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TECHNICAL REPORT

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

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Third external quality assessment on antimicrobial susceptibility testing and detection of ESBL-, acquired AmpC-, and carbapenemase-production of *Salmonella*, 2017

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Therese Westrell, Food- and Waterborne Diseases and Zoonoses Programme, ECDC and produced by Mia Torpdahl, Gitte Sørensen, Kristoffer Kiil, Malgorzata Ligowska-Marzeta and Jeppe Boel, Unit of Foodborne Infections, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark.

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
DD	disk diffusion
ECOFF	Epidemiological cut-off value
EQA	External Quality Assessment
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

This report presents the results of the third round of the external quality assessment (EQA) on antimicrobial susceptibility testing (AST) for national public health laboratories on *Salmonella* (hereafter *Salmonella* EQA3-AST). The *Salmonella* EQA3-AST covered testing of antimicrobial susceptibility and detection and confirmation of ESBL-, acquired AmpC-, and carbapenemase-producing *Salmonella*. Twenty-seven National Public Health Reference Laboratories (NPHRLs) in the EU/EEA participated in the EQA which took place between February 2017 and January 2018. In addition, six EU candidate/potential candidate countries (EU enlargement countries) participated in the EQA. This report focuses only on the results from the EU/EEA countries.

Since 2008, it has been possible for EU/EEA countries to report antimicrobial resistance (AMR) data to the European Surveillance System (TESSy) as part of the routine surveillance for salmonellosis and campylobacteriosis. In 2014, the European Centre for Disease Prevention and Control (ECDC) published an EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates. In addition, ECDC launched an EQA scheme for AST on *Salmonella* and *Campylobacter* to support implementation of the EU protocol in EU/EEA countries and to obtain an overview of the quality of the AMR data reported to ECDC. The objectives of the EQA3-AST were to determine the accuracy of quantitative AST results reported by the participating laboratories, to identify common laboratory problems related to the guidance in the EU protocol, and to assess the overall comparability of routinely collected AST data from NPHRLs across Europe.

Eight *Salmonella* test strains were selected according to their current relevance to public health in Europe. Testing and reporting of four mandatory antimicrobials: ampicillin, ciprofloxacin (when using dilution methods)/pefloxacin (when using disk diffusion), cefotaxime and tetracycline, was required for participation in the EQA and an additional ten optional antimicrobials could also be reported. Results of pheno- and genotypic characterisation of ESBL-, AmpC- and carbapenamse-producing *Salmonella* could also be reported. Test results from all participants were evaluated and feedback was provided individually.

Laboratories reported results either as values based on disk diffusion (DD) or, when using dilution method or gradient strip, as the minimum inhibitory concentration (MIC). Test results were analysed using two different approaches. The reported DD (mm) and MIC (mg/L) results were compared with values established by the EQA provider, either by calculating mm difference for DD or calculating the number of dilution differences for MIC values. Reported quantitative results were further interpreted as wild-type (WT) or non-wild type (NWT) based on the available European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values (ECOFFs).

Nine of the 27 participating laboratories applied only DD methodology, seven used MIC and eleven laboratories reported results from both methods for all, or a combination of the mandatory antimicrobials. All laboratories except one submitted results for all eight tests strains for the mandatory antimicrobials, thereby fulfilling the requirement for participation in the EQA3-AST. The laboratory that did not fulfil this requirement did not report results for tetracycline.

Overall, there was good correspondence between the expected results established by the EQA provider and the results reported by the participating laboratories. For all antimicrobials, the relative accuracy (i.e. the percentage of DD and MIC results that were within ± three millimetres or ± one dilution step from the expected results) was 88% (1550/1764) for DD results, 93% (213/249) for MIC results generated with gradient strips and 97% (1031/1062) for MIC results generated using broth dilution methods. For the mandatory antimicrobials, 90% (60/584) of the DD results were correct and 99% (427/432) of the MIC results. However, it should be noted that 10% of the MIC results could not be assessed in terms of dilution differences as six laboratories did not comply with the recommended concentration ranges in the harmonised EU AST protocol. When the reported quantitative data were interpreted using EUCAST ECOFFs, where available, 99% of the DD results for the mandatory antimicrobials were in accordance with the category established by the EQA provider, and 97% of the DD results from the optional antimicrobials. The corresponding numbers for MIC results were 97% and 98%.

The panel of test strains included two ESBL-, one acquired AmpC-, and one carbapenemase-producing strain. Twenty-four of the 27 participating laboratories were able to assign the correct phenotype in ≥88% (up to 100%) of the strains but some of the results could not entirely be derived from the submitted phenotypic test-results. Eighteen laboratories reported genotypic results and overall assigned the correct genotypes to the ESBL-, acquired AmpC, and carbapenemase-producing strains. The four laboratories that submitted results based on sequence data could specify the correct gene rather than just the 'group' or 'type'.

The main conclusion from this EQA is that it is generally possible to compare routinely collected AST results from NPHRLs across Europe. The harmonised EU AST protocol recommends (micro-) broth dilution as the preferred testing method for monitoring purposes and the EQA results support this recommendation. It is, however, important to also apply the concentration ranges recommended in the protocol. A few laboratories had problems with the pheno- and genotypic characterisation of the ESBL-, acquired AmpC and carbapenemase-producing test strains and the reported genotypic data reflected the fact that there is no standardised protocol for performing and reporting such analyses.

1. Introduction

1.1 Background

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to operate the infectious disease networks and 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 part of a quality management systems where laboratory performance 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 for improvement in laboratory diagnostic capacities and further characterisation relevant to surveillance of diseases listed in Decision No 2000/96/EC [2] (repealed in June 2018 by Commission Implementing Decision (EU) 2018/945) and to ensure the reliability and comparability of results in laboratories from all EU/EEA countries.

In June 2014, ECDC tendered a framework service contract covering two lots relating to 'External quality assessment on antimicrobial susceptibility testing (AST) for national public health laboratories for *Salmonella* and *Campylobacter'* for the period 2014–2018. The unit of Foodborne Infections at Statens Serum Institut (SSI) won the two lots covering *Salmonella* and *Campylobacter*. The contract covers the organisation of an EQA exercise to test antimicrobial susceptibility and detect ESBL-, acquired AmpC and carbapenemase-producers in *Salmonella* and species designation and to test antimicrobial susceptibility in *Campylobacter*. The present report presents the *Salmonella spp.* results of the third EQA exercise under this contract (*Salmonella* EQA3-AST).

1.2 Surveillance of *Salmonella* AMR

Antimicrobial resistance (AMR) is a serious threat to public health in Europe, leading to mounting healthcare costs, treatment failure and deaths. The issue calls for concerted efforts at Member State level and 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 and campylobacteriosis are the two leading causes of zoonotic foodborne diseases in the EU/EEA, with around 300 000 laboratory confirmed cases reported annually. Although most infections are self-limiting, the more severe cases may require antibiotic treatment.

EU surveillance of AMR in foodborne human infections is carried out within the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net), led by the ECDC. Since 2008, the EU Member States and 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) is also collecting AMR data from zoonoses and zoonotic agents in food-producing animals and food according to Directive 2003/99/EC [3] and Implementing Decision 2013/652/EU [4]. Since 2012, both EFSA and ECDC have 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 [5] which was further updated in 2016 [6]. The EU 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 for *Salmonella* spp. and *Campylobacter* spp., are [5,6]:

- 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);
- 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 EQA3-AST scheme

The aim of the EQA3-AST on *Salmonella* was to support the implementation of the harmonised EU AST protocol for monitoring antimicrobial resistance in human *Salmonella* and to assess the quality of AST data obtained using minimum inhibitory concentration (MIC) determinations and/or measurement of disk diffusion inhibition zones (DD, mm) in NPHRLs across Europe.

