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TECHNICAL REPORT

Fourth external quality assessment on antimicrobial susceptibility testing and detection of ESBL-, acquired AmpC-, and carbapenemase-production of *Salmonella*

2018

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Fourth external quality assessment on antimicrobial susceptibility testing and detection of ESBL-, acquired AmpC-, and carbapenemase-production of *Salmonella*, 2018

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Abbreviations

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
DD	Disk Diffusion inhibition zone
ECOFF	Epidemiological Cut-Off Value
EQA	External Quality Assessment
EU/EEA	European Union/European Economic Area
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
FWD	Food- and Waterborne Diseases and Zoonoses
FWD-Net	European Food- and Waterborne Diseases and Zoonoses Network
MIC	Minimum Inhibitory Concentration
NA	Not Applicable
ND	Not Determined
NPHRL	National Public Health Reference Laboratory
NWT	Non-wild type
R	Resistant
S	Susceptible
SSI	Statens Serum Institut
TESSy	The European Surveillance System
WT	Wild type

Executive summary

Since 2008, the countries of the European Union and European Economic Area (EU/EEA) have been able to report antimicrobial resistance (AMR) data to the European Surveillance System (TESSy) as part of the routine surveillance for salmonellosis and campylobacteriosis. In 2014, ECDC published an EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates (updated in 2016). In addition, ECDC launched an external quality assessment (EQA) scheme for antimicrobial susceptibility testing (AST) for *Salmonella* and *Campylobacter*, with the aim of supporting the implementation of the EU protocol in EU Member States and EEA countries and to obtain an overview of the quality of the AMR data reported to ECDC.

This report presents the results of the fourth round of the EQA on AST for national public health laboratories on *Salmonella* (hereafter *Salmonella* EQA4-AST) within the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net). The objectives of the EQA were to:

- determine the accuracy of quantitative AST results reported by participants;
- identify common laboratory problems related to the guidance in the EU protocol; and
- assess the overall comparability of routinely collected AST data from National Public Health Reference Laboratories across Europe.

An additional aim of the EQA was to evaluate the capacity to determine ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC), and carbapenemase pheno and genotypes following the EU protocol for phenotypic characterisation and in-house methods for genotypic characterisation.

Twenty-five National Public Health Reference Laboratories (NPHRLs) in the EU/EEA participated in the EQA, whichtook place between March 2018 and December 2018. Six EU candidate/potential candidate countries (EU enlargement countries) also participated in the EQA. This report focuses only on the results and evaluation from the EU/EEA countries.

Strains for the EQA were selected according to their current relevance to public health in Europe. The EQA included eight *Salmonella* test strains. Testing and reporting of four antimicrobials was mandatory for participation in the EQA:– ampicillin, pefloxacin (when using disk diffusion)/ciprofloxacin (when using dilution methods), cefotaxime and tetracycline. The EQA also included the possibility of submitting results for all antimicrobials specified in the harmonised EU protocol. Only one laboratory did not fulfil the requirements for participation.

Overall, there was good correspondence between the expected results established by the EQA provider and the results reported by the participating laboratories. For all tested antimicrobials the relative accuracy (i.e. the percentage of disk diffusion (DD) and minimum inhibitory concentration (MIC) results that were within the accepted range of the expected result) was 86% (1 248/1 457) for DD results, 83% (176/212) for MIC results generated with gradient strips, and 94% (1 073/1 145) for results generated by micro-broth dilution methods.

For the mandatory antimicrobials, 96% (453/472) of the DD results and 91% (353/386) of the MIC results were correct and in accordance with the expected results. Most of the incorrect MIC results for the mandatory antimicrobials were for ciprofloxacin generated by gradient strips. These MIC values were at a lower concentration than the expected values but were correct when evaluated using the ECOFFs provided by EUCAST. For the optional antimicrobials, 81% (795/985) of the DD results and 92% (896/971) of the MIC results were in accordance with the expected results. When the reported quantitative results were interpreted using the ECOFFs, more than 98% of the DD and MIC results were correct.

Twenty-three of the 25 participating laboratories reported 131 results for the phenotypic characterisation of the eligible test strains for ESBL, acquired AmpC and carbapenemase production. All results, except two, were correct. Twenty-two laboratories reported genotypic results for the carbapenemase producing test strain and 12 laboratories reported results for some or all the ESBL- and pAmpC-producing test strains. Generally, the laboratories were able to assign the correct genotype groups – CTX-M, OXA-48, and CMY-2 – to the test strains, and the laboratories that used partial sequencing or WGS were generally able to assign the correct genotypes to the test strains. Four laboratories submitted genotypic results based on WGS, and all results submitted by these laboratories were correct.

No common laboratory problems related to the guidance in the harmonised EU AST protocol were identified. However, some laboratories did not entirely comply with the protocol, using concentration ranges that did not follow the recommendations set to cover both the EUCAST ECOFF and the clinical breakpoints, while some used disk contents that deviated from those recommended by EUCAST (the latter results were excluded from the report).

The surveillance system implemented as part of TESSy relies on the capacity of the FWD-Net laboratories to produce comparable AST results. The overall results from this *Salmonella* EQA4-AST indicated that it is possible to compare AST results from the European NPHRLs when applying ECOFFs. However, improvements were warranted in a few laboratories. The results from the EQA further showed that many laboratories had the capacity to correctly characterise ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella* strains pheno- and genotypically.

1. Introduction

1.1 Background

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with the mandate to operate the infectious disease networks and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. As part of its mission, ECDC fosters the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre maintains and extends this cooperation and support to the implementation of quality assurance schemes [1].

External quality assessment (EQA) is a part of quality management systems in which the performance of laboratories is evaluated by an external evaluator for material specifically supplied for the purpose.

ECDC supports a series of EQAs for EU/EEA countries within the disease networks. The aim of the EQAs is to identify needs and areas for improvement in laboratory diagnostic capacities and further characterisation relevant to the surveillance of diseases listed in European Commission Implementing Decision 2018/945/EU [2], as well as to ensure the reliability and comparability of results in laboratories from all EU/EEA countries. The main objectives of EQA schemes include:

- assessing the general standard of performance ("state of the art");
- assessing the effects of analytical procedures (method principle, instruments, reagents, calibration);
- evaluating individual laboratory performance;
- identifying and explaining problem areas;
- providing continuing education; and
- identifying needs for training activities.

The unit of Foodborne Infections at Statens Serum Institut (SSI) in Denmark was awarded the framework service contract 'External quality assessment on antimicrobial susceptibility testing (AST) for national public health laboratories for *Salmonella* and *Campylobacter* for the two lots covering *Salmonella*, and *Campylobacter*, for the period 2014–2018. The contract covers the organisation of an EQA exercise for the testing of antimicrobial susceptibility and detection of ESBL-, acquired AmpC and carbapenemase producers in *Salmonella* and species identification and the testing of antimicrobial susceptibility in *Campylobacter* species. This report presents the results of the fourth EQA exercise under this contract (*Salmonella* EQA4-AST).

1.2 Surveillance of *Salmonella* AMR

Antimicrobial resistance (AMR) is a serious threat to public health in Europe, leading to mounting healthcare costs, failures in treatment and deaths. The issue calls for concerted efforts in Member States and also close international cooperation in order to preserve future antimicrobial effectiveness and access to effective treatment for bacterial infections. Surveillance of AMR is a fundamental part of an effective response to this threat, and surveillance results are an essential source of information on the magnitude and trends of resistance.

Salmonellosis is one of the leading causes of zoonotic foodborne diseases in the EU/EEA, with approximately 93 000 laboratory-confirmed cases reported in 2017 [3].

Surveillance of AMR in foodborne human infections in the EU is carried out within the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net), led by ECDC. Since 2008, EU/EEA countries have been able to report AMR data to The European Surveillance System (TESSy) as part of the routine surveillance data for salmonellosis and campylobacteriosis. The European Food Safety Authority (EFSA) also collects AMR data from zoonoses and zoonotic agents in food-producing animals and food, in accordance with Directive 2003/99/EC [4] and Implementing Decision 2013/652/EU [5].

Since 2012, EFSA and ECDC have both strived to harmonise the AMR monitoring in zoonoses and zoonotic agents within their respective areas but also between the areas in order to obtain data that can be compared across the sectors. This work has also been requested by the European Commission as part of the Commission Action Plan on AMR. In connection with this, in 2014 ECDC published an EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates [6], which was updated in 2016 [7] (hereafter harmonised EU AST protocol). The harmonised EU AST protocol is primarily directed towards the National Public Health Reference Laboratories or other nationally recognised public health laboratories to guide the susceptibility testing needed for EU surveillance and the reporting to ECDC.

EU surveillance objectives for antimicrobial resistance in zoonotic bacteria, specifically *Salmonella* spp. and *Campylobacter* spp. are [6,7]:

- to monitor, in human clinical isolates, trends in the occurrence of resistance to antimicrobial agents relevant for treatment of human *Salmonella* and *Campylobacter* infections, including comparison with food/animal isolates;
- to monitor, in human clinical isolates, trends in the occurrence of resistance to other antimicrobial agents of public and animal health importance, including comparison with food/animal isolates;
- to monitor, in human clinical isolates, the prevalence of ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC) and carbapenemase phenotypes;
- to use antimicrobial resistance patterns to characterise human clinical isolates, i.e. as an epidemiological marker, to support identification of outbreaks and related cases;
- to identify and monitor, in human clinical isolates, genetic determinants of resistance that are important for public health e.g. to aid recognition of epidemic cross-border spread of multi-drug resistant *Salmonella* strains; and
- to monitor, in human clinical isolates, trends in the occurrence of resistance to antimicrobial agents that may be important for future therapeutic use.

1.3 Objectives of the EQA4-AST on *Salmonella*

The aim of the EQA4-AST on *Salmonella* was to support the implementation of the harmonised EU AST protocol for monitoring antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates and to assess the quality of AST data obtained using minimum inhibitory concentration (MIC) determinations and/or measurement of disk diffusion inhibition zones (DD) in National Public Health Reference Laboratories (NPHRLs) across Europe.

The *Salmonella* EQA4-AST covered the laboratory procedure when producing AST data on a well characterised test strain. The objectives of the exercise were:

- to determine the relative accuracy of quantitative AST results reported by participating laboratories;
- to identify common laboratory problems related to testing of individual antimicrobials and the guidance in the harmonised EU AST protocol; and
- to assess the overall comparability of routinely collected AST results from NPHRLs across Europe based on the results of the EQA.

