
Ampicillin, AMP	≥ 16	< 14
Azithromycin, AZI[a]	≥ 32*	≤ 12*
Cefepime, FEP[a]	≥ 0.50	< 24
Cefotaxime, FOT	≥ 0.50	< 21
Cefotaxime, FOT + clavulanic acid	NA	NA
Cefoxitin, FOX	≥ 16	< 19
Ceftazidime, TAZ	≥ 1	< 20
Ceftazidime, TAZ + clavulanic acid	NA	NA
Chloramphenicol, CHL	≥ 32*	≤ 12*
Ciprofloxacin, CIP	≥ 0.25	< 22
Colistin, COL	≥ 4	NA
Ertapenem, ETP[a]	≥ 0.06	< 24
Gentamicin, GEN	≥ 4	< 17
Imipenem, IMI	≥ 2	< 23
Meropenem, MERO	≥ 0.25	< 25
Nalidixic acid, NAL	≥ 16	< 16
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 16*	≤ 11*
Tigecycline, TIG[a]	≥ 4	< 18
Trimethoprim, TMP	≥ 4	< 18

Reference values are based on *K. pneumoniae* and/or *E. coli* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on Enterobacterales breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

Beta-lactam and carbapenem resistance

The following tests for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes for ***Klebsiella 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, E-test or Disc 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 (E-test 3 dilution steps difference; MIC FOT : FOT/Cl or TAZ : TAZ/Cl ratio ≥ 8). A positive synergy testing for Disc 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.

The classification of the phenotypic results should be based on the adaptation of the most recent EFSA recommendations (**Figure 1** below) – 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 and DD
- FOT or TAZ: R AND
- MERO: S AND
- FOX: S AND
- SYN FOT/CLV and/or TAZ/CLV

2. AmpC-Phenotype
MIC and DD
- FOT or TAZ: R AND
- MERO: S AND
- FOX: R AND
- No SYN FOT/CLV nor TAZ/CLV

(Does not exclude presence of ESBLs)

3. ESBL + AmpC-Phenotype
MIC and DD
- FOT or TAZ: R AND
- MERO: S AND
- FOX: R AND
- SYN FOT/CLV and/or TAZ/CLV

4. Carbapenemase-Phenotype
MIC and DD
- MERO: R

5. Other Phenotypes
1) MIC and DD
- FOT or TAZ: R AND
- MERO: S AND
- FOX: S AND
- No SYN FOT/CLV nor TAZ/CLV

2) MIC and DD
- FOT and TAZ: S AND
- MERO: S AND
- FOX: R

3) MIC and DD
- MERO: S BUT
- ETP: R AND/OR
- IMI: R

Susceptible
MIC and DD
- FOT, TAZ, FOX, MERO: S

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.

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 EQAS.

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

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK[a]	≥ 16	< 17
Ampicillin, AMP	≥ 16	< 14
Azithromycin, AZI[a]	≥ 32*	≤ 12*
Cefepime, FEP[a]	≥ 0.50	< 28
Cefotaxime, FOT	≥ 0.50	< 21
Cefoxitin, FOX	≥ 16	< 17
Ceftazidime, TAZ	≥ 1	< 20
Chloramphenicol, CHL	≥ 32*	≤ 12*
Ciprofloxacin, CIP	≥ 0.12	< 25
Colistin, COL	≥ 4	NA
Ertapenem, ETP[a]	≥ 0.06	< 24
Gentamicin, GEN	≥ 4	< 17
Imipenem, IMI	≥ 1	< 24
Meropenem, MERO	≥ 0.12	< 25
Nalidixic acid, NAL	≥ 16	< 19
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 16*	≤ 11*
Tigecycline, TIG[a]	≥ 1	< 18
Trimethoprim, TMP	≥ 4	< 20

Reference values are based on *E. coli* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on Enterobacterales breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

Table 3. Interpretive criteria for *Acinetobacter* spp. antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Amikacin, AMK	≥ 16	< 19
Cefotaxime, FOT	≥ 64*	≤ 14*
Ceftazidime, TAZ	≥ 32*	≤ 14*
Ciprofloxacin, CIP	≥ 2	< 21
Colistin, COL	≥ 4	NA
Doripenem, DOR	≥ 4	< 22
Gentamicin, GEN	≥ 8	< 17
Imipenem, IPM	≥ 8	< 21
Levofloxacin, LVX	≥ 2	< 20
Meropenem, MERO	≥ 16	< 15
Minocycline, MIN	≥ 16*	≤ 12*
Piperacillin/tazobactam, P/T4	≥ 128/4*	≤ 17*
Tigecycline, TGC	≥ 1	NA
Tobramycin, TOB	≥ 8	< 17

Reference values are based on *Acinetobacter* spp. clinical breakpoint values from www.eucast.org on June 2021.
*Reference values are based on *Acinetobacter* spp. breakpoint values from CLSI M100, 30th Ed.