The objectives of the EQA3-AST scheme were:

- to determine the relative accuracy of quantitative AST results reported by participating laboratories;
- to identify common laboratory problems related to the guidance in the EU protocol and testing of individual antimicrobials;
- to assess the overall comparability of routinely collected AST data 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

2.1 Organisation

The EQA3-AST was funded by ECDC and organised by SSI. The *Salmonella* EQA3-AST was conducted between February 2017 and January 2018.

On 17 March 2017, SSI e-mailed invitations to the laboratories in the FWD-Net (27 laboratories) that had been nominated as contact points for the EQA by ECDC's national focal points for FWD. In addition, ECDC circulated invitations to EU candidate and potential candidate countries (EU enlargement countries).

Twenty-seven NPHRL in EU/EEA countries and six EU enlargement countries accepted the invitation to participate. The list of participants is presented in Figure 1 and Annex 1. The EQA test-strains were sent to the participating laboratories on 10 May 2017. The participants were asked to submit their results using the reporting scheme by 7 July 2017. All laboratories were assigned an arbitrary laboratory number by the EQA provider and this number is used throughout the report when referring to the results from individual laboratories.

2.2 Selection of strain panel

Strains were selected for the *Salmonella* EQA3-AST programme based on the following criteria:

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

Initially, 16 *Salmonella* strains were tested and eight of them were selected as EQA test strains. The strain represented different antimicrobial susceptibility patterns that were relevant for the epidemiological situation in Europe, including recent outbreak strains. The characteristics of the *Salmonella* test strains are shown in Table 1. The genotype was 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/).

In addition to the eight test strains, the laboratories could request a control reference strain used for susceptibility testing of *Enterobacteriaceae* (*Escherichia coli* ATCC 25922) and a reference strain used to characterise low level colistin resistance encoded by *mcr-1* (*Escherichia coli* NCTC 13846).

The EQA provider established expected results for MIC and DD values for the eight test strains in accordance with the harmonised EU AST protocol [5]. The DD values were determined using disks from Oxoid and the MIC values were determined using the micro-broth dilution-based MIC system from Thermo Scientific's TREK diagnostic systems©.

Table 1. Serotype and resistance profile of the *Salmonella* EQA3-AST test strains

Strain	Serotype	Microbiological resistance profile[1](NWT)	Genotype Selected resistance genes
EQA_AST.S17.0001	*S.* O:4,12; H:i: -	AMP, CHL, COL, SMX, TCY, TMP	*mcr-1*
EQA_AST.S17.0002	*S.* Tyhimurium	AMP, CTX, CAZ, FOX, CHL, SMX, TCY	*bla*CMY-2
EQA_AST.S17.0003	*S.* Infantis	AMP, CTX, CAZ, FEP, CIP, NAL, SMX, TCY, TMP	*bla*CTX-M-1
EQA_AST.S17.0004	*S.* Infantis	AMP, CIP, NAL	
EQA_AST.S17.0005	*S.* Senftenberg		
EQA_AST.S17.0006	*S.* Chester	CHL, CIP, SMX, TCY, TMP	*qnrS1*
EQA_AST.S17.0007	*S.* O:4,5,12; H:i: -	AMP, CTX, CAZ, FEP, CHL, GEN, SMX, TCY, TMP	*bla*CTX-M-55
EQA_AST.S17.0008	*S.* Senftenberg	AMP, CTX, CAZ, FEP, FOX, CIP, ETP, GEN, MEM, NAL, SMX, TEM	*bla*SHV-12, *bla*CMY-4, *bla*NDM-1

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

AMP: ampicillin, CTX: cefotaxime, CAZ: ceftazidime, CIP: ciprofloxacin, CHL: chloramphenicol, COL: colistin, ETP: ertapemen, FEP: cefepime, FOX: cefoxitin, GEN: gentamycin, MEM: meropenem, NAL: nalidixic acid, SMX: sulfamethoxazole, 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 10 May 2017 and labelled in accordance with the IATA regulations (UN 3373 Biological Substance, Category B).

2.4 Testing and reporting

The EQA3-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 EQA3-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 given 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 the EUCAST website [7]. 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 the test strains were dispatched the laboratories received an email with a link to an electronic submission form. The form was constructed using Enalyzer software (www.enalyzer.com) in order to ensure that the results were reported in a fixed format. The deadline for submission of results was 7 July 2017 but this deadline was extended to 28 July 2017 because of delays in delivery of strains to a few countries.

Data reporting included quantitative DD and/or MIC results for antimicrobials, along with results for ESBL screening and characterisation purposes. It was also 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

The reported *Salmonella* test results were analysed using two different approaches:

- Laboratories reported their results as test values that were compared to the value obtained by the EQA provider, either by calculating mm difference for DD values or the number of dilution differences for MIC values. MIC dilution differences between reported and EQA provider's values were calculated taking into consideration several factors:
  - in situations when the operator of the reported value was >, we approximated the result to = the next dilution step;
  - when the operator of the reported value was <=, we approximated the results to = the same dilution step;
  - in cases where 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 the EQA provider's range, we noted the dilution difference as '0';
  - when the EQA provider's range was wider than that of the participant's and the expected result was within this wider range, the dilution difference could not be calculated.

  MIC gradient strip values were transformed on a $\log_2$ base scale, rounded to the nearest two-fold dilution and then retransformed to enable comparison with the results from micro-dilution methods. The quantitative results were further categorised into three groups. The first group includes DD results that were within the accepted 3mm difference from the expected result and one dilution difference for MIC results (correct). The second group were results that deviated from the expected results (incorrect) and the third group included the MIC results that were not within the relevant range for comparison with the EQA provider's results (not determined - ND).
- Reported results were used to make an interpretation based on the EUCAST ECOFF when available. This interpretation (WT or NWT) was compared with the expected result established by the EQA provider. These qualitative results where then categorised into four 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), the third group included strains where this comparison was not possible because no ECOFF existed for the antimicrobial (NA) and the fourth group included the reported MIC values that were not within the relevant range for interpretation using ECOFF (ND). The EUCAST ECOFFs applied can be found in Annex 2.

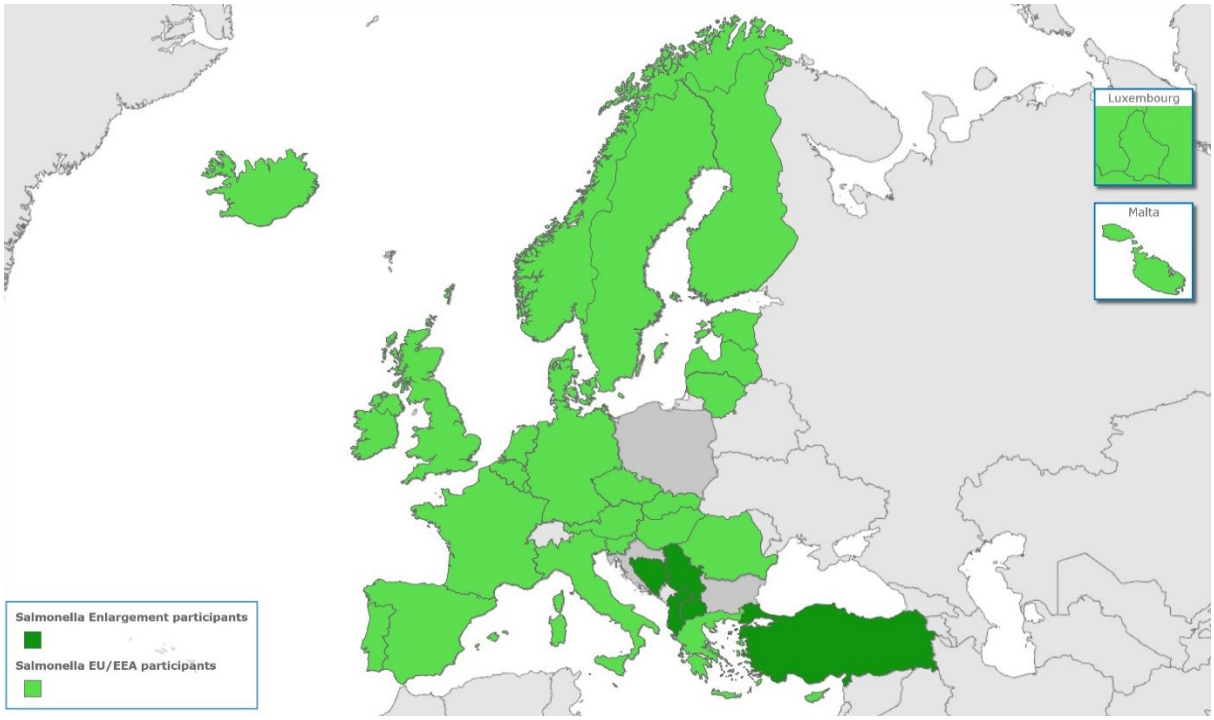
The genotypic results were evaluated on a case-by-case basis.