The term "relative accuracy" of the quantitative result means that the results from the participating laboratories are compared with an expected result established by the EQA provider. An additional aim of the EQA was to provide an opportunity for the laboratories to evaluate the capacity to determine ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC), and carbapenemase pheno- and genotypes following the harmonised EU AST protocol for phenotypic characterisation and in-house methods for genotypic characterisation.

2. Study design and methods

2.1 Organisation

The EQA, from planning to final reporting, was conducted between March 2018 and December 2018 and included AST of eight *Salmonella enterica* strains.

On 30 April 2018, SSI emailed invitations to the laboratories in the FWD-Net (27 laboratories) that had been nominated as contact points for the EQA by the national focal points for FWD in the FWD-Net. Twenty-five NPHRL in EU/EEA countries accepted the invitation to participate. In addition, six laboratories from EU candidate and potential candidate countries (EU enlargement countries) participated in the EQA. The list of participants is presented in Figure 1 and Annex 1.

The EQA test-strains were sent to the laboratories on 12 July 2018. The participants were asked to submit their results using a web-based submission form. All laboratories were assigned an arbitrary laboratory number by the EQA provider, and these numbers are used throughout this report to ensure the anonymity of the participating laboratories.

2.2 Selection of strain panel

Strains were selected for the EQA4-AST based on the following criteria:

- that they should represent commonly reported strains in the EU/EEA; and
- that they should remain stable during the preliminary testing period in the organising laboratory.

The EQA provider tested 16 *Salmonella* strains and selected eight of these strains with different resistance attributes for the study. In order to determine the accuracy of the reported results, the EQA provider established expected results for MIC (mg/L) and DD (mm) for the test strains. The expected values were established following the harmonised EU AST protocol [6]. The DD values were determined using disks from Oxoid, while the MIC values were determined using the micro-broth dilution-based MIC system from Thermo Scientific's TREK diagnostic systems©. The expected results were verified by the EUCAST Development Laboratory for Antimicrobial Susceptibility Testing of bacteria, Clinical Microbiology, Central Hospital, Växjö, Sweden. The genotypes were established by whole genome sequencing and subsequent mapping using the ARIBA tool (https://github.com/sanger-pathogens/ariba) and the CGE ResFinder database (https://cge.cbs.dtu.dk/services/ResFinder). The characteristics of the *Salmonella* test strains are presented in Table 1.

Table 1. Serotype and resistance profiles of the *Salmonella* EQA4-AST test strains

Strain	Serotype	Microbiological resistance profile[1] (Non-wild type)	Genotype, selected resistance genes
EQA_AST.S18.0001	Monophasic Typhimurium	AMP, CAZ, CHL, COL, CTX, TCY	*bla*CTX-M55*, mcr1*
EQA_AST.S18.0002	Kentucky	AMP, CHL, CIP/PEF, NAL, TCY	
EQA_AST.S18.0003	Enteritidis	SMX	
EQA_AST.S18.0004	Heidelberg	AMP, AZM, CAZ, CHL, CIP/FEP, CTX, SXT, TCY, TMP	*bla*CTX-M123, *qnr*S1
EQA_AST.S18.0005	Kentucky	AMP, CAZ, CIP/PEF, CTX, GEN, NAL, TCY	*bla*CTX-M14b
EQA_AST.S18.0006	Saintpaul	AMP, AZM, CAZ, CHL, CIP/PEF, CTX, GEN, NAL, SMX, SXT, TCY, TMP	*bla*CTX-M55
EQA_AST.S18.0007	Seftenberg	AMP, CTX, ETP, FEP, MEM, TCY, TEM	*bla*OXA48
EQA_AST.S18.0008	Typhimurium	AMP, CTX, TCY, CAZ, SMX, CHL	*bla*CMY2

[1] Based on MIC and according to EUCAST ECOFFs with the exceptions of colistin and cefepime, where the clinical breakpoint was used. For sulfamethoxazole and temocillin, no ECOFF or clinical breakpoints are available from EUCAST.

AMP: ampicillin, AZM: Azithromycin, CTX: cefotaxime, CAZ: ceftazidime, CIP: ciprofloxacin, CHL: chloramphenicol, COL: colistin, ETP: ertapenem, FEP: cefepime, FOX: cefoxitin, GEN: gentamycin, MEM: meropenem, NAL: nalidixic acid, PEF: pefloxacin, SMX: sulfamethoxazole, STX: Trimethoprim + sulfamethoxazole (co-trimoxazole), TEM: temocillin, TCY: tetracycline, TMP: trimethoprim

2.3 Preparation and shipment of the strains

Cultures of the test strains were grown on blood agar and transferred to Stuart's transport medium using cotton swabs. The parcels with the strains in Stuart's transport medium were shipped from SSI on 12 July 2018 and labelled in accordance with the IATA regulations (UN 3373 Biological Substance, Category B).

2.4 Testing and reporting

The EQA4-AST included AST of 16 first-priority and optional antimicrobials listed in the EU protocol [6]. Testing of four of the first priority antimicrobials (ampicillin, ciprofloxacin (MIC)/pefloxacin (DD), cefotaxime and tetracycline) was mandatory and a requirement for participation in the EQA4-AST. There was also an option to test and report pheno- and genotypic characteristics of ESBL-, acquired AmpC- and carbapenemase-producing Salmonella.

Instructions for AMR testing were provided in the invitation letter, in an email following shipment of strains, and in the reporting forms. Participants were asked to follow the harmonised EU AST protocol which, to a large extent, refers to the methods/guidelines recommended by EUCAST, available on EUCAST's website [8]. For MIC determination, it was possible to report results generated with gradient strips and broth dilution methods. No instructions were given regarding genotypic characterisation, as it was anticipated that the laboratories would use their own standard method.

At the same time as test strains were dispatched, the laboratories received an email with a link to an electronic submission form built using Enalyzer software (https://www.enalyzer.com), in which the results could be reported in a fixed format. The deadline for submitting the results was 28 July 2018. This deadline was later extended to 3 August 2018 due to delays in the delivery of strains to some countries. Data reporting included quantitative DD and/or MIC results for antimicrobials, including results for ESBL screening and characterisation purposes. In addition, it was possible to report the predicted phenotype (positive/negative for ESBL, AmpC, carbapenemase) and the resistance genotype (free text field). Data reporting also included information about DD or MIC methods, growth media, brand of disks for DD and brand of gradient strips or panels for MIC determination and methodology used for genotypic characterisation.

2.5 Data analysis

For the phenotypic characterisation, the participating laboratories provided test results, i.e. inhibition zones measured as diameter in mm for disk diffusion methods and MIC values in mg/L for broth dilution and gradient strip methods.

These test results were analysed using different approaches:

1. The laboratories reported their results and these values were compared to the expected results established by the EQA provider, either by calculating mm difference for DD values or the number of dilution differences for MIC values.

2. DD results (values in mm) that were generated with disk loads that deviated from the recommended disk loads were classified as 'not determined' (ND).

3. MIC dilution differences between the reported and expected results were calculated considering several factors:

- If the operator of the reported value was >, results were approximated to = the next dilution step.
- If the operator of the reported value was <=, results were approximated to = the same dilution step.
- If the operator of both the reported value and the expected value were > and the participant's range for a given antimicrobial was wider than that of EQA provider's range, the dilution difference was denoted as "0".
- If the expected result was outside of the range tested by the participant but within the EQA provider's range, the dilution difference could not be calculated.

MIC values generated using gradient strips for MIC determination were transformed on a log2 base scale, rounded to the nearest two-fold dilution, and then retransformed to enable comparison with the results from dilution methods.

The quantitative results were categorised into three groups. The first group, designated correct, included DD results that were within ±3 mm difference from the expected result and MIC results that were within one dilution difference. The second group were results outside the accepted area (incorrect), and the third group included MIC results that were not within the relevant range for comparison with expected results and were therefore classified as ND.

Reported qualitative results were interpreted based on the available EUCAST ECOFFs. This interpretation (Wild type (WT) or Non-wild type (NWT) was compared to the expected result established by the EQA provider. These qualitative results were categorised into three groups. The first group included results that were in compliance with the expected interpretation (correct), the second group included the interpreted results not in compliance with the expected interpretation (incorrect), and the third group included results where this comparison was impossible due to the lack of EUCAST ECOFFs for the antimicrobial (NA). The EUCAST ECOFFs applied can be found in Annex 2.

Eight MIC results were reported as zero, and these results were excluded from the dataset.