Table 4. Interpretive criteria for *Staphylococcus aureus* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)	Reference value Disk diffusion (mm)
	Resistant	Resistant
Cefoxitin, FOX	≥ 8	< 22
Chloramphenicol, CHL	≥ 32	< 18
Ciprofloxacin, CIP	≥ 2	< 20
Clindamycin, CLI	≥ 0.50	< 22
Erythromycin, ERY	≥ 2	< 21
Fusidate, FUS[a]	≥ 1	< 24
Gentamicin, GEN	≥ 4	< 18
Kanamycin, KAN[a]	≥ 16	< 18
Linezolid, LZD	≥ 8	< 19
Mupirocin, MUP[a]	≥ 2	< 30
Penicillin, PEN	≥ 0.25	< 26
Quinupristin/Dalfopristin, SYN[a]	≥ 2	< 21
Rifampin, RIF	≥ 0.03	< 25
Streptomycin, STR[a]	≥ 32	NA
Sulfamethoxazole, SMX	≥ 512*	≤ 12*
Tetracycline, TET	≥ 2	< 22
Tiamulin, TIA[a]	≥ 4	NA
Trimethoprim, TMP	≥ 4	< 19
Vancomycin, VAN	≥ 4	NA

Reference values are based on *S. aureus* epidemiological cut off values from www.eucast.org on June 2021.
*Reference values are based on *S. aureus* breakpoint values from CLSI M100, 30th Ed.
[a] Optional.

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 web database. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The web database 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 '1' (correct), while results deviating from the expected interpretation are categorised as '0' (incorrect).

Results must be submitted no later than September 15th 2021.

If you have trouble entering your results, please contact the EQAsia Project Manager directly, explaining the issues that you encountered:

Rikke Braae
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK
E-mail: rikb@food.dtu.dk

Direct communication with the EQAsia Project Manager must be in English.

5 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

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 webtool using this address. See below how to login to the webtool.

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 troubles with the webtool.

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 webtool:

When first given access to login to the webtool, 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 rikb@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	Chloramphenicol CHL		Ciprofloxacin CIP	
Kp EQASIA 21.2	16	R	> 32	R	> 64	R	> 32	R	> 64	R	≤ 0.06/4	4	S	64	R	0.5/4	≤ 8	S	> 8	R
Kp EQASIA 21.3	≤ 4	S	> 32	R	> 64	R	> 32	R	> 64	R	≤ 0.25	S	R	> 128	R	> 128/4	≤ 8	S	2	R
Kp EQASIA 21.4	> 128	R	> 32	R	> 64	R	32	R	> 64	R	> 64/4	> 64	R	> 128	R	> 128/4	16	S	> 8	R
Kp EQASIA 21.7	32	R	> 32	R	> 64	R	16	R	16	R	16/4	64	R	64	R	64/4	> 64	R	> 8	R
Kp EQASIA 21.8	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	2/4	> 64	R	> 128	R	2/4	> 64	R	> 8	R
Kp EQASIA 21.9	> 128	R	> 32	R	> 64	R	> 32	R	> 64	R	2/4	> 64	R	> 128	R	2/4	> 64	R	> 8	R
Kp EQASIA 21.10	≤ 4	S	32	R	16	S	≤ 0.06	S	≤ 0.25	S	≤ 0.06/4	4	S	≤ 0.25	S	0.25/4	≤ 8	S	0.06	S
Kp EQASIA 21.11	≤ 4	S	> 32	R	> 64	R	16	R	> 64	R	1/4	8	S	64	R	2/4	> 64	R	2	R