3. Results

3.1 Participation

All invited 27 laboratories from EU/EEA countries participated in the *Salmonella* EQA (Figure 1). In addition, six EU candidate/potential candidate countries (EU enlargement countries) also took part. Test results from all participants have been evaluated and feedback provided individually. This report will focus only on the results and evaluation of data from EU/EEA countries.

Figure 1. EU/EEA countries and EU candidate/potential candidate countries participating in the *Salmonella* EQA3-AST, 2017



3.2 Applied methods at participating laboratories

A total of 21 laboratories reported DD results for the mandatory and/or optional antimicrobials. For the antimicrobials azithromycin, cefotaxime, ceftazidime and gentamicin, one of the 21 laboratories used a disk load that deviated from the EUCAST recommended disk load and for sultamethoxazole, seven laboratories did not follow the recommendation. For all other antimicrobials, the laboratories used the EUCAST recommended disk load.

All laboratories used Mueller Hinton agar as growth medium for establishing DD diffusion results.

Disks from Oxoid were widely used; 59% of the DD results were generated using this brand. Bio-Rad- and Becton Dickinson disks were used for 16% and 14% of the results, respectively, and disks from i2A diagnostics, Mast and Rosco Diagnostica were used to generate 5%, 3% and 3% of the results, respectively.

Nineteen laboratories reported MIC results. A total of 59% of the MIC results were produced with TREK sensititre equipment. The remaining results were produced with consumables from Liofilchem (13%), in-house assays (11%), Bell Miditech (8%), bioMérieux-ETEST (5%), bioMérieux-Vitek 2 (2%), Umic (2%) and other unreported equipment (2%). For ESBL characterisation most laboratories used TREK Sensititre and Liofilchem.

3.3 Antimicrobial susceptibility testing of *Salmonella*

The participation rate for all laboratories with DD- and MIC results and the percentage of correct qualitative and quantitative results are presented in Table 2. Results classified as ND are included in the denominator. Nine of 27 laboratories tested the mandatory antimicrobials using only DD, seven used only MIC determinations and eleven reported results from both methods for all, or a combination of, the mandatory antimicrobials (Table 2). All laboratories except one, L006, reported results for all mandatory antimicrobials.

The number of optional antimicrobials reported from the individual laboratories varied (Table 2). DD results were reported for one to 13 of the optional antimicrobials and MIC results were reported for one to 14 of the optional antimicrobials.

Disk diffusion

The laboratories reported a total of 1764 DD results. For the mandatory antimicrobials, 90% of the DD results were within the accepted three mm difference from the expected value established by the EQA provider and therefore evaluated as correct. For the optional antimicrobials this figure was 87% (Table 2). The range by laboratory for the mandatory antimicrobials was 69–100% and for the optional antimicrobials 63–100% (Table 2).

After interpretation of DD results using EUCAST ECOFFs, 99% of the results for the mandatory antimicrobials were in accordance with the category established by the EQA provider and 97% of the results for the optional antimicrobials. Three laboratories had less than 100% correct qualitative results for the mandatory antimicrobials (all reporting 97%) (Table 2).

Table 2. EU/EEA laboratories participating (represented by an arbitrary number) in the *Salmonella* EQA, participation of mandatory antimicrobials by method and percentage correct results*

	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	Correct qualitative results	Number of antimicrobials reported	Correct quantitative results	Correct qualitative results
L002					91%	100%	12	85%	96%	B	B	B	B	100%	100%	14	99%	100%
L004					75%	100%	1	63%	100%	B	B	B	B	100%	100%	10	96%	100%
L006					88%	100%				B	B			25%	75%	4	16%	96%
L007					94%	100%	4	69%	96%	B	B	B		54%	100%	8	66%	98%
L008							2	100%	100%	B	B	B	B	100%	100%	10	98%	100%
L009					100%	100%	6	98%	98%									
L010					97%	100%	13	81%	99%	B	B	G	B	100%	100%	14	99%	99%
L012										B	B	B	B	41%	88%	6	50%	96%
L013					72%	100%	7	80%	95%									
L014					84%	100%	7	95%	98%							1	0%	
L015					100%	100%	4	97%	100%			G		100%	100%			
L016										B	B	B	B	100%	100%	10	100%	98%
L017					88%	97%	9	82%	95%									
L019					91%	100%	8	85%	98%									
L020					100%	100%	7	95%	100%			G		100%	100%	1	100%	
L021					88%	100%	8	81%	98%			G		100%	100%	1	100%	
L022					69%	97%	7	96%	96%			G		100%	100%			
L024					88%	100%	11	81%	97%									
L028					72%	97%	10	80%	96%									
L029										B	B	B	B	47%	94%	10	60%	91%
L031					96%	100%	7	95%	100%			G		100%	100%			
L032					97%	100%	10	88%	98%									
L033					97%	100%	2	94%	88%									
L034										B	B	B	B	100%	100%	14	98%	98%
L037										G	G	G	G	94%	100%	10	84%	96%
L039										G	G	G	G	97%	97%	6	96%	100%
L040					100%	100%	13	95%	93%	B	B	B	B	100%	100%	14	100%	99%
Total					**90%**	**99%**		**87%**	**97%**					**86%**	**97%**		**87%**	**98%**

** Results categorised as ND (not determined) are included, while results categorised as NA (not applicable) are excluded.*
B: Broth microdilution, G: Gradient strip
In green: all test strains reported. In yellow: not all test strains reported.

Dilution and gradient strip

The laboratories reported 1 461 MIC results for the mandatory (432) and optional (1029) antimicrobials. For the mandatory antimicrobials, 86% of the MIC results were within one dilution of the expected value established by the EQA provider and therefore evaluated as correct. For the optional antimicrobials this figure was 87% (Table 2). This varied by laboratory from 25% to 100% for the mandatory antimicrobials, and from 0% to 100% for the optional antimicrobials (Table 2).

Overall, 10% (150 MIC results) of the 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.

The 49 MIC results (five from mandatory antimicrobials) which differed by more than one dilution from the expected were reported by 11 of 19 laboratories submitting MIC results. Two of these laboratories reported 16 (L029) and 15 (L037) incorrect results and the remaining nine laboratories all reported four or less incorrect results. L029 used a microdilution assay from Bell Miditech and L037 used gradient strips from Liofilchem for MIC determination.

Of the 1 461 MIC results, 1 212 were generated using broth dilution methods and 249 using gradient strips. Ninety-seven percent of the results from broth dilution were correct while for gradient strips, this figure was 93%. All 150 MIC results classified as ND were generated using broth dilution methods.

After interpretation of MIC results using EUCAST ECOFFs, 97% of the results for the mandatory antimicrobials were in accordance with the category established by the EQA provider and for the optional antimicrobials this figure was 98%. Only four laboratories reported less than 100% correct qualitative results for the mandatory antimicrobials, and these laboratories reported correct results ranging from 75% to 97%. Two laboratories reported eight ND MIC results for cefotaxime that were impossible to evaluate qualitatively due to the ECOFF value being outside of the range tested.