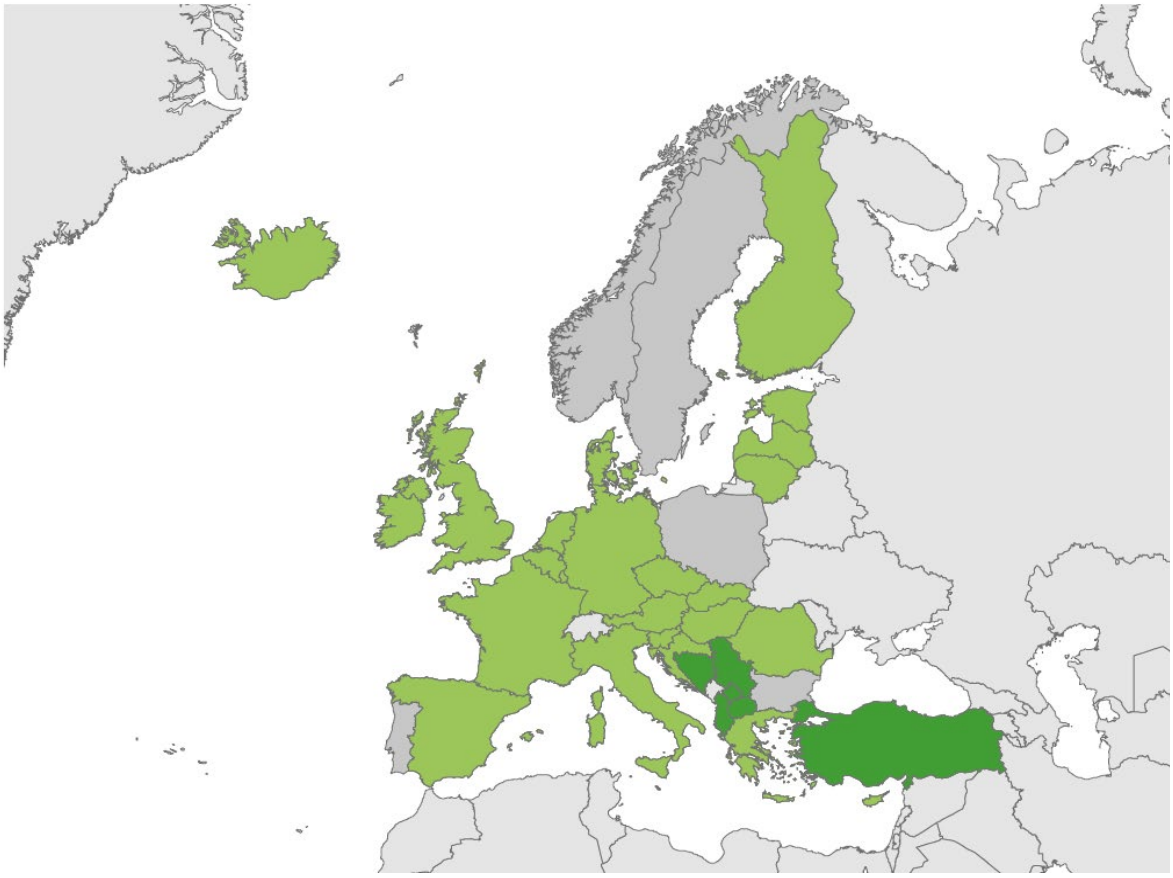
The genotypic results were evaluated on a case-by-case basis. Some laboratories reported genotypes that were meaningless, e.g. "ESBL", and such results were excluded from the dataset.

3. Results

3.1 Participation

Twenty-five laboratories from EU/EEA countries participated in the *Salmonella* EQA4-AST (Figure 1). In addition, six EU candidate/potential candidate countries (EU enlargement countries) also participated. The test results from all participants were evaluated and feedback provided individually on 12 October 2018. All participants also received a file sent by e-mail on 19 October 2018 with the distributions of all reported MIC and DD (mm) values for all test strains/antimicrobials included in the EQA4-AST. This feedback was given to provide the participating laboratories with an opportunity to evaluate their own results and, if needed, make the appropriate corrective measures. This report focuses solely on the results and evaluation of data from the EU/EEA countries.

Figure 1. EU/EEA countries (light green) and EU candidate/potential candidate countries (dark green) participating in the *Salmonella* EQA4-AST, 2018



3.2 Applied methods

3.2.1 Disk diffusion

Eighteen laboratories reported DD results. Most used the disk loads recommended in the harmonised EU AST protocol. For cefotaxime, four laboratories used a disk content of 30 micrograms instead of the recommended five micrograms while for ceftazidime, two used a disk content of 30 micrograms instead of the recommended 10 micrograms. For sulfamethoxazole, several different disk contents were used, and only two laboratories used the recommended concentration of 100 micrograms. The results generated with disk contents that deviated from the recommended concentrations were excluded from the dataset. All laboratories used Mueller Hinton agar for DD susceptibility tests.

Disks from Oxoid were used to produce 63% of the DD results and disks from Becton Dickinson and Bio-Rad were used to generate 12% and nine percent of the results, respectively. Disks from Mastdiscs, i2A diagnostics and Rosco were used to make nine, six, and one percent of the DD results, respectively.

3.2.2 Broth dilution and gradient strip

Twenty-one laboratories reported MIC results. Eighty-five percent of the results were produced using broth dilution methods (BD). Seventy-one percent of the BD MIC results were generated using the Trek Sensititre system: 61% using the EUVSEC 1 or 2 plate format and ten percent using other plate formats from Trek Sensititre. Thirteen percent of the BD results were produced by in-house methods and nine and five percent were produced using materials from Bel-Miditech and VITEK 2 from bioMerieux, respectively. Materials from Biocentric and Micronauts were each used to produce one percent of the results. Gradient strips were used to produce 15% of the total number of MIC results. Of these, gradient strips from Liofilchem were used to generate 63% of the results and the remaining 37% were produced with Etest from bioMerieux. Four laboratories applied MIC concentration ranges that deviated from the recommendations in the harmonised EU AST protocol. This meant that it was impossible to calculate the dilution difference for the quantitative MIC results applying the principles described in section 2.5 and consequently some of the results from these four laboratories were classified as ND.

3.2.3 Genotyping of ESBL, acquired AmpC and carbapenemases

Genotypic characterisation of ESBL-, acquired AmpC- and carbapenemase-producing test strains was performed using PCR, PCR in combination with sequencing, and whole genome sequencing (WGS). One laboratory used an in-house-developed Luminex array-based assay, one laboratory reported a pAmpC genotype generated with Check-Points, and one laboratory reported a pAmpC type established by an "NG lateral flow test". The applied methods are presented in Table 8.

3.3 Antimicrobial susceptibility testing of *Salmonella*

The participation rate for all laboratories with DD and MIC results, as well as the percentage of correct qualitative and quantitative results and the percentage of results classified as ND, are presented in **Table** 2. **Table** 3 gives an overview of the DD and MIC results by antimicrobial, excluding ND results.

All laboratories except one submitted AST results for the mandatory antimicrobials and thus fulfilled the criteria for participating in the EQA (Table 2).

Disk diffusion

Overall, 1 248 of 1 457 (86%) of DD results for the test strains were evaluated as correct and within the accepted 3 mm difference from the expected value (Table 3). The number of correct DD results for the mandatory antimicrobials was 453/472 (96%) and varied by laboratory from 81% to 100% (Table 2). The corresponding numbers for the optional antimicrobials was 795/985 (81%), varying from 50% to 100% by laboratory (Table 2). The proportion of correct qualitative DD results after the interpretation of results using EUCAST ECOFFs was 99% for both the mandatory and the optional antimicrobials, ranging from 97% to 100% for the mandatory antimicrobials and from 95% to 100% for the optional antimicrobials (Table 2 and 3).

Dilution and gradient strip

A total of 1 249 of the 1 357 (92%) quantitative MIC results (excluding ND) were evaluated as correct within one dilution difference from the expected value (Table 3). The number of correct MIC results for the mandatory antimicrobials were 353/386 (91%) and for the optional antimicrobials 896/971 (92%) (Table 3). Another 160 results, reported by six laboratories, were classified as ND because the test ranges did not comply with the recommended test ranges in the harmonised EU AST protocol. Incorrect results, deviating more than one dilution from expected, were reported by 16 laboratories. Four laboratories reported between 10 and 16 incorrect results, and the remaining 17 laboratories reported less than seven incorrect results.

Broth dilution methods were used to generate 1 300 MIC results, of which 1 145 could be evaluated; 1 073 (94%) were evaluated as correct when compared with the expected results. Sulfamethoxazole (14 incorrect of 63, 22%), temocillin (5 of 28, 18%) and trimethoprim (11 of 74, 15%) were the antimicrobials most frequently reported with incorrect broth dilution MIC results.

Gradient strips were used to generate 217 MIC results, of which 212 could be evaluated; 176 (83%) of these results were evaluated as correct. Cefepime (seven incorrect of 16, 44%) and ciprofloxacin (21 of 68, 31%) were the antimicrobials that were most frequently reported with incorrect gradient strip MIC results.

The majority (97%) of 160 results classified as ND were produced with broth dilution methods, and of these 127 of 132 results (96%) were qualitatively correct when interpreted with the EUCAST ECOFF (excluding 23 ND results for which an ECOFF was not available). The five gradient strip results that were classified as ND were all for sulfamethoxazole and from the same laboratory, and when evaluated with EUCAST ECOFFs, were all in accordance with the expected result.

Overall, the proportion of correct qualitative MIC results after the interpretation of results using EUCAST ECOFF was 97% for the mandatory antimicrobials, ranging from 50 to 100% by laboratory, and 99% for the optional antimicrobials, ranging from 75 to 100% by laboratory (Table 2).

Table 2. Laboratories participating (represented by an arbitrary number) from the EU/EEA countries in the *Salmonella* EQA, participation with mandatory antimicrobials by method and percentage correct results for the test strains*

	Disk diffusion									MIC													
	Mandatory						Optional			Mandatory								Optional					
Laboratory number	Ampicillin	Cefotaxime	Pefloxacin	Tetracycline	Correct quantitative results	Correct qualitative results	Number of antimicrobials reported	Correct quantitative results	Correct qualitative results	Ampicillin	Cefotaxime	Ciprofloxacin	Tetracycline	Correct quantitative results	ND quantitative results	Correct qualitative results	ND qualitative results	Number of antimicrobials reported	Correct quantitative results	ND quantitative results	Correct qualitative results	ND qualitative results
L002					97%	100%	11	78%	99%	BD	BD	BD	BD	100%	0%	100%	0%	14	95%	0%	100%	0%
L004										BD		BD	BD	94%	0%	100%	0%	10	94%	0%	100%	0%
L006										BD	BD	GS		33%	38%	88%	4%	5	23%	63%	75%	22%
L007					94%	94%	1	100%		BD	BD	BD		46%	54%	100%	0%	8	63%	28%	100%	0%
L008					100%	100%	4	94%	100%	BD	BD	BD	BD	100%	0%	100%	0%	10	95%	0%	100%	0%
L009					100%	97%	5	90%	97%													
L010					100%	100%	12	77%	99%	BD	BD	BD	BD	100%	0%	100%	0%	15	98%	0%	100%	0%
L012										BD	BD	BD	BD	41%	56%	88%	6%	6	54%	38%	100%	0%
L013					96%	96%	10	82%	98%													
L014					91%	100%	7	89%	100%			GS		100%	0%	100%	0%	1	0%	100%		
L015					97%	97%	3	83%	100%			GS		38%	0%	100%	0%					
L016										BD	BD	BD	BD	100%	0%	100%	0%	12	90%	5%	100%	0%
L017					100%	100%	8	67%	98%									1	100%	0%		
L019					81%	97%	6	48%	100%									1	100%	0%	100%	0%
L020					100%	100%	7	91%	98%			GS		88%	0%	50%	50%	1	100%	0%		
L021					97%	100%	7	75%	100%			GS		100%	0%	100%	0%	1	100%	0%		
L028					94%	100%	8	78%	100%													
L029							1	50%		BD	BD	BD	BD	41%	56%	97%	0%	10	60%	29%	98%	0%
L031					100%	97%	6	92%	100%			GS		100%	0%	100%	0%					
L032					94%	97%	10	90%	98%													
L034										BD	BD	BD	BD	100%	0%	100%	0%	13	93%	0%	100%	0%
L037										GS	GS	GS	BD	78%	0%	97%	0%	10	83%	6%	100%	0%
L039										BD	GS	BD	BD	78%	0%	97%	0%	4	75%	0%	100%	0%
L040					100%	100%	12	89%	95%	GS		GS	GS	100%	0%	100%	0%	15	95%	0%	100%	0%
L043					96%	100%	7	75%	100%			GS		75%	0%	100%	0%	1	100%	0%		
Total					96%	99%		81%	99%					80%	13%	97%	2%		84%	10%	99%	1%

** Results classified as NA were excluded from the total number of results. In a few cases the laboratories did submit results for all eight test strains. L004, L020, L040 and L043 used other disk loads than the recommended, and these results have been excluded.*

: Antimicrobial tested. BD: Broth dilution, GS: Gradient strip.