R, Resistant; S, Susceptible

	Colistin COL		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Meropenem MERO		Nalidixic acid NAL		Sulfamethoxazole SMX		Tetracycline TET		Tigecycline TGC		Trimethoprim TMP	
Kp EQASIA 21.2	1	S	0.12	R	1	S	0.25	S	0.06	S	> 64	R	> 512	R	> 32	R	1	S	> 16	R
Kp EQASIA 21.3	4	R	> 2	R	≤ 0.5	S	> 16	R	> 16	R	≤ 4	S	≤ 8	S	> 32	R	0.5	S	> 16	R
Kp EQASIA 21.4	> 16	R	> 2	R	> 16	R	16	R	> 16	R	> 64	R	> 512	R	> 32	R	2	S	> 16	R
Kp EQASIA 21.7	≤ 1	S	> 2	R	1	S	8	R	4	R	> 64	R	> 512	R	8	S	1	S	> 16	R
Kp EQASIA 21.8	≤ 1	S	> 2	R	> 16	R	0.25	S	4	R	> 64	R	> 512	R	8	S	1	S	2	S
Kp EQASIA 21.9	≤ 1	S	> 2	R	> 16	R	0.25	S	2	R	> 64	R	> 512	R	8	S	1	S	2	S
Kp EQASIA 21.10	≤ 1	S	≤ 0.015	S	≤ 0.5	S	≤ 0.12	S	≤ 0.03	S	≤ 4	S	≤ 8	S	≤ 2	S	0.5	S	1	S
Kp EQASIA 21.11	≤ 1	S	2	R	> 16	R	1	S	1	R	16	R	> 512	R	2	S	0.5	S	> 16	R

R, Resistant; S, Susceptible

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

	Amikacin AMK		Ampicillin AMP		Azithromycin AZI		Cefepime FEP		Cefotaxime FOT		Cefoxitin FOX		Ceftazidime TAZ		Chloramphenicol CHL		Ciprofloxacin CIP	
Shi EQASIA 21.1	≤ 4	S	4	S	8	S	≤ 0.06	S	≤ 0.25	S	2	S	≤ 0.25	S	≤ 8	S	8	R
Shi EQASIA 21.2	≤ 4	S	> 32	R	> 64	R	2	R	16	R	2	S	0.5	S	≤ 8	S	8	R
Shi EQASIA 21.3	8	S	4	S	≤ 2	S	≤ 0.06	S	≤ 0.25	S	8	S	≤ 0.25	S	≤ 8	S	0.12	R
Shi EQASIA 21.5	≤ 4	S	> 32	R	> 64	R	0.25	S	≤ 0.25	S	4	S	≤ 0.25	S	> 64	R	≤ 0.015	S
Shi EQASIA 21.6	≤ 4	S	> 32	R	> 64	R	4	R	64	R	4	S	4	R	≤ 8	S	0.25	R
Shi EQASIA 21.7	≤ 4	S	> 32	R	> 64	R	4	R	64	R	8	S	4	R	> 64	R	8	R
Shi EQASIA 21.9	≤ 4	S	> 32	R	> 64	R	≤ 0.06	S	≤ 0.25	S	8	S	≤ 0.25	S	≤ 8	S	0.25	R
Shi EQASIA 21.11	≤ 4	S	> 32	R	4	S	1	R	8	R	4	S	1	R	> 64	R	> 8	R

R, Resistant; S, Susceptible

	Colistin COL		Ertapenem ETP		Gentamicin GEN		Imipenem IMI		Meropenem MERO		Nalidixic acid NAL		Sulfamethoxazole SMX		Tetracycline TET		Tigecycline TGC		Trimethoprim TMP	
Shi EQASIA 21.1	≤ 1	S	≤ 0.015	S	1	S	≤ 0.12	S	≤ 0.03	S	> 64	R	> 512	R	> 32	R	0.5	S	> 16	R
Shi EQASIA 21.2	1	S	≤ 0.015	S	≤ 0.5	S	≤ 0.12	S	≤ 0.03	S	> 64	R	> 512	R	> 32	R	≤ 0.25	S	> 16	R
Shi EQASIA 21.3	≤ 1	S	≤ 0.015	S	2	S	0.25	S	≤ 0.03	S	64	R	≤ 8	S	≤ 2	S	≤ 0.25	S	> 16	R
Shi EQASIA 21.5	≤ 1	S	≤ 0.015	S	1	S	0.25	S	≤ 0.03	S	≤ 4	S	> 512	R	> 32	R	≤ 0.25	S	> 16	R
Shi EQASIA 21.6	≤ 1	S	≤ 0.015	S	1	S	0.25	S	≤ 0.03	S	64	R	> 512	R	> 32	R	0.5	S	> 16	R
Shi EQASIA 21.7	≤ 1	S	≤ 0.015	S	1	S	0.25	S	≤ 0.03	S	> 64	R	≤ 8	S	> 32	R	≤ 0.25	S	> 16	R
Shi EQASIA 21.9	≤ 1	S	≤ 0.015	S	1	S	≤ 0.12	S	≤ 0.03	S	≤ 4	S	> 512	R	> 32	R	≤ 0.25	S	> 16	R
Shi EQASIA 21.11	≤ 1	S	≤ 0.015	S	≤ 0.5	S	0.25	S	≤ 0.03	S	> 64	R	> 512	R	> 32	R	≤ 0.25	S	> 16	R