3.3.1 Results by antimicrobial and strain

Table 3 provides an overview of the DD and MIC results reported by all participating laboratories. The table shows the number of laboratories performing DD and MIC testing for the four mandatory antimicrobials and the optional antimicrobials and summarises the number of correct quantitative and qualitative results.

Disk diffusion

Between 84% and 99% of the DD results were correct for the mandatory antimicrobials (Table 3). The highest proportion of correct results were reported for ampicillin, with 99% (143 of 144) of the reported results falling within the accepted difference of 3 mm from the expected value (Table 3). All results for the mandatory antimicrobials ampicillin, cefotaxime and pefloxacin were correct after interpretation using the EUCAST ECOFF and for the reported tetracycline results this figure was 98% (141/144) (Table 3).

The laboratories submitted DD results for a varying number of the optional antimicrobials. Only one laboratory submitted DD results for temocillin, whereas 17 laboratories reported DD results for ceftazidime (Table 3). Between 80% and 100% of the reported DD results for the optional antimicrobials were correct (Table 3). For the nine optional antimicrobials with ECOFFs established by EUCAST, between 91% and 100% of the interpreted results were correct. For cefoxitin, ceftazidime and nalidixic acid, two (C17.0003 and C17.0004), one (C17.0003) and one (C17.0006) of the test strains respectively exhibited expected DD zones that were close to the ECOFF. Some results evaluated as quantitatively correct were evaluated qualitatively as incorrect since they were on the 'wrong side' of the ECOFF.

Dilution and gradient strip

Thirteen laboratories submitted MIC results for the mandatory antimicrobials ampicillin and cefotaxime, 11 laboratories for tetracycline and 17 laboratories for ciprofloxacin (Table 3). For the optional antimicrobials, the number of laboratories submitting MIC results also varied by antimicrobial, ranging from four laboratories reporting on temocillin to 13 reporting on ceftazidime (Table 3).

Between 86% and 92% of the results for cefotaxime, ciprofloxacin and tetracycline were within the accepted one dilution difference, while 74% of the MIC results for ampicillin were evaluated as correct (Table 3). The low proportion of correct results for ampicillin was due to a high number of results being classified as ND. After interpreting results with ECOFFs, 99% (103/104) of the ampicillin results were correct. The corresponding numbers for cefotaxime, ciprofloxacin and tetracycline were 92%, 99% and 100% respectively. The eight incorrect cefotaxime results were submitted by two laboratories, L006 and L012, who reported the MIC values as ≤1, while the ECOFF is ≤0.5, and therefore the results could not properly be assessed. A total of 63 out of 64 (98%) MIC results reported for ciprofloxacin and generated using gradient strips were correct.

Between 70% and 100% of the reported MIC results for the optional antimicrobials were correct (Table 3). The antimicrobials that were most frequently reported with incorrect MIC results were azithromycin (11%), sulfamethoxazole (8%) and cefoxitin (8%). Nine percent of the MIC results were classified as ND since the tested range was too narrow. For the nine antimicrobials with ECOFFs established by EUCAST, 95–100% of the MIC results were correct after interpretation (Table 3). The incorrect results for cefoxitin and ceftazidime were all

reported for the same strain, C17.0003 (Annex 4). Six of the incorrect results with ECOFFs were quantitatively evaluated as being correct (Annex 4).

Table 3. Performance per antimicrobial for DD and MIC

Antimicrobials	Number of laboratories performing DD	Numbers of DD results within accepted 3 mm difference of the total tested	Number of correct results when using EUCAST ECOFF	Number of laboratories performing MIC	Numbers of MIC results within accepted 1-dilution difference of the total tested	Number of correct results when using EUCAST ECOFF
Mandatory						
Ampicillin	18	143/144 (99%)	144/144 (100%)	13	77/104 (74%)	103/104 (99%)
Cefotaxime	19	132/152 (87%)	152/152 (100%)	13	96/104 (92%)	96/104 (92%)
Ciprofloxacin	-	-	-	17	121/136 (89%)	134/136 (99%)
Pefloxacin	18	121/144 (84%)	144/144 (100%)	-	-	-
Tetracycline	18	128/144 (89%)	141/144 (98%)	11	76/88 (86%)	88/88 (100%)
Optional						
Azithromycin	5	32/40 (80%)	40/40 (100%)	8	57/64 (89%)	NA
Cefepime	7	46/56 (82%)	NA	8	52/57 (91%)	NA
Cefoxitin	12	83/96 (86%)	87/96 (91%)	9	54/65 (83%)	60/65 (95%)
Ceftazidime	17	113/136 (83%)	125/136 (92%)	13	93/104 (89%)	102/104 (98%)
Chloramphenicol	16	122/128 (95%)	128/128 (100%)	11	77/88 (88%)	87/88 (99%)
Colistin	-	-	-	11	80/89 (90%)	NA
Ertapenem	7	47/52 (90%)	NA	8	54/57 (95%)	54/57 (95%)
Gentamicin	16	102/128 (80%)	128/128 (100%)	12	82/96 (85%)	96/96 (100%)
Meropenem	16	94/128 (73%)	127/128 (99%)	11	62/88 (70%)	88/88 (100%)
Nalidixic acid	11	81/88 (92%)	83/88 (94%)	8	59/64 (92%)	63/64 (98%)
Sulfamethoxazole	10	75/80 (94%)	NA	9	61/72 (85%)	NA
Temocillin	1	8/8 (100%)	NA	4	25/25 (100%)	NA
Tigecycline	4	29/32 (91%)	32/32 (100%)	9	68/72 (94%)	69/72 (96%)
Trimethoprim	13	96/104 (92%)	102/1004 (98%)	11	68/88 (77%)	87/88 (99%)
Trimethoprim-sulfamethoxazole	13	98/104 (94%)	NA	-	-	-

NA: Not applicable due to EUCAST ECOFF not being available

Distribution of test results by antimicrobial and strain

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 (mandatory) and Annex 3 (optional) for DD and Table 5 (mandatory) and Annex 4 (optional) for MIC.

EUCAST has defined acceptance criteria for the size of the inhibition zones and MIC values for the control strain *E.coli* ATCC 25922 [8] for all antimicrobials tested except tetracycline, sulfamethoxazole and temocillin. For these antimicrobials, the reported result for the control strain was compared with the expected result established by the EQA provider. Overall, the reported DD inhibition zones for the control strain were within the accepted range, both for the mandatory and the optional antimicrobials (Table 4 and Annex 3). One laboratory, L24, made an exception to this and reported incorrect inhibition zones of 6 mm for several antimicrobials for the fully susceptible control strain. Overall, the reported MIC results for the control stain were also in accordance with the expected values (Table 5 and Annex 4).

Disk diffusion results were mostly within the accepted range for strains that were resistant to the antimicrobial in question (Table 4 and Annex 3). One exception was for pefloxacin, where many of the results for the resistant strains S17.0003, S17.0004 and S17.0006 were lower than the expected values established by the EQA provider and a few reported results were 4–9 mm below the expected range. For susceptible strains, results were often more widely distributed and differences of up to 18 mm from the expected value (azithromycin) were reported.

Most DD results classified as incorrect when interpreted with the EUCAST ECOFFs were reported for cefoxitin (S17.003 and S17.0004), ceftazidime (S17.0003) and nalidixic acid (S17.0006). However, most of these results were within the expected 3 mm range (Annex 3).