3.4.1 Results by antimicrobial and strain

Table 3 gives an overview of the DD and MIC results by antimicrobial. The distribution of reported *Salmonella* DD and MIC results from all laboratories for each test strain and the control strain *Escherichia coli* ATCC 25922 are presented in Table 4 and Annex 3 (DD distributions) and Table 5 and Annex 4 (MIC distributions).

EUCAST has defined acceptance criteria for the size of the inhibition zones and MIC values for the control strain *E. coli* ATCC 25922 [9] for all antimicrobials tested except tetracycline, sulfamethoxazole and temocillin. For these

antimicrobials, the reported result for the control strain was compared with expected result established by the EQA provider. Overall, the reported inhibition zones for the control strain were within the accepted range, both for the mandatory and the optional antimicrobials. The reported MIC results for the control strain were also overall in accordance with the expected values with the exceptions of colistin, for which all laboratories reported MIC results that were too high, and the mandatory antimicrobial cefotaxime, for which 10 of 12 laboratories reported values that were too high.

For DD, the lowest scores for the quantitative DD results were observed for nalidixic acid, ertapenem and azithromycin, for which 66%, 67%, and 71% of the results were correct, respectively. The highest scores were achieved for ampicillin, pefloxacin and cefoxitin, with 99% correct results. For nalidicix acid and azithromycin, most of the reported values were higher than the value established by the EQA provider, and for ertapenem the deviating values were rather dispersed. A single laboratory was responsible for five of the seven incorrect DD values reported for tetracycline.

For MIC, the lowest score for the quantitative results was observed for sulfamethoxazole, with 79% correct results, while for eight of 18 antimicrobials, 98–100% of the quantitative results were correct. Twenty-three incorrect MIC results were reported for ciprofloxacin, of which 21 were generated by gradient strips. The incorrect gradient strip MIC results were all two or three dilution steps lower than the expected value, but correctly evaluated when interpreted when using the EUCAST ECOFFs.

The overall proportions of correct results when interpreted with EUCAST ECOFFs were 99% for DD and 100% for MIC respectively, ranging from 95–100% for all antimicrobials except nalidixic acid, for which the interpretation of DD values only resulted in 71% correct results.

Table 3. Performance per antimicrobial for DD and MIC

Antimicrobials	Number of laboratories performing DD	Numbers of DD results within the accepted 3 mm difference of the total tested	Number of correct results using EUCAST ECOFF	Number of laboratories performing MIC	Numbers of MIC results within the accepted 1 dilution difference of the total tested	Number of correct results using EUCAST ECOFF
Ampicillin	15	119/120 (99%)	120/120 (100%)	13	75/75 (100%)	104/104 (100%)
Cefotaxime	13	94/104 (90%)	103/104 (99%)	13	99/101 (98%)	99/101 (98%)
Ciprofloxacin		-	-	19	110/133 (83%)	147/148 (99%)
Pefloxacin	15	119/120 (99%)	120/120 (100%)	-	-	-
Tetracycline	16	121/128 (95%)	122/128 (95%)	11	69/77 (90%)	84/88 (95%)
Total mandatory		453/472 (96%)	465/472 (99%)		353/386 (91%)	434/441 (98%)
Azithromycin	3	17/24 (71%)	24/24 (100%)	8	64/64 (100%)	NA
Cefepime	9	55/72 (76%)	NA	9	48/58 (83%)	NA
Cefoxitin	11	87/88 (99%)	88/88 (100%)	9	59/65 (91%)	70/71 (99%)
Ceftazidime	13	86/104 (83%)	104/104 (100%)	13	90/96 (94%)	104/104 (100%)
Chloramphenicol	12	90/96 (94%)	96/96 (100%)	10	69/70 (99%)	80/80 (100%)
Colistin	-	-	-	15	111/112 (99%)	NA
Ertapenem	8	42/63 (67%)	NA	8	46/54 (85%)	NA
Gentamicin	14	84/112 (75%)	112/112 (100%)	12	81/82 (99%)	96/96 (100%)
Meropenem	15	99/120 (83%)	120/120 (100%)	12	60/68 (88%)	89/89 (100%)
Nalidixic acid	8	42/64 (66%)	17/24 (71%)	7	54/54 (100%)	56/56 (100%)
Sulfamethoxazole	2	14/16 (88%)	NA	9	52/66 (79%)	NA
Temocillin	3	9/10 (90%)	NA	4	23/28 (82%)	NA
Tigecycline	3	21/24 (88%)	23/24 (96%)	9	71/72 (99%)	72/72 (100%)
Trimethoprim	11	75/88 (85%)	86/88 (98%)	11	68/82 (83%)	87/88 (99%)
Trimethoprim-sulfamethoxazole	13	74/104 (71%)	NA		-	NA
Total optional		795/985 (81%)	670/680 (99%)		896/971 (92%)	654/656 (100%)
Total (mandatory + optional)		1 248/1 457 (86%)	1 135/1 152 (99%)		1249/1357** (92%)	1 088/1 097 (99%)

** Results classified as NA and ND excluded*
*** The laboratories reported 1 517 MIC results of which 1 357 could be evaluated using the principles described in section 2.5*

Table 4. Distribution of *Salmonella* DD values (mm) of the participating laboratories in the EQA4-AST

Antimicrobial	Strain	Disk diffusion results for the *Salmonella* strains tested / mm																																		
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Ampicillin	ATCC 25922										2		2	2	4	3	1	1																		
	S18.0001	15																																		
	S18.0002	15																																		
	S18.0003															1			6	5	2	1														
	S18.0004	15																																		
	S18.0005	15																																		
	S18.0006	15																																		
	S18.0007	15																																		
	S18.0008	15																																		
Cefotaxime	ATCC 25922																				2	2	1	2	3	2	1									
	S18.0001	13																																		
	S18.0002																	1		1	2	1	1	3	2	1				1						
	S18.0003																			2	2	1	1		2	2	3									
	S18.0004	13																																		
	S18.0005	13																																		
	S18.0006	13																																		
	S18.0007	1								1	3	2	2	2	1	1																				
	S18.0008	9	1	1	1	1																														
Pefloxacin	ATCC 25922																						1	4	4	2	2	2								
	S18.0001																					1	7	4	1	1	1									
	S18.0002	15																																		
	S18.0003																			1	2	1	3	5	1	2										
	S18.0004										1	1	2	6	4		1																				
	S18.0005	15																																		
	S18.0006									1	1	3	4	4	2																					
	S18.0007																					3	5	4	1	2										
	S18.0008																					1	6	4	1	3										
Tetracycline	ATCC 25922	1															1	2	3	3	3	1														
	S18.0001	14		2																																
	S18.0002	11	4	1																																
	S18.0003	1												1		2	3	4	2	2	1															
	S18.0004						1			3	3	4		3	1	1																				
	S18.0005	15	1																																	
	S18.0006	14	2																																	
	S18.0007	16																																		
	S18.0008	16																																		

*: Expected value *: Accepted range*

The red line indicates the ECOFF according to EUCAST for the respective antimicrobial; WT strains to the right of the red line.
Values for ATCC 25922 from EUCAST, except for tetracycline, where the expected value was established by the EQA provider.

Table 5. Distribution of MIC values (mg/L) of participating laboratories in the EQA4-AST

Antimicrobial	Strain	ND	MIC results / mg/L																
			0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Ampicillin	ATCC 25922	32										2	6	4						
	S18.0001															1	3	7		2
	S18.0002															1	3	8		1
	S18.0003										4	9								
	S18.0004															1	3	7		2
	S18.0005															1	3	7		2
	S18.0006															1	3	7		2
	S18.0007															1	3	7		2
	S18.0008															1	3	8		1
Cefotaxime	ATCC 25922	3				1	1		8		2									
	S18.0001													4		1	3	5		
	S18.0002							3	7	1	1							1		
	S18.0003						2	1	8		2									
	S18.0004													4		1	3	5		
	S18.0005													4		1	5	3		
	S18.0006													4		1	4	4		
	S18.0007									1		8	4							
	S18.0008													5	2	6				
Ciprofloxacin	ATCC 25922	15		4	10	2	1		1											
	S18.0001			3	3	11	1													
	S18.0002										1	1	2	8	6		1			
	S18.0003		1	3	3	9	2													
	S18.0004						1	3	4	10				1						
	S18.0005											1		12	5	1				
	S18.0006							3	6	10										
	S18.0007		1	2	3	11	1													
	S18.0008		1	3	5	8	1													
Tetracycline	ATCC 25922	11								1	2	7								
	S18.0001														1	2		7		1
	S18.0002														1	2	4	3		1
	S18.0003										2	9								
	S18.0004												1	2	3	3	1			1
	S18.0005														1	2	1	6		1
	S18.0006														1	2		7		1
	S18.0007													1		1		8		1
	S18.0008														1	1	1	7		1

*: Expected value *: Accepted range*

The red line indicates ECOFF according to EUCAST for the respective antimicrobial; WT strains to the left of the red line