R, Resistant; S, Susceptible

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

	Amikacin AMK		Cefotaxime FOT		Ceftazidime TAZ		Ciprofloxacin CIP		Colistin COL		Doripenem DOR		Gentamicin GEN	
Aci EQASIA 21.1	≤ 4	S	8	S	4	S	≤ 0.25	S	0.5	S	0.25	S	≤ 1	S
Aci EQASIA 21.2	≤ 4	S	16	S	4	S	> 2	R	0.5	S	> 2	R	8	R
Aci EQASIA 21.3	16	R	> 32	R	> 16	R	> 2	R	≤ 0.25	S	> 2	R	> 8	R
Aci EQASIA 21.4	≤ 4	S	≤ 1	S	2	S	≤ 0.25	S	≤ 0.25	S	≤ 0.12	S	≤ 1	S
Aci EQASIA 21.8	≤ 4	S	16	S	8	S	≤ 0.25	S	0.5	S	> 2	R	≤ 1	S
Aci EQASIA 21.9	> 32	R	> 32	R	> 16	R	> 2	R	> 4	R	> 2	R	> 8	R
Aci EQASIA 21.10	≤ 4	S	4	S	2	S	≤ 0.25	S	≤ 0.25	S	≤ 0.12	S	≤ 1	S
Aci EQASIA 21.11	> 32	R	> 32	R	> 16	R	> 2	R	0.5	S	> 2	R	> 8	R

R, Resistant; S, Susceptible

	Imipenem IMI		Levofloxacin LEVO		Meropenem MERO		Minocycline MIN		Piperacillin/tazobactam P/T4		Tigecycline TGC		Tobramycin TOB	
Aci EQASIA 21.1	≤ 1	S	≤ 1	S	≤ 1	S	≤ 2	S	≤ 8/4	S	≤ 0.25	S	≤ 1	S
Aci EQASIA 21.2	> 8	R	8	R	> 8	R	4	S	> 64/4	R	≤ 0.25	S	8	R
Aci EQASIA 21.3	> 8	R	> 8	R	> 8	R	8	S	> 64/4	R	4	R	> 8	R
Aci EQASIA 21.4	≤ 1	S	≤ 1	S	≤ 1	S	≤ 2	S	≤ 8/4	S	≤ 0.25	S	≤ 1	S
Aci EQASIA 21.8	> 8	R	≤ 1	S	> 8	R	≤ 2	S	32/4	S	≤ 0.25	S	≤ 1	S
Aci EQASIA 21.9	> 8	R	> 8	R	> 8	R	16	R	> 64/4	R	2	R	> 8	R
Aci EQASIA 21.10	≤ 1	S	≤ 1	S	≤ 1	S	≤ 2	S	≤ 8/4	S	0.25	S	≤ 1	S
Aci EQASIA 21.11	> 8	R	> 8	R	> 8	R	> 16	R	> 64/4	R	4	R	> 8	R

R, Resistant; S, Susceptible

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

	Cefoxitin FOX		Chloramphenicol CHL		Ciprofloxacin CIP		Clindamycin CLI		Erythromycin ERY		Fusidate FUS		Gentamicin GEN		Kanamycin KAN		Linezolid LZD		Mupirocin MUP	
Sa EQASIA 21.1	> 16	R	8	S	> 8	R	> 4	R	> 8	R	≤ 0.25	S	> 16	R	> 32	R	≤ 1	S	≤ 0.5	S
Sa EQASIA 21.2	2	S	≤ 4	S	0.5	S	≤ 0.12	S	≤ 0.25	S	≤ 0.25	S	≤ 0.5	S	≤ 4	S	≤ 1	S	≤ 0.5	S
Sa EQASIA 21.3	4	S	8	S	0.5	S	≤ 0.12	S	0.5	S	≤ 0.25	S	≤ 0.5	S	≤ 4	S	2	S	≤ 0.5	S
Sa EQASIA 21.5	4	S	8	S	0.5	S	≤ 0.12	S	0.5	S	≤ 0.25	S	≤ 0.5	S	≤ 4	S	2	S	≤ 0.5	S
Sa EQASIA 21.6	4	S	8	S	0.5	S	≤ 0.12	S	> 8	R	≤ 0.25	S	≤ 0.5	S	≤ 4	S	2	S	≤ 0.5	S
Sa EQASIA 21.7	> 16	R	8	S	> 8	R	0.25	S	> 8	R	≤ 0.25	S	≤ 0.5	S	> 32	R	2	S	≤ 0.5	S
Sa EQASIA 21.8	16	R	64	R	2	R	≤ 0.12	S	0.5	S	> 4	R	≤ 0.5	S	≤ 4	S	2	S	≤ 0.5	S
Sa EQASIA 21.9	> 16	R	8	S	> 8	R	> 4	R	> 8	R	≤ 0.25	S	> 16	R	> 32	R	2	S	≤ 0.5	S