Table 4. Distribution of DD values (mm) of participating laboratories for mandatory 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
Ampicillin	ATCC 25922	1									1	1	3	6	3	3														
	S17.0001	18																												
	S17.0002	18																												
	S17.0003	18																												
	S17.0004	18																												
	S17.0005																		4	6	3	3	1	1						
	S17.0006																	1	7	4	4		1	1							
	S17.0007	18																												
	S17.0008	18																												
Cefotaxime	ATCC 25922																				2	1	7	4	1	3		1		
	S17.0001																		1	1	2	2	4	2		4	1	1	1	
	S17.0002	9	1	4	2	1	1						1																	
	S17.0003	18			1																									
	S17.0004															1		1		4	2	3	4	1	1		1	1		
	S17.0005																	1		2	2	1	2	3	4	4				
	S17.0006															1			1			2	4	1	3	6	1			
	S17.0007	18			1																									
	S17.0008	18			1																									
Pefloxacin	ATCC 25922	1																					2	5	1	6	1	1		1
	S17.0001																			1	3	4	5	3	1	1				
	S17.0002																				3	2	2	5	1	3	2			
	S17.0003	1					2	1	1	1	2	2	5	2	1															
	S17.0004	2			1		3	4	3	1	1	2	1																	
	S17.0005																			2	1	4	5	5	1					
	S17.0006	2						1	1	2	1	7	3	1																
	S17.0007																			1	2	4	4	3	2	2				
	S17.0008	18																												
Tetracycline	ATCC 25922	1														1		5	3	3	4	1								
	S17.0001	18																												
	S17.0002	16	1	1																										
	S17.0003	18																												
	S17.0004									1	1	1	3	2	4	4		1				1								
	S17.0005													1	6	2	3	2	1			2		1						
	S17.0006	17			1																									
	S17.0007	18																												
	S17.0008												2	2		4	2	4	2		1			1						

Expected value *Accepted range*

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

MIC results for the mandatory antimicrobials were mostly within the accepted range of plus/minus one dilution difference from those expected (Table 5) and only one result for ampicillin and one for ciprofloxacin was incorrect when interpreted with EUCAST ECOFFs. However, for all mandatory antimicrobials there were several results that were classified as ND because the reported MIC values were outside of the range for comparison with the EQA provider's results.

With regard to the optional antimicrobials, all MIC results for temocillin, ceftazidime and gentamicin were correct (Annex 4). The largest deviations from the expected results in terms of the number of dilution steps were for

sulfamethoxazole, where three results were seven dilution steps lower than the expected MIC. Of the MIC results classified as incorrect when interpreted with EUCAST ECOFFs, three were for ertapenem, three for tigecycline and one each for chloramphenicol and nalidixic acid. All of these were at a higher MIC than expected and would result in an NWT instead of a WT result.

Table 5. Distribution of MIC values (mg/L) of participating laboratories for mandatory antimicrobials

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											2	8	3						
	S17.0001	4																7		2
	S17.0002	4																7		2
	S17.0003	4																7		2
	S17.0004	4																8		1
	S17.0005	1								1	6	4					1			
	S17.0006	1							1		8	3								
	S17.0007	4																7		2
	S17.0008	4																7		2
Cefotaxime	ATCC 25922	2					2	1	8											
	S17.0001	2						3	8											
	S17.0002													6	2	2	3			
	S17.0003													5		3	4	1		
	S17.0004	2						1	10											
	S17.0005	2				1		2	8											
	S17.0006	2				1	2		8											
	S17.0007													5		1	4	3		
	S17.0008													5		1	3	4		
Ciprofloxacin	ATCC 25922	3	2	4	8															
	S17.0001	3			4	10														
	S17.0002	3		2	11	1														
	S17.0003							4	8	5										
	S17.0004								5	10	2									
	S17.0005	2			6	8			1											
	S17.0006								6	10	1									
	S17.0007	3			5	8			1											
	S17.0008	2													6	1	8			
Tetracycline	ATCC 25922								1		2	8								
	S17.0001	2															1	7	1	
	S17.0002	2														1	1	7		
	S17.0003	2															2	7		
	S17.0004								1		1	5	4							
	S17.0005							1				10								
	S17.0006	2														1	1	7		
	S17.0007	2															1	8		
	S17.0008								1			9	1							

ND: Not Determined, the reported MIC result was not in range for comparison with EQA provider's result

Expected value *Accepted range*

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

3.3.2 Individual laboratory results

A comparison of the performance of each laboratory is shown for DD results reported for mandatory antimicrobials in Figure 2. The data are shown as a percentage of results within 0–1, 2–3 (correct) or >3 mm difference from the expected value established by the EQA provider. Four laboratories (L009, L015, L020 and L040) reported 100% correct DD results and another seven laboratories had over 90% correct results (Figure 2). Eight laboratories reported from 72% to 87% correct results and only one laboratory (L022) reported less than 70% correct results.

Figure 2. Distribution of DD (mm) differences for results reported for mandatory antimicrobials shown for each laboratory

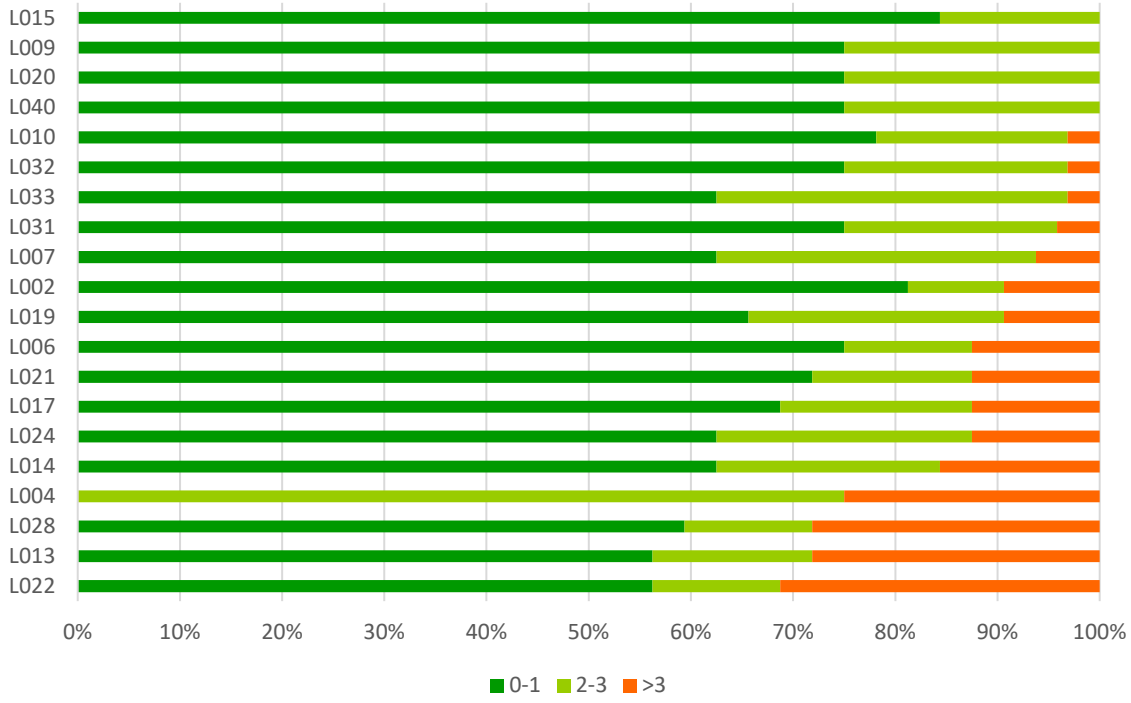
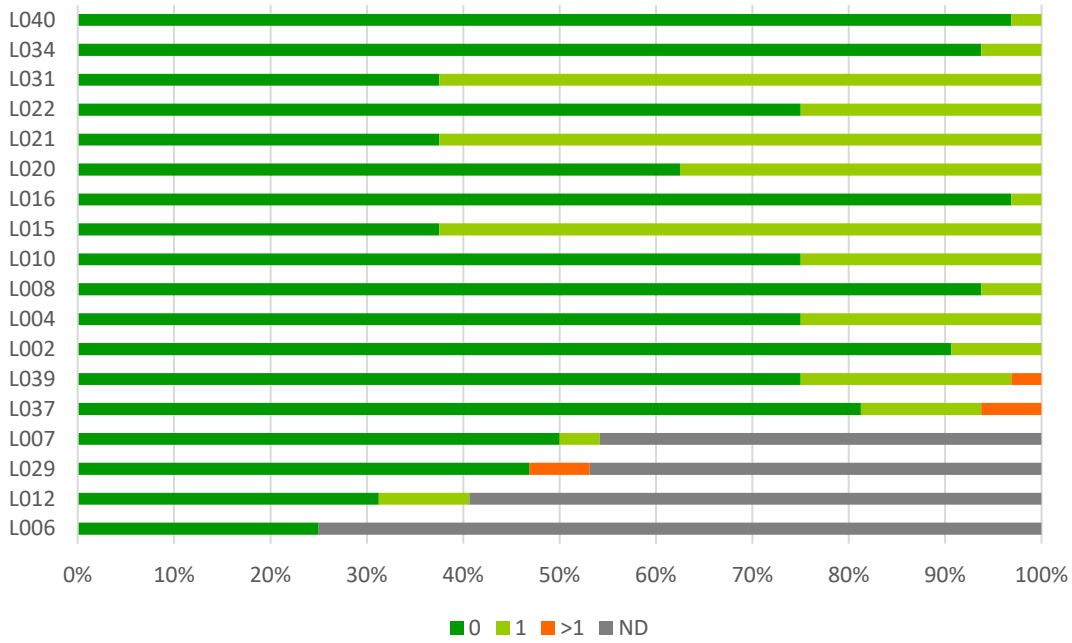