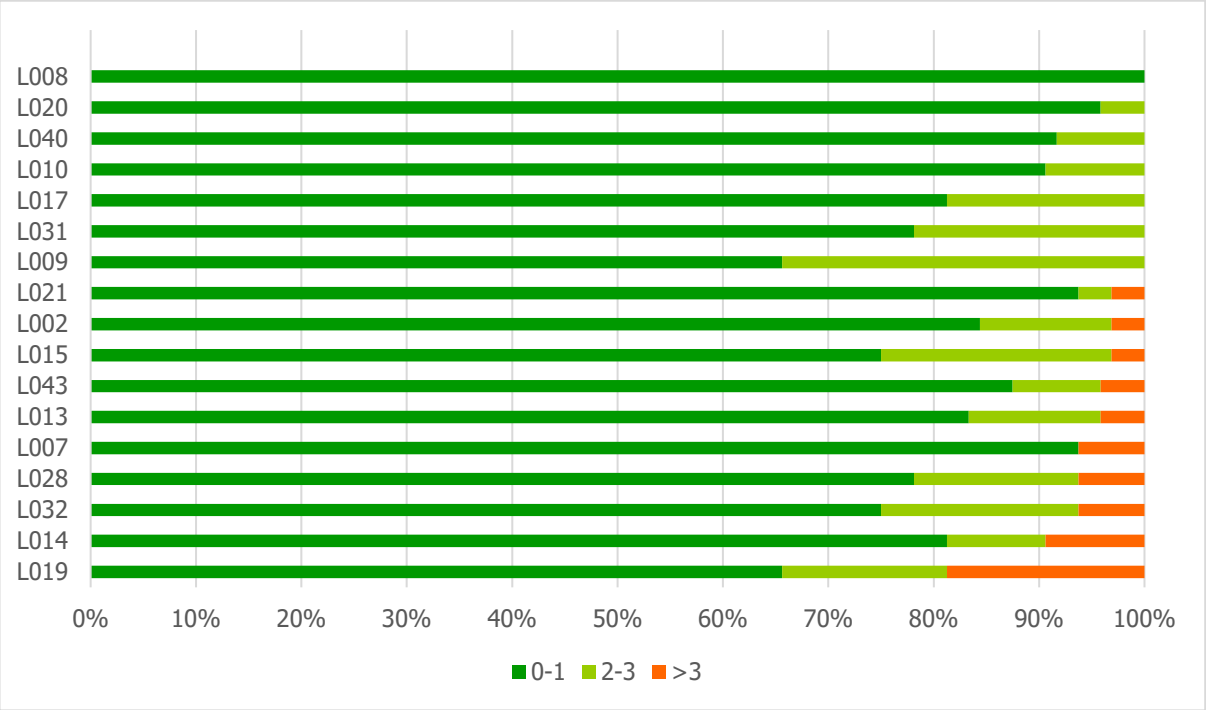
Values for ATCC 25922 from EUCAST, except for tetracycline, where the expected value was established by the EQA provider

3.4.2 Individual laboratory results

3.4.2.1 Disk diffusion

The performance of each of the 17 laboratories that reported DD results for mandatory antimicrobials is presented in Figure 2. Seven laboratories reported 100% correct DD results, and only one laboratory (L019) reported less than 90% correct results, i.e. results that deviated more than three millimetres from the expected value.

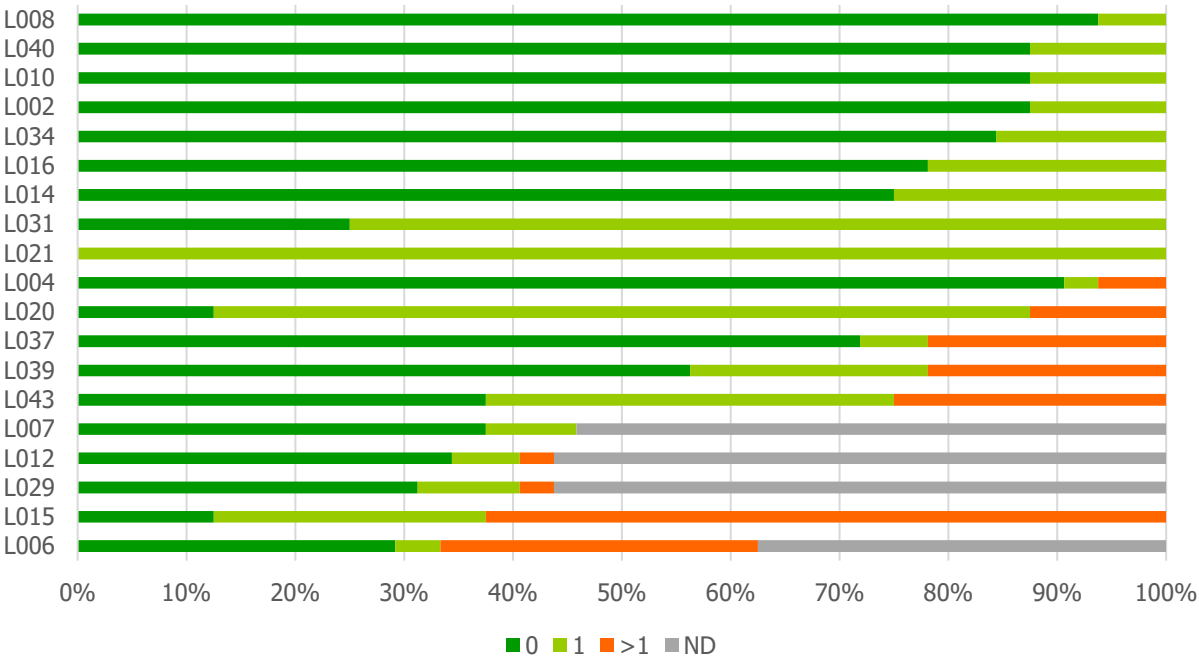
Figure 2. Distribution of DD (mm) differences compared to the expected results for the mandatory antimicrobials for *Salmonella* by laboratory



3.4.2.2 Dilution and gradient strip

The performance of each of the 19 laboratories reporting MIC results for the mandatory antimicrobials is shown in Figure 3. Nine laboratories reported MIC results that were all in accordance with the expected value and five laboratories reported between 75% and 94% correct results. Laboratory L015 reported MIC results using gradient strips for ciprofloxacin and five of eight results were incorrect. Four laboratories reported between nine and 18 MIC results that could not be evaluated as the expected value was outside the concentration range tested (ND).

Figure 3. Distribution of MIC dilution differences compared to the expected results for the mandatory antimicrobials for *Salmonella* by laboratory



3.4.3 ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella*

Twenty-three of the 25 participating laboratories reported phenotypic results for some or all the test strains on ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella*. Ninety-eight percent (129/131) of the reported results were correct (Table 6). One laboratory incorrectly identified the test strains S18.0004 and S18.0008 as both ESBL and pAmpC.

The phenotypes were established in part by using the results of the synergy testing, where the antimicrobials cefotaxime, ceftazidime and cefepime were tested in combination with clavulanic acid. The reported results of the synergy testing for both DD and MIC are presented in Table 7, where values to the right of the dashed lines are considered as a positive synergy tests. The highest number of synergy tests were performed for cefotaxime/clavulanic acid on the four ESBL test strains, where 16 synergy tests were reported. For cefotaxime/clavulanic acid and ceftazidime/clavulanic acid, one and six results, respectively, were incorrect when compared with the results established by the EQA provider. All reported results for cefepime/clavulanic acid were correct.

Table 6. Laboratories reporting phenotypic prediction of ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella*

Strain	Expected phenotype	Number of laboratories reporting correct phenotype	AmpC	Carbapene-mase	ESBL	ESBL, AmpC
EQA_AST.S18.0001	ESBL	22/22 (100%)			22	
EQA_AST.S18.0002	Negative					
EQA_AST.S18.0003	Negative					
EQA_AST.S18.0004	ESBL	21/22 (95%)			21	1
EQA_AST.S18.0005	ESBL	22/22 (100%)			22	
EQA_AST.S18.0006	ESBL	22/22 (100%)			22	
EQA_AST.S18.0007	Carbapenemase (ESBL, pAmpC)	22/22 (100%)		22		
EQA_AST.S18.0008	pAmpC	20/21 (95%)	20			1

: *Expected phenotype*

Table 7. Distribution of synergy test results of the participating laboratories

		Synergy test (+/-clavulanic acid)							
		DD - Zone difference (mm)				MIC ratio			
Strain		0-1	2-4	5-7	>7	< 2	2-7	8-16	> 16
Cefotaxime	S18.0001				8			3	5
	S18.0002								
	S18.0003								
	S18.0004				8			2	6
	S18.0005				8			1	7
	S18.0006				7			2	7
	S18.0007						2		
	S18.0008	1	2		1	4	2		
Ceftazidime	S18.0001				6		1	2	5
	S18.0002								
	S18.0003								
	S18.0004				6		1	2	5
	S18.0005			4	2		1	6	
	S18.0006				6		1	2	5
	S18.0007					2	0		
	S18.0008	1	3	1		4	0	1	
Cefepime	S18.0001				1			1	2
	S18.0002								
	S18.0003								
	S18.0004				1			1	1
	S18.0005				1				2
	S18.0006				1			1	2
	S18.0007								
	S18.0008								

: Expected value : Accepted value
Values to the right of the dashed line are considered as a positive synergy test
The EQA provider did not establish expected MIC values for cefepime/clavulanic acid

The laboratories could also report the predicted genotypes of the test strains, including what methods that were used to determine the genotypes, see Table 8. The genotypes of the four ESBL strains (S18.0001 and S18.0004-6) were reported by 12 laboratories and the genotypes of the pAmpC-producing strain (S18.0008) and the carbapenemase-producing strain (S18.0007) were reported by 11 and 22 laboratories, respectively. The reported results are a reflection of the resolution of the applied methods. The laboratories that used PCR were able to identify that the ESBL-producing strains contained CTX-M genes, whereas the correct assignment of the specific CTX-M number could only be achieved if the PCR method was supplemented with DNA sequencing or DNA arrays. All PCR-based methods were able to identify the OXA-48 and CMY-2 genes present in S18.0007 and S18.0008. The expected genotypes established by the EQA provider were based on WGS, and the four laboratories that applied WGS were all able to correctly identify the genotypes of the test strains. The laboratories that used "Check-Points'" (Check-Points BV, Wageningen, NL) and "NG lateral flow test" (bioTRADING Benelux B.V, Mijdrecht, NL) were able to correctly assign the genotype OXA-48 to the test strain S18.0007. One laboratory reported S18.0005 as positive for the OXA-48 genotype, and this result was incorrect.