R, Resistant; S, Susceptible

	Penicillin PEN		Quinupristin/dalfopristin SYN		Rifampin RIF		Streptomycin STR		Sulfamethoxazole SMX		Tetracycline TET		Tiamulin TIA		Trimethoprim TMP		Vancomycin VAN	
Sa EQASIA 21.1	> 1	R	≤ 0.5	S	≤ 0.015	S	> 32	R	128	S	≤ 0.5	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.2	1	R	≤ 0.5	S	≤ 0.015	S	8	S	≤ 64	S	≤ 0.5	S	≤ 0.5	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.3	≤ 0.06	S	≤ 0.5	S	≤ 0.015	S	8	S	≤ 64	S	≤ 0.5	S	1	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.5	1	R	≤ 0.5	S	≤ 0.015	S	8	S	≤ 64	S	≤ 0.5	S	1	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.6	> 1	R	≤ 0.5	S	≤ 0.015	S	8	S	≤ 64	S	≤ 0.5	S	1	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.7	> 1	R	≤ 0.5	S	≤ 0.015	S	≤ 4	S	≤ 64	S	≤ 0.5	S	1	S	≤ 1	S	≤ 1	S
Sa EQASIA 21.8	1	R	≤ 0.5	S	≤ 0.015	S	8	S	≤ 64	S	≤ 0.5	S	1	S	2	S	≤ 1	S
Sa EQASIA 21.9	> 1	R	≤ 0.5	S	≤ 0.015	S	> 32	R	512	R	> 16	R	≤ 0.5	S	> 16	R	≤ 1	S

R, Resistant; 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 + clavulanic acid, F/C	--	--
Cefoxitin, FOX	2-8	23-29
Ceftazidime, TAZ	0.06-0.5	25-32
Ceftazidime + clavulanic acid, T/C	--	--
Chloramphenicol, CHL	2-8	21-27
Ciprofloxacin, CIP	0.004-0.016	29-38
Ertapenem, ETP	0.004-0.016	29-36
Gentamicin, GEN	0.25-1	19-26
Imipenem, IMI	0.06-0.25	26-32
Meropenem, MERO	0.008-0.06	28-35
Nalidixic acid, NAL	1-4	22-28
Sulfamethoxazole, SMX	8-32	15-23
Tetracycline, TET	0.5-2	18-25
Tigecycline, TGC	0.03-0.25	20-27
Trimethoprim, TMP	0.5-2	21-28

MIC ranges and disk diffusion ranges are according to CLSI M100 31st 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	18-26
Cefotaxime, FOT	8-32	18-22
Ceftazidime, TAZ	1-4	22-29
Ciprofloxacin, CIP	0.12-1	25-33
Colistin, COL	0.5-4	11-17
Doripenem, DOR	0.12-0.5	28-35
Gentamicin, GEN	0.5-2	17-23
Imipenem, IMI	1-4	20-28
Levofloxacin, LEVO	0.5-4	19-26
Meropenem, MERO	0.12-1	27-33
Minocycline, MIN	--	--
Piperacillin/tazobactam, P/T4	1-8	25-33
Tigecycline, TGC	--	9-13
Tobramycin, TOB	0.25-1	20-26

MIC ranges and disk diffusion ranges are according to CLSI M100 31st 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
Mupirocin, MUP	--	--
Penicillin, PEN	0.25-2	26-37
Quinupristin/dalfopristin	0.25-1	21-28
Rifampin, RIF	0.004-0.016	26-34
Streptomycin, STR	--	14-22
Sulfamethoxazole, SMX	32-128	--
Tetracycline, TET	0.12-1	24-30
Tiamulin, TIA	--	--
Trimethoprim, TMP	1-4	19-26
Vancomycin, VAN	0.5-2	17-21

MIC ranges and disk diffusion ranges are according to CLSI M100 31st edition, Tables 4A-1 and 5A-1

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