Figure 3 shows a comparison of the performance of each laboratory for MIC results reported for mandatory antimicrobials. The data are presented as the percentage of results within 0, 1 or >1 dilution difference to the expected value established by the EQA provider. In addition, results categorised as ND are presented. Five of the laboratories (L015, L020, L021, L022, and L031), all using gradient strips, only reported MIC results for ciprofloxacin (Table 2).

Twelve laboratories submitted MIC results that were all evaluated to be correct and two laboratories reported more than 90% correct results (Figure 3). For four laboratories (L006, L007, L012, and L029), 46–75% of the MIC results for the mandatory antimicrobials could not be assessed quantitatively as the tested concentration range was too narrow (results categorised as ND) (Figure 3 and Table 2).

Figure 3. Distribution of MIC dilution differences for the reported *Salmonella* results for mandatory antimicrobials, shown for each laboratory



3.3.3 ESBL-, acquired AmpC- and carbapenemase-producing Salmonella

The set of test strains included one AmpC-, two ESBL- and one carbapenemase-producing strain (Table 1 and Table 6). Twenty-four laboratories reported results on ESBL-, acquired AmpC and carbapenemase-producing *Salmonella* strains for all or some of the test strains (Table 6) and three laboratories (L012, L014 and L024) did not participate in this part of the EQA. The proportions of correctly identified phenotypes ranged from 88% to 100% by strain. Overall, 176/183 (96%) results were designated correctly. Three laboratories (L017, L022 and L033) only reported some of their negative results.

Twenty-two laboratories reported strain S17.0001 and S17.0006 correctly as negative for ESBL-, acquired AmpC and carbapenemase-production and the strains S17.0004 and S17.0005 were reported correctly as negative by 21 laboratories. One laboratory (L039) incorrectly reported the negative strain S17.0004 as AmpC (Table 6).

Twenty-one laboratories reported strain S17.0002 correctly as AmpC, two laboratories (L020 and L029) misclassified this strain as ESBL and one laboratory (L032) reported it as both ESBL and AmpC (Table 6). Twenty-three laboratories correctly identified strain S17.0003 as ESBL and one laboratory (L040) incorrectly classified the strain as both ESBL and AmpC. The ESBL-producing strain S17.0007 was classified correctly by all 24 laboratories reporting results for this strain.

The expected phenotype for strain S17.0008 was carbapenemase positive, but the phenotype carbapenemase in combination with ESBL and/or AmpC was also accepted as a correct result. Twenty-two laboratories identified the strain correctly (one of these laboratories (L033) reported with the comment 'Possible carbapenemase, meropenem 17 mm. Not confirmed'). Two laboratories (L017 and L019) reported the strain incorrectly as ESBL and AmpC (Table 6).

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

Strain	Expected phenotype	Number of laboratories reporting correct phenotype	AmpC	AmpC, Carbapenemase	Carbapenemase	ESBL	ESBL, AmpC	ESBL, AmpC, Carbapenemase	ESBL, Carbapenemase
S17.0001	Negative	22/22 (100%)							
S17.0002	pAmpC	21/24 (88%)	21			2	1		
S17.0003	ESBL	23/24 (96%)				23	1		
S17.0004	Negative	21/22 (95%)	1						
S17.0005	Negative	21/21 (100%)							
S17.0006	Negative	22/22 (100%)							
S17.0007	ESBL	24/24 (100%)				24			
S17.0008	Carbapenemase (ESBL, pAmpC)	22[1]/24[1] (92%)		4	10		2	5	2

Expected value *Accepted range*

[1]One lab reported only as comments: 'Possible carbapenemase, meropenem 17 mm. Not confirmed'

Three laboratories reported an incorrect phenotype for strain S17.0002. One of these laboratories (L020) did not report results for cefoxitin and cefepime, and was unable to assign the correct AmpC phenotype. Two other laboratories (L029, L032) reported incorrect phenotypic results but did report phenotypic testing results that enabled assignment of the correct phenotype (Table 6). L029 reported an incorrect positive synergy test with clavulanic acid for cefotaxime and ceftazidime, which could explain the misclassification of the strain (Table 6).

One laboratory (L040) reported an incorrect phenotype for strain S17.0003. The reported cefoxitin DD result for this strain was 20 mm. According to the harmonised EU AST protocol, the standard EUCAST ECOFF for cefoxitin is ≥21 mm but when screening for ESBL production a breakpoint of ≥19 mm should be used. The laboratory overlooked this detail.

One laboratory (L039) reported the strain S17.0004 incorrectly as AmpC, despite not reporting results indicating this phenotype (Table 7). The presumptive carbapenemase phenotype for strain S17.0008 was identified by 21 laboratories, while one further laboratory (L033) made the comment 'Possible carbapenemase, meropenem 17 mm. Not confirmed'. Two laboratories (L017 and L019) did not identify the carbapenemase phenotype although both laboratories reported the strain as resistant to meropenem (Table 2). Eleven laboratories reported carbapenemase in combination with ESBL and/or AmpC.

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

		Synergy test (+/-clavulanic acid)							
		MIC ratio				DD - Zone difference (mm)			
Strain		< 2	2-7	8-16	> 16	0-1	2-4	5-7	>7
Cefotaxime	S17.0001	1	2			2			
	S17.0002	3	1	1		5	6		1
	S17.0003			3	4	1	1	1	8
	S17.0004	2	1			3			
	S17.0005	2	1			2			
	S17.0006	1	2			3			
	S17.0007				7	1		2	8
	S17.0008	4				7	3	1	
Ceftazidime	S17.0001	1	2				2		
	S17.0002	2	2		1	5	6		
	S17.0003		2	5			5	5	
	S17.0004	3				1	2		
	S17.0005	3				1	1		
	S17.0006	1	2			2	1		
	S17.0007				7				10
	S17.0008	4				7	2	1	
Cefepime	S17.0001	1							
	S17.0002		2			1			
	S17.0003			1	3				
	S17.0004		1						
	S17.0005		1						
	S17.0006	1							
	S17.0007			1	2				
	S17.0008	1				2			

Expected value *Accepted range*

Values to the right of the dashed line are considered as a positive synergy test

No expected MIC ratio was established by the EQA provider for cefepime/clavunilanic acid.