Table 8. Genotypes predicted and method used for prediction of ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella*

Strain	Expected genotype	Method used for genotype prediction	Genotype predicted (number of laboratories)
S18.0001	CTX-M55	PCR	CTX-M (6)
		PCR/sequencing	CTX-M-55 (1)
		WGS	CTX-M-55 (4)
		In-house Luminex assay	CTX-M-15 (1)
S18.0004	CTX-M123	PCR	CTX-M (3)
		PCR	CTX-M1 (1)
		PCR	CTX-M9 (2)
		PCR/sequencing	CTX-M-123 (1)
		WGS	CTX-M-123 (3)
		WGS	CTX-M-123, CTX-M-132[v] (1)
		In-house Luminex assay	CTX-M-15 (1)
S18.0005	CTX-M14	PCR	CTX-M (1)
		PCR	CTX-M-9 (3)
		PCR	OXA48 (1)
		PCR/sequencing	CTX-M-14b (1)
		WGS	CTX-M-14 (4)
		In-house Luminex assay	CTX-M-9/14 group (1)
S18.0006	CTX-M55	PCR	CTX-M (6)
		PCR/sequencing	CTX-M-55 (1)
		WGS	CTX-M-55 (4)
		In-house Luminex assay	CTX-M-1 (1)
S18.0007	OXA-48	PCR	OXA-48 (15)
		Check-Points	OXA-48 (1)
		PCR/sequencing	OXA-48 (1)
		WGS	OXA-48 (3)
		NG lateral flow test*	OXA-48 (1)
		In-house Luminex assay	OXA-48 (1)
S18.0008	CMY-2	PCR	CMY-2 (2)
		PCR	CMY (1)
		PCR	CIT (2)
		PCR/sequencing	CMY-2 (2)
		WGS	CMY-2 (3)
		In-house Luminex assay	CMY-2 (1)

** Immunologically based assay*

4. Discussion

In 2014, ECDC published a harmonised EU AST protocol (updated in 2016) with guidance on laboratory procedures and the interpretation of antimicrobial susceptibility data [6,7]. The purpose of the EQA4-AST on *Salmonella* was to evaluate the quality of the AST data generated in the FWD laboratory network when following the harmonised EU AST protocol. The EQA also gave the laboratories the opportunity to test and report the detection and confirmation of ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella* following the guidance provided in the harmonised EU AST protocol or following in-house methods for genotypic characterisation. An additional aim of the EQA was to collect information on the methods used by each laboratory to produce data on antimicrobial susceptibility.

Twenty-five of 27 invited laboratories from EU/EEA countries participated in the EQA and all laboratories, except one, submitted results for the mandatory antimicrobials, ampicillin, ciprofloxacin (MIC)/pefloxacin (DD), cefotaxime and tetracycline, thereby fulfilling the requirement for participation in the EQA4-AST. The laboratory that did not fulfil this requirement did not report results for ciprofloxacin/pefloxacin. Twenty-three laboratories reported phenotypic characterisation for ESBL, acquired AmpC and carbapenemase production for some or all of the test strains and 18 laboratories reported genotypic characterisation for the genes encoding ESBL, acquired AmpC and carbapenemase production. The participation rate was in line with the EQA3-AST performed in 2017, in which 27 laboratories participated.

The logistics of the EQA were a success. All the laboratories were able to recover the test strains, and all were able to submit their results using the Enalyzer platform. Furthermore, there was good overall correspondence between the quantitate results reported for the different antimicrobials and the expected results established by the EQA provider. With a few exceptions, the test strains exhibited DD zones and MIC values that were distinct from the ECOFF values, and consequently the interpreted qualitative results were generally better than the quantitative results. One of the exceptions was strain S18.005, where the expected result for tetracycline was close to the ECOFF and two incorrect qualitative MIC results were correct when interpreted quantitatively.

A broadly equal number of DD and MIC results were reported by the participating laboratories. Some laboratories used disk loads that deviated from that recommended, most prominently for sulfamethoxazole, but a few laboratories also used deviating disk loads for cefotaxime and ceftazidime. For the cephalosporins, it is important that the laboratories use the recommended disks with low content for ESBL-screening purposes and use the recommended higher disk content for synergy-testing. Two laboratories reported MIC values for cefotaxime and meropenem, respectively, where the ECOFFs were not covered by the applied concentration range. In general, the reported results for the E. coli control strain ATTC25922 were in accordance with the accepted range specified by EUCAST.

For both the mandatory and the optional antimicrobials, 98% to 100% of the results were correct after interpretation using the EUCAST ECOFFs. This indicates that the overall quality of the reported AST results are acceptable, and in line with the results obtained in the most recent EQA on antimicrobial resistance organised by the EU Reference Laboratory for the national reference laboratories in the food/animal field in 2017 [10].

When excluding MIC results that were classified as ND, the results produced by broth dilution methods had a higher accuracy than results made with gradient strips and disks, with 94% (1073/1145) of the quantitative MIC results from broth dilution being correct compared to 83% (176/212) of the results generated with gradient strips and 86% (1248/1457) of the DD results (mandatory and optional antimicrobials combined). The harmonised EU AST protocol recommends (micro-) broth dilution as the preferred testing method for monitoring purposes but validated methods of gradient strip diffusion or DD in accordance with EUCAST protocols are also accepted. The data from this EQA support the EUCAST recommendation on choice of methods.

The expected MIC results were determined using a micro-broth dilution method applying the two-fold dilution range recommended in the harmonised EU AST protocol. Six laboratories reported 160 MIC results that were classified as ND because the test range was narrower than recommended. This meant that it was impossible to calculate the dilution difference. However, most of the reported ND MIC results were meaningful and evaluated as qualitatively correct when interpreted using the EUCAST ECOFFs. It can nevertheless be concluded that six laboratories did not follow the recommendations for the concentration range as specified in the harmonised EU AST protocol.

It is always important that the EUCAST-defined acceptance criteria for the control strain, *E. coli* ATCC 25922, are fulfilled since the validity of the susceptibility results can otherwise be hampered. Overall, the reported results for the control strain were within the accepted criteria for inhibition zones and MIC values, but deviations were observed, especially for MIC values for cefotaxime and colistin.

It could be argued that gradient strip and disk diffusion results are more related than gradient strip and broth dilution methods, as they both rely on diffusion of the antimicrobial into agar-based media. This could also lead to the assumption that it would be problematic to use ciprofloxacin in gradient strip-based MIC assays, as EUCAST recommends pefloxacin disks to test for (low-level) quinolone susceptibility. For this EQA, the incorrect ciprofloxacin results produced by gradient strips all had a MIC that was too low, but that were correct when interpreted using ECOFFs. This indicates that gradient strips are acceptable for testing the strains included in this EQA.

The percentage of correct results for sulfamethoxazole when using MIC was lower than for DD. This could indicate that it is difficult to determine the minimal inhibitory concentration when using broth dilution methods for sulfamethoxazole.

The fact that several DD and MIC results deviated slightly from the expected results, most notably for the susceptible strains, did not lead to incorrect results when interpreted with EUCAST ECOFFs.

The EQA included one meropenem-resistant strain, S18.0007, and all the laboratories were able to identify this strain as resistant. This indicates that the laboratories are capable of monitoring for carbapenemase-producing *Salmonella*.

The number of DD and MIC results evaluated as correct were overall in line with the results seen in the EQA3-AST performed in 2017. Some variation was observed in the number of reported correct MIC and DD results from the different laboratories. A few laboratories reported DD and MIC results that all were in accordance with the expected results and this indicates that there is a best practice and it is feasible to improve the quality of AST data generated by the FWD laboratories.

In the harmonised EU AST protocol [6], phenotypic testing is proposed on isolates resistant to either cefotaxime, ceftazidime or meropenem for detection and confirmation of ESBL-, acquired AmpC- and carbapenemase-producing *Salmonella*. The proposed phenotypic testing includes testing of cefoxitin, cefepime and meropenem, as well as synergy testing with clavulanic acid for cefotaxime, ceftazidime and cefepime, to assess the inhibitory effect of clavulanic acid on beta-lactamase activity. Synergy is observed if the presence of clavulanic acid increases zone diameters by at least 5 mm or if the MIC ratio is ≥ 8, i.e. the MIC result when testing the antimicrobial agent alone against testing it in combination with clavulanic acid (e.g. MIC CTX / MIC CTX+clavulanic acid).

The *Salmonella* test strains in this EQA included four strains that were ESBL producers, one strain that was AmpC-producing, and one strain that was carbapenemase-producing. Twenty-three of the 25 participating laboratories reported results for phenotypic characterisation for the six eligible strains, and all results, except two, were correct. However, the number of laboratories that reported results for synergy testing were considerably lower, and it was not possible to derive all the reported phenotypes from the test results reported to the EQA provider.

Twenty-two laboratories reported results of the genotypic characterisation for some or all of the six strains eligible for further characterisation. The laboratories used different methods with different specificity. Generally, the laboratories were able to assign the correct genotype group, CTX-M, OXA-48, and CMY-2, to the test strains and the laboratories that did further characterisation of amplified DNA sequences (sequencing or array analysis) were overall able to assign the correct genotypes to the test strains. Four laboratories submitted results based on WGS and these genotype results were all correct.

In conclusion, the majority of participating laboratories succeeded in identifying ESBL-, acquired AmpC- and carbapenemase-producing test strains, however not all laboratories participated in this part of the EQA and some participants did not characterise the genotype to the extent needed to identify and monitor such strains nationally and at the EU level. The use of different methodologies for genotypic characterisation made it difficult to compare results, but it seems evident that especially WGS and PCR in combination with further characterisation of the amplified DNA are strong tools for genotypic characterisation.

5. Conclusions

Twenty-five laboratories from EU/EEA countries participated in the EQA4-AST and all laboratories, except one, submitted results on all eight tests strains for the mandatory antimicrobials, ampicillin, pefloxacin (DD)/ciprofloxacin (MIC), cefotaxime and tetracycline, thereby fulfilling the requirement for participation in the EQA. The laboratory that did not fulfil the requirement did not report results for pefloxacin/ciprofloxacin.

Overall, there was good correspondence between the expected results established by the EQA provider and the results reported by the participating laboratories. The relative accuracy, i.e. the percentage of DD and MIC results that were within the accepted range of the expected result, were 86% (1 248/1 457) for DD results, 83% (176/212) for MIC results generated with gradient strips and 94% (1 073/1 145) for results generated by micro-broth dilution methods, when including all antimicrobials and excluding ND results.

For the mandatory antimicrobials, 96% (453/472) of the quantitative DD results and 91% (353/386) of the MIC results were correct and in accordance with the expected results. Most of the incorrect MIC results for the mandatory antimicrobials were for ciprofloxacin generated by gradient strips. These results were at a lower concentration than the expected results but were all correct when evaluated using the EUCAST ECOFFs. For the optional antimicrobials, 81% (795/985) of the quantitative DD results and 92% (896/971) of the MIC results were in accordance with the expected results. When the reported quantitative results for the mandatory antimicrobials were interpreted using ECOFFs more than 98% of the DD and MIC results were correct. These results indicate that it is possible to compare routinely collected DD and MIC AST results from NPHRLs across Europe.

Twenty-three of the 25 participating laboratories reported 131 results for the phenotypic characterisation of the eligible test strains for ESBL, acquired AmpC and carbapenemase production. All results, except two, were correct. However, the number of laboratories that reported results for synergy testing were considerably lower, and it was not possible to derive all the reported phenotypes from the results submitted to the EQA provider.

Twenty-two laboratories reported genotypic results for the carbapenemase-producing test strain and 12 laboratories reported results for some or all the ESBL and pAmpC-producing test strains. The laboratories used several different methods with different specificities. In general, the laboratories were able to assign the correct genotype groups, CTX-M, OXA-48, and CMY-2, to the test strains, and those laboratories that used partial sequencing or WGS were generally able to assign the correct genotypes to the test strains. Four laboratories submitted genotypic results based on WGS, and all results submitted by these laboratories were correct.

No common laboratory problems related to the guidance in the harmonised EU AST protocol were identified, but some laboratories did not comply entirely to the protocol, as many laboratories established MIC results using concentration ranges that did not follow the recommendations and some used disk contents that deviated from the recommended (the latter results were excluded from the report). The reported results of the genotypic characterisation reflect the fact that there is no common protocol for genotypic characterisation of ESBL-, acquired AmpC and carbapenemase-producing test strains.

6. Recommendations

6.1 Laboratories

Some laboratories are not strictly following the recommendations in the harmonised EU AST protocol [6], e.g. several are testing too narrow a concentration range when establishing MIC values, while some use other disk contents than those recommended in disk diffusion. This EQA shows that, when following the recommended concentration range, results generated by MIC broth dilution methods are more accurate than the diffusion-based methods and if possible, laboratories should consider implementing broth dilution methods. Several laboratories, both for DD (7/17) and MIC (9/19), submitted results that were 100% in accordance with the expected values established by the EQA provider. This indicates that it should be possible for other FWD laboratories to improve the quality of their AST data. It would be preferable if all laboratories participated in the identification and characterisation of ESBL-, acquired AmpC- and carbapenemase-producing test strains, and participating laboratories should report results that can justify the designated phenotype.

6.2 ECDC and FWD-Net

It is important to support the use of standardised testing methods and standardised interpretation of data in the Member States in order to enhance the comparability of AST data reported to TESSy. Furthermore, there is a need to further develop and standardise the reporting scheme for the characterisation of ESBL-, AmpC- and carbapenemase-producing *Salmonella*.

6.3 The EQA provider

The current reporting scheme should be further developed for a more detailed and uniform collection of relevant information about the applied material and methods. The reporting scheme also needs further development to accommodate the data on pheno- and genotypic characterisation of ESBL-, AmpC- and carbapenemase-producing *Salmonella*, both to improve feedback to participants and for the extraction and comparison of results in the final report.

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Annex 1. List of participants

Country	EU status	Name of laboratory	Name of institution
Albania	Enlargement	Laboratory of Enterobacteriology	Institute of Public Health
Austria	EU/EEA	NRC *Salmonella* Austria	Institute for Medical Microbiology and Hygiene, Graz
Belgium	EU/EEA	Bacterial Diseases	Sciensano
Croatia	EU/EEA	Department for clinical microbiology	University Hospital for Infectious Diseases
Cyprus	EU/EEA	Reference Laboratory for *Salmonella* and other Enteric Pathogens	Nicosia General Hospital
Czechia	EU/EEA	National reference laboratory for antibiotics	National Institute of Public Health
Denmark	EU/EEA	Diagnostic and Typing of Gastrointestinal Bacteria	Statens Serum Institut
England	EU/EEA	Gastrointestinal Bacteria Reference Unit	National Infection Service
Estonia	EU/EEA	Laboratory of Communicable Diseases	Health Board
Finland	EU/EEA	Expert Microbiology	National Institute for Health and Welfare
France	EU/EEA	NRC *E. coli*, *Shigella* and *Salmonella*	Institut Pasteur
Germany	EU/EEA	FG11	Robert Koch Institute
Greece	EU/EEA	National Reference Centre for *Salmonella*	National School of Public Health
Hungary	EU/EEA	Department of Phagetyping and Molecular Epidemiology	National Health Institute
Iceland	EU/EEA	Dept. of Clinical Microbiology	Landspítali University Hospital
Ireland	EU/EEA	National *Salmonella*, *Shigella* and *Listeria* Reference Lab (NSSLRL)	NSSLRL
Italy	EU/EEA	Antimicrobial resistance and special pathogens	Istituto Superiore di Sanità
Kosovo	Enlargement	Microbiology	National Institute of Public Health of Kosovo
Latvia	EU/EEA	National Microbiology Reference Laboratory of Latvia	Riga East University Hospital
Lithuania	EU/EEA	National Public Health Surveillance Laboratory	National Public Health Surveillance Laboratory
Luxembourg	EU/EEA	Bacteriologie-Mycologie-Antibiorésistance-Hygiéne hospitalière	Laboratoire National de Santé
Macedonia	Enlargement	Laboratory of bacteriology and AMR	Institute of public health of Macedonia
Malta	EU/EEA	Bacteriology Laboratory	Mater Dei Hospital
Republic of Serbia	Enlargement	Reference Laboratory for *Salmonella*, *Shigella*, *Vibrio cholerae* and *Yersinia enterocolitica*	Institute of Public Health of Serbia
Republic of Srpska, Bosnia and Herzegovina	Enlargement	Department of Microbiology	Public Health Institute of The Republic of Srpska
Romania	EU/EEA	Bacterial Enteric Infections Laboratory	National Institute of Medico-Military Research and Development Cantacuzino
Slovak Republic	EU/EEA	NRC for Salmonelloses, NRC for ATB resistance monitoring	Public Health Authority of the Slovak Republic, Dpt. for Medical Microbiology
Slovenia	EU/EEA	Department for medical microbiology, Celje	National Laboratory of Health, Environment and Food
Spain	EU/EEA	Unidad de Enterobacterias	Centro Nacional de Microbiología
The Netherlands	EU/EEA	NRL on AMR in animals	Wageningen Bioveterinary Research
Turkey	Enlargement	National Reference Laboratory for Enteric Pathogens	General Directorate of Public Health

Annex 2. EUCAST ECOFFs and clinical breakpoints used for EQA4-AST, 2018

	MIC determination (µg/mL)				Disk Diffusion (mm)			
Antimicrobial agent	EUCAST ECOFF		EUCAST Clinical breakpoint		EUCAST ECOFF		EUCAST Clinical breakpoint	
Mandatory	S/WT ≤	R/NWT >	S ≤	R >	S/WT ≥	R/NWT <	S ≥	R <
Ampicillin	8	8	8	8	18	18	14	14
Cefotaxime	0.5	0.5	1	2	20	20	20	17
Ciprofloxacin	0.064	0.064	0.06	0.06				
Pefloxacin					24	24	24	24
Tetracycline	8	8			17	17		
Optional								
Azithromycin					12	12		
Ceftazidime	2	2	1	4	20	20	22	19
Cefepime			1	4			27	24
Cefoxitin	8	8			21	21	19	19
Colistin			2	2				
Chloramphenicol	16	16	8	8	19	19	17	17
Ertapenem			0.5	1			25	22
Gentamicin	2	2	2	4	16	16	17	14
Meropenem	0.125	0.125	2	8	27	27	22	16
Nalidixic acid	16	16			16	16		
Sulfamethoxazole								
Temocillin								
Tigecycline	2	2	1	2	16	16		
Trimethoprim	2	2	2[A]	4[A]	23	23	18[A]	15[A]
Trimethoprim-sulfamethoxazole	1	1	2	4			14	11

A. Uncomplicated UTI only

Annex 3. Distribution of DD (mm) values for optional antimicrobials

Disk diffusion results for the *Salmonella* strains tested / mm

Antimicrobial	Strain	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Azithromycin	ATCC 25922											1		1							1															
	S18.0001													1				1					1													
	S18.0002												1			1				1																
	S18.0003													1				1				1														
	S18.0004	3																																		
	S18.0005													1				1		1																
	S18.0006	1	1			1																														
	S18.0007											1						1	1																	
	S18.0008													1		1			1																	
Cefepime	ATCC 25922																										1	2	2	2	1					
	S18.0001				2	1		3		2													1													
	S18.0002	1																								4			2		1	1				
	S18.0003																							2	1	1	1			1	2	1				
	S18.0004								3	3	1	1			1																					
	S18.0005	1								1		2	1	3		1																				
	S18.0006					1	1			1	1	2	1	2																						
	S18.0007																					2		4	2	1										
	S18.0008																						2		1	3	1	2								
Cefoxitin	ATCC 25922																		1		3	3	3													
	S18.0001																		1	2	2	1	3	2												
	S18.0002																		3	4	1	2	1													
	S18.0003																			1	5	2	3													
	S18.0004																			2	2		1	4	1	1										
	S18.0005																		1	3		5	1	1												
	S18.0006																			2	1	2	4	2												
	S18.0007																	1	1	2	5	1		1												
	S18.0008				3	2	2		1	3																										
Ceftazidime	ATCC 25922																			1		3	4	2	1	1										
	S18.0001	10	1	1	1																															
	S18.0002																	2	4		4	3														
	S18.0003																		1	3		4	2	2	1											
	S18.0004	11			1	1																														
	S18.0005									3	1	1	6	2																						
	S18.0006	3	1	2	3	3		1																												
	S18.0007																1	1	1	2	3	3	1	1												
	S18.0008	9	1	2		1																														