Twelve, 14, 14 and 18 laboratories respectively reported genotype for strains S17.0002, S17.0003, S17.0007 and S17.0008 and the results and applied methods are presented in Table 8. The laboratories applied different methods for genotypic characterisation. Several laboratories used PCR either alone or in combination with array and/or sequencing and four laboratories used sequencing methods – either whole genome sequencing (WGS) or sequencing not further specified. One laboratory used the BD-Max system and another the Check-Points assay (Table 8).

The genotype results were reported in several different ways and reflected the different methodologies used to identify the genotypes.

Genotypes of strain S17.0002 were correctly reported by all 12 laboratories. Laboratories doing PCR or RT-PCR reported CIT-type, CMY-group or CMY-2 and laboratories that used sequencing alone or in combination with other methods correctly identified CMY-2. A few laboratories identified additional genes, OXA and CMY-33, that were not identified by the EQA provider in this strain (Table 8).

The genotype was also correctly identified for strain S17.0003 by the 14 laboratories reporting data (Table 8). Laboratories using PCR or RT-PCR reported CTX-M-group and the correct genotype CTX-M-1 was reported by laboratories that performed sequencing (Table 8). One laboratory reported several genes in strain S17.003 that were not identified by the EQA provider. This laboratory also reported the OXA genotype for S17.0002 and identified other genes in strain S17.0007 and S17.0008 that were not identified by the EQA provider.

The genotype for S17.0007 was reported as TEM-type ESBL, CTX-M-group when using PCR or RT-PCR, and the same methods - alone or in combination with sequencing - were used to incorrectly report CTX-M-1 or CTX-M15 (Table 8). Laboratories doing sequencing correctly reported the genotype CTX-M-55 (Table 8).

The genotype for strain S17.0008 was reported as NDM-type or NDM by laboratories using PCR, RT-PCR, BD-Max or Check-points (Table 8). A few laboratories using PCR alone, in combination with sequencing or sequencing alone, correctly reported NDM-1 (Table 8). Some laboratories correctly reported SHV-12 and CMY-4 or SHV and CMY-group, or CMY (Table 8). One laboratory using sequencing also incorrectly reported NDM-18 in addition to the correct genes. The laboratory that used Check-points reported the presence of the SHV variants '238S' and '240K' (Table 7).

Table 8. Genotypes predicted and methods used for prediction of ESBL 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*)
S17.0002	CMY-2	PCR	CIT-type (1)
			CMY-group (1)
			OXA, CMY-2 (1)
			CMY-2 (1)
		Array/PCR/sequencing	CMY-2 (3)
		Real-time PCR	CIT-type (1)
		Sequencing/WGS	CMY-2, CMY-33 (1)
			CMY-2 (3)
S17.0003	CTX-M-1	PCR (BD-Max)	CTX-M-1 (1)
		PCR	CTX-M group (2)
			OXA, CTX-1, CTX-2, CMY-2 (1)
			CTX-M-1 (3)
		Array/PCR/sequencing	CTX-M-1 (2)
		Real-time PCR	CTX-M Group 1 (1)
		Sequencing/WGS	CTX-M-1 (4)
S17.0007	CTX-M-55	PCR (BD-Max)	CTX-M-1 (1)
		PCR	TEM type ESBL (1);
			CTX-M-group (2)
			CTX-M-1 (2)
			TEM, SHV, OXA, CTX-1, CTX-2, CMY-2 (1)
		Array/PCR/sequencing	CTX-M-15 (2)
		Real-time PCR	CTX-M Group (1)
			CTX-M-15 (1)
		Sequencing/WGS	CTX-M-55 (3)
.S17.0008	SHV-12, CMY-4, NDM-1	PCR (BD-Max)	SHV, NDM (1)
		Check-Points	SHV 238S, SHV 240K, CMY II,NDM (1)
		PCR	NDM-type (2)
			NDM-1 (2)
			NDM, CIT (1)
			SHV-1, CMY-2 (1)
			SHV, TEM, CMY-group, NDM-1 (1)
			TEM, SHV, CTX-1, CTX-2, DHA/NDM (1)
		Array/PCR/sequencing	SHV, TEM, CMY-4, NDM (1)
			NDM-1, CMY-4, SHV-12 (1)
			NDM-1, CMY-4, SHV-12, TEM-1a (1)
		Real-time PCR	CIT-type, NDM (1)
			NDM (1)
		Sequencing/WGS	NDM-1, CMY-4 (1)
			CMY-4, SHV-12, NDM-1 (1)
			SHV-12, CMY-4, NDM-1, NDM-18 (1)

4. Discussion

Since 2008, EU/EEA countries could report AMR data to TESSy as part of the routine surveillance data for salmonellosis. In 2014, ECDC published the harmonised EU AST protocol (updated in 2016) with guidance on laboratory procedures and the interpretation of data [4]. The purpose of the EQA3-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 submitted data were used to determine the relative accuracy of quantitative and qualitative AST data and to assess the overall comparability of AST data. Furthermore, laboratories had the option to test and report detection and confirmation of ESBL-, acquired AmpC and carbapenemase-producing *Salmonella*. This could be done phenotypically, following the guidance provided in the harmonised EU AST protocol, or genotypically. For the genotypic characterisation there were no recommendations regarding methodology. An additional aim of the EQA3-AST was to collect information on the methods used by each laboratory to produce data on antimicrobial susceptibility.

Twenty-seven 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 EQA3-AST. The laboratory that did not fulfil this requirement did not report results for tetracycline.

Twenty-four laboratories reported phenotypic characterisation for ESBL-, acquired AmpC and carbapenemase-production and up to 18 laboratories reported genotypic characterisation for the genes encoding ESBL-, acquired AmpC and carbapenemase- production. The participation rate for this part of the EQA had improved against the EQA2-AST performed in 2016, where 17 and 10 laboratories reported pheno- and genotypic data respectively.

The logistics of the EQA went well. All laboratories were able to recover the test strains and successfully submit the results on the Enalyzer platform. Furthermore, there was an overall agreement between the quantitative results reported for the different antimicrobials and the expected results established by the EQA provider, especially for the reported MIC results. With few exceptions, the test strains exhibited DD zones and MIC values that were distinct from the ECOFF values and this meant that the interpreted qualitative results (ECOFF interpretation) were generally better than the quantitative results.

When the 150 results classified as ND were excluded from the total 1 461 MIC results, 96% (1 213/1 262) of the quantitative MIC results were evaluated as correct, compared to 88% (1 550/1 764) of the reported DD results. Gradients strips were used to generate 249 of the MIC results and 213 (93%) of these results were correct when compared to the expected results. The corresponding numbers for results generated using broth-dilution methods were 1 031/1 062 (97%). The harmonised EU AST protocol recommends (micro-) broth dilution as the preferred testing method for monitoring purposes, but, disk diffusion or validated methods of gradient strip diffusion are also accepted. The data from this EQA supports 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 method. Six laboratories reported 150 MIC results that were classified as ND because the test range deviated from the recommended range in the harmonised EU AST protocol. 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. Two laboratories reported eight MIC results for the mandatory antimicrobial cefotaxime that were impossible to evaluate qualitatively, as the range tested did not cover the ECOFF value. This highlights the importance of selecting the right concentration range, and it should be noted that six laboratories did not follow the recommendations for concentration ranges specified in the harmonised EU AST protocol.

Eighteen laboratories submitted MIC results for the mandatory antimicrobials and 427/432 (99%) were evaluated as correct. The five incorrect MIC results were reported by three laboratories. Two laboratories were responsible for 15 and 16 of the 49 incorrect reported MIC results respectively, one using a micro-dilution assay and one using gradient strips. The reason for this relatively poor performance has not been clarified. However, the overall quality of the MIC results, and in particular results generated by broth dilution methods were very good.