Disk diffusion results for the *Salmonella* strains tested / mm

Antimicrobial	Strain	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Chloramphenicol	ATCC 25922																	1	4	2	3	2														
	S18.0001	12																																		
	S18.0002	6	1	1	1	1	1				1																									
	S18.0003																			1	5	2	2	1	1											
	S18.0004	12																																		
	S18.0005																			4	3	2	1		1	1										
	S18.0006	12																																		
	S18.0007																		2	2	2	4		1				1								
	S18.0008	12																																		
Ertapenem	ATCC 25922																											2	2	1	1	1				
	S18.0001							1														1		1	2	1	1		1							
	S18.0002														1											1		1		1		1	2		1	
	S18.0003															1										1	1	1			1	1		1		
	S18.0004											1											1	2				1	2	1						
	S18.0005												1										1			2	1	1	1			1				
	S18.0006										1													1	1	1	1		1	2						
	S18.0007	1						1					2	1	2	1																				
	S18.0008									1														3		2		2								
Gentamicin	ATCC 25922														1	3	1	1	3	4	1														
	S18.0001														1	2	2	1	2	1	3	1	1													
	S18.0002													1	3	1	2	1	1	2	1	1	1													
	S18.0003														2	3		2	1	3	3															
	S18.0004														2	3		2	1	3	2			1												
	S18.0005			2	3	4	3		1	1																										
	S18.0006	10	1	1	1	1																														
	S18.0007													3	1	1	1	2	2	3	1															
	S18.0008													1	2	2	1	2	1	2	3															
Meropenem	ATCC 25922																								1	2	7	1	2	1	1					
	S18.0001																								1	7	1	3		2	1					
	S18.0002																									2	4	3	1	2	1	2				
	S18.0003																									4	3	2	2	1	2		1			
	S18.0004																								1		4	3		4	2			1		
	S18.0005																									1	5	2	1	1	2	2	1			
	S18.0006																										6	1	2	1	1	4				
	S18.0007											1		1	3	2	2	2	3	1																
	S18.0008																										6	2	3	1	1	1		1		

Disk diffusion results for the *Salmonella* strains tested / mm

Antimicrobial	Strain	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Nalidixic acid	ATCC 25922																		2	1	1	1	1	1	1											
	S18.0001															1		1	1		3	1		1												
	S18.0002	8																																		
	S18.0003																1	3	1	1	1	1														
	S18.0004												1			4		2		1																
	S18.0005	8																																		
	S18.0006							1					1	1	3		1	1																		
	S18.0007												1					3	1	1	2															
	S18.0008									1						1			3	1	1	1														
Sulfamethoxazole	ATCC 25922														1		1																			
	S18.0001	2																																		
	S18.0002	2																																		
	S18.0003											1				1																				
	S18.0004	2																																		
	S18.0005	2																																		
	S18.0006	1															1																			
	S18.0007	1																	1																	
	S18.0008	2																																		
Temocillin	ATCC 25922													1	1																					
	S18.0001										1																									
	S18.0002														1																					
	S18.0003																	1																		
	S18.0004																	1																		
	S18.0005															1																				
	S18.0006														1																					
	S18.0007	3																																		
	S18.0008															1																				
Tigecycline	ATCC 25922																		1			2														
	S18.0001																1	1							1											
	S18.0002															1	1	1																		
	S18.0003																2		1																	
	S18.0004																	2		1																
	S18.0005													1			2																			
	S18.0006												1				2																			
	S18.0007										1					2																				
	S18.0008													1		1	1																			

Disk diffusion results for the *Salmonella* strains tested / mm

Antimicrobial	Strain	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Trimethoprim	ATCC 25922														1				1	2	4	1	2													
	S18.0001																					2	1	1	1		2	2	1		1					
	S18.0002																	1					3	4	1	1	1									
	S18.0003																				1		3	2	2	2		1								
	S18.0004	11																																		
	S18.0005																					2	3	1	2	1	1		1							
	S18.0006	11																																		
	S18.0007																					2	1	2	3	2		1								
	S18.0008														1						1	2		2	1		4									
Trimethoprim-sulfamethoxazol	ATCC 25922																			1	3	3	4	1			1									
	S18.0001																	3	2	1	1	2	1		2	1										
	S18.0002													1			2	1	2	1	5					1										
	S18.0003																			1		1	1	2	2	4	2									
	S18.0004	12																								1										
	S18.0005															3	1	2		3		2			1	1										
	S18.0006	10				1	1																				1									
	S18.0007														1			1				1	2	4	2					2						
	S18.0008									1			1	1				2	2	2	1	2				1										

*: Reference value *: Accepted range*

The red line indicates the ECOFF according to EUCAST for the respective antibiotic, WT strains right of the line

EUCAST acceptance criteria used for ATCC 25922, except for sulfamethoxazole and temocillin, where the expected value was established by the EQA provider

Annex 4. Distribution of MIC (mg/L) values for optional antimicrobials

Antimicrobial	Strain	ND	MIC results																			
			0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Azithromycin	ATCC 25922										1		6	1								
	S17.0001												4	4								
	S17.0002												4	4								
	S17.0003												3	5								
	S17.0004																	7		1		
	S17.0005												2	5	1							
	S17.0006																8					
	S17.0007												2	6								
	S17.0008												6	2								
Cefepime	ATCC 25922	8					6		1		1											
	S17.0001													1		2	6					
	S17.0002				1		1	3	1													
	S17.0003				1		3	1	1													
	S17.0004													1	1	3	4					
	S17.0005												1	2	5		1					
	S17.0006													1	2	2	4					
	S17.0007									4	3	2										
	S17.0008							1	3	2	3											
Cefoxitin	ATCC 25922	6										1	8									
	S17.0001											2	7	1								
	S17.0002											2	3	1								
	S17.0003										1	3	2									
	S17.0004											6	3		1							
	S17.0005											3	4	3								
	S17.0006											3	7									
	S17.0007											2	4	3								
	S17.0008															1	7	2				
Ceftazidime	ATCC 25922	8						5	5	1	1											
	S17.0001													1	3	1	7	1				
	S17.0002								7	5	1											
	S17.0003							3	8	1	1											
	S17.0004													1	4	4	3	1				
	S17.0005												5	8								
	S17.0006													1	8	3	1					
	S17.0007								8	4	1											
	S17.0008													1	5	4	3					

Antimicrobial	Strain	ND	MIC results																		
			0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Chloramphenicol	ATCC 25922	10											3	7								
	S17.0001																2		7	1		
	S17.0002																8	1		1		
	S17.0003										1		2	7								
	S17.0004																2		7	1		
	S17.0005												3	6	1							
	S17.0006																2		7	1		
	S17.0007												3	7								
	S17.0008																2		7	**1**		
Colistin	ATCC 25922	8											7	1								
	S17.0001												7	7	1							
	S17.0002								1	5	7	2										
	S17.0003								1	2	7	5										
	S17.0004							1	2	3	8	1										
	S17.0005								2	3	8	2										
	S17.0006								1	5	7	2										
	S17.0007								2	3	9	1										
	S17.0008								1	4	9	1										
Ertapenem	ATCC 25922	6			5	2																
	S17.0001					5	3															
	S17.0002		1	1	3	1																
	S17.0003			2	3	1																
	S17.0004			1	3	4																
	S17.0005			1	6	1																
	S17.0006				2	6																
	S17.0007									1		7										
	S17.0008			1		4	3															
Gentamicin	ATCC 25922	14								10	2											
	S17.0001									6	6											
	S17.0002									7	4	1										
	S17.0003							1		10	1											
	S17.0004							1		6	5											
	S17.0005														4	**8**						
	S17.0006														1	5	6					
	S17.0007							1		8	3											
	S17.0008									7	5											

Antimicrobial	Strain	ND	MIC results																			
			0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Meropenem	ATCC 25922	28				7	2	1	1													
	S17.0001					2	8	1	1													
	S17.0002				1	7	2	1	1													
	S17.0003					7	3	1	1													
	S17.0004					5	5	1	1													
	S17.0005				1	6	3	1	1													
	S17.0006					8	2	1	1													
	S17.0007								3	5	1	1	2									
	S17.0008					5	5	1	1													
Nalidixic acid	ATCC 25922	2											7									
	S17.0001												6	1								
	S17.0002																1		6			
	S17.0003												6	1								
	S17.0004												1	6								
	S17.0005																1		6			
	S17.0006													6	1							
	S17.0007												7									
	S17.0008												5	2								
Sulfamethoxazole	ATCC 25922	6												1	1	6	1					
	S17.0001														1					1		7
	S17.0002														1					1		7
	S17.0003													1	1	2	3	1				1
	S17.0004																			2		7
	S17.0005														1					1		7
	S17.0006														2	1	1	3	1			1
	S17.0007													2	3	3						1
	S17.0008														1					1		7
Temocillin	ATCC 25922													1	2	1						
	S17.0001													1	2	1						
	S17.0002														1	1						
	S17.0003													2								
	S17.0004													4								
	S17.0005														4							
	S17.0006													3	1							
	S17.0007																		4			
	S17.0008													4								

Antimicrobial	Strain	ND	MIC results																		
			0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Tigecycline	ATCC 25922						2	7														
	S17.0001									4	5											
	S17.0002								1	8												
	S17.0003							1	5	3												
	S17.0004							1	5	3												
	S17.0005									6	2	1										
	S17.0006								2	6		1										
	S17.0007									3	6											
	S17.0008									6	3											
Trimethoprim	ATCC 25922	6							1	6	3	1										
	S17.0001								7	3	1											
	S17.0002								6	3	2											
	S17.0003								8	1	2											
	S17.0004															3	8					
	S17.0005								7	2	2											
	S17.0006										1					2	8					
	S17.0007								8	2	1											
	S17.0008								6	4	1											

*: Reference value *: Accepted range*

The red line indicates the ECOFF according to EUCAST for the respective antibiotic; WT strains are to the left of the line

EUCAST acceptance criteria used for ATCC 25922, except for sulfamethoxazole and temocillin, where the expected value was established by the EQA provider

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