EUCAST recommend pefloxacin disks to test for fluoroquinolone susceptibility with disk diffusion as results with ciprofloxacin are difficult to interpret due to an overlap of the wild type and non-wild type populations [9]. It could be argued that gradient strip and disk diffusion results are related as they both rely on diffusion of the antimicrobial into agar based media. This could lead to the assumption that it would be problematic to use ciprofloxacin in gradient-based MIC assays. In this EQA, 63 out of 64 (98%) of the reported ciprofloxacin MIC values generated with gradient strips were correct, indicating that ciprofloxacin strips are suitable for testing the eight strains included in this EQA. The proportion of correct pefloxacin results generated by DD was 84% (121/144). The distribution of the reported pefloxacin DD values showed that many laboratories report DD values that were below the expected value established by the EQA provider, particularly for three resistant test strains. The reason for this finding has not been clarified.

A number of DD results deviated from the accepted range, most notably for meropenem 34/128 (27%), gentamicin 26/128 (20%), azithromycin 8/40 (20%), cefepime 10/56 (18%), ceftazidime 23/136 (17%), cefoxitin 13/96 (14%), pefloxacin 23/144 (16%), cefotaxime 20/152 (13%), and ertapenem 5/52 (10%). For meropenem and azithromycin there was a general tendency for the incorrect reported values to be higher than the expected value for the susceptible strains, whereas the reported values for pefloxacin and gentamicin were lower than the expected value. For the remaining antimicrobials there were no obvious patterns in the results that deviated from the expected values. Two of the deviating results for the mandatory antimicrobial cefotaxime were only one mm from the ECOFF for the susceptible strains S17.0004 and S17.0006. Following interpretation with EUCAST ECOFFs the overall proportion of correct qualitative DD results was close to 100% for all antimicrobials except for cefoxitin (87/96, 91%), ceftazidime (125/136, 92%) and nalidixic acid (83/88, 94%). Part of the reason for these low scores was that there was overlap between the ECOFF values and the range for correctly reported inhibition zones for two (cefoxitin) and one (ceftazidime and nalidixic acid) of the test strains.

The proportion of correctly evaluated DD results reported by the 20 laboratories varied from 69% to 100% for the mandatory antimicrobials. Overall, these figures are in line with the results seen in the EQA2-AST and indicate that it is feasible to improve the quality of the DD AST data generated by some of the FWD laboratories.

In the harmonised EU AST protocol [6] phenotypic testing for detection and confirmation of ESBL-, acquired AmpC and carbapenemase-producing *Salmonella* is proposed for isolates resistant to either cefotaxime, ceftazidime or meropenem. 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 rather than in combination with clavulanic acid – e.g. MIC CTX / MIC CTX+clavulanic acid).

The eight *Salmonella* test strains included two strains that were ESBL producers, one strain that was AmpC positive and one strain that was carbapenemase-producing. Based on the results for cefotaxime screening, all laboratories were able to correctly assign the test strains into presumptive AmpC/ESBL positive and negative strains and the 24 laboratories that reported results for meropenem were also able to identify the test strain S.17.008 as a presumptive carbapenemase producer. Twenty-four of the 27 laboratories reported 88–100% correct phenotypical identification of ESBL-, acquired AmpC and carbapenemase production. The incorrect results were most often caused by laboratories that assigned more than one phenotype to the same strain - e.g. AmpC in combination with ESBL. A few laboratories had difficulties in assigning the correct phenotypes to the test strains and some of the reported phenotypes could not be justified from the data provided by the laboratories.

Eighteen laboratories reported genotypic results for all or some of the four test strains that were eligible for genotypic characterisation. The laboratories used a number of different methods and the results were reported in several different ways, reflecting the methodologies that had been used to identify the genotypes. In general, the laboratories were able to assign the correct genotypes to the strains. The expected genotypes established by the EQA provider were based on WGS, and the four laboratories that applied WGS/sequencing methods were all able to correctly identify the genotypes of the test strains.

In conclusion, the reported results on the characterisation of the ESBL-, acquired AmpC and carbapenemase-producing test strains indicate that it is possible to improve the understanding of the protocols for detection, conformation and reporting of ESBL-, acquired AmpC and carbapenemase-producing *Salmonella* in the network of laboratories, especially when it comes to genotypic characterisation.

5. Conclusion

Twenty-seven laboratories from EU/EEA countries participated in the EQA3-AST and all laboratories, except one, submitted results for eight tests strains for the mandatory antimicrobials, thereby fulfilling the requirement for participation in the EQA3-AST. The laboratory that did not fulfil this requirement did not report results for tetracycline.

Overall, there was good correspondence between the expected results established by the EQA provider and the results reported by the participating laboratories. For all antimicrobials, the relative accuracy (i.e. the percentage of DD and MIC results that were within the accepted range of the expected result) was highest for results generated using broth dilution methods, followed by gradient strip and disk diffusion. The harmonised EU AST protocol recommends (micro-) broth dilution as the preferred testing method for monitoring purposes and the data from this EQA supports the EUCAST recommendation on choice of methodology. However, it should be noted that 10% of the MIC results could not be assessed in terms of dilution differences as the concentration range tested was not wide enough. Six laboratories did not comply with the recommended concentration ranges in the harmonised EU AST protocol. There were also a few laboratories using DD that achieved results 100% in accordance with the expected values established by the EQA provider. This indicates that it is possible to improve the quality of AST data in other laboratories using this methodology.

After interpreting DD results using EUCAST ECOFFs, 99% of the results for the mandatory antimicrobials were in accordance with the category established by the EQA provider and for the optional antimicrobials the figure was 97%. The corresponding numbers for MIC results were 97% and 98%.

With regard to phenotypical identification of ESBL-, acquired AmpC and carbapenemase, the results were good overall. The most common errors involved the assigning of more than one phenotype to the same strain. For the genotypic results, the laboratories used a number of different nomenclatures to report because of the different methods used. In general, the laboratories were able to assign the correct genotypes though the most accurate results were obtained by laboratories using whole genome sequencing or sequencing.

No common laboratory problems were identified in relation to the guidance in the harmonised EU AST protocol, but some laboratories did not comply entirely with the protocol (e.g. not following the recommendations on the concentration ranges for MIC testing). A few laboratories had problems with the pheno- and genotypic characterisation of the ESBL-, acquired AmpC and carbapenemase-producing test strains and the reported genotypic data reflected the fact that there is no standardised protocol for performing such analyses.

The main conclusion from this EQA is that, in general, it is possible to compare routinely collected AST results from NPHRLs across Europe.

6. Recommendations

6.1 Laboratories

A number of laboratories did not test within the concentration range recommended in the harmonised EU AST protocol [5,6] when establishing MIC values. However, when following the recommendations, the results generated by MIC broth dilution methods were superior to those using diffusion based methods. If possible, laboratories should therefore implement the use of broth dilution methods. There were also a few laboratories using DD that achieved results that were 100% in accordance with the expected values established by the EQA provider. This indicates that it is possible to improve the quality of AST data in the laboratories using this methodology.

6.2 ECDC and FWD-Net

In order to enhance the comparability of AST data reported to TESSy it is important to support the use of standardised testing and standardised interpretation of data in the Member States. In order to ensure a better understanding of the standard methods for antimicrobial susceptibility testing, one option could be to provide hands-on training courses. Further development of standardised reporting for pheno- and genotypic characterisation of ESBL, AmpC and carbapenemase producing *Salmonella* would facilitate the comparability of data.

6.3 The EQA provider

The current reporting scheme should be further developed for a more detailed and uniform collection of method, manufacturer and growth medium used by the participating laboratories. Furthermore, reporting of ESBL, AmpC and carbapenemase producing *Salmonella* needs to be harmonised, to improve feedback to participants and extraction and comparison of results in the final report.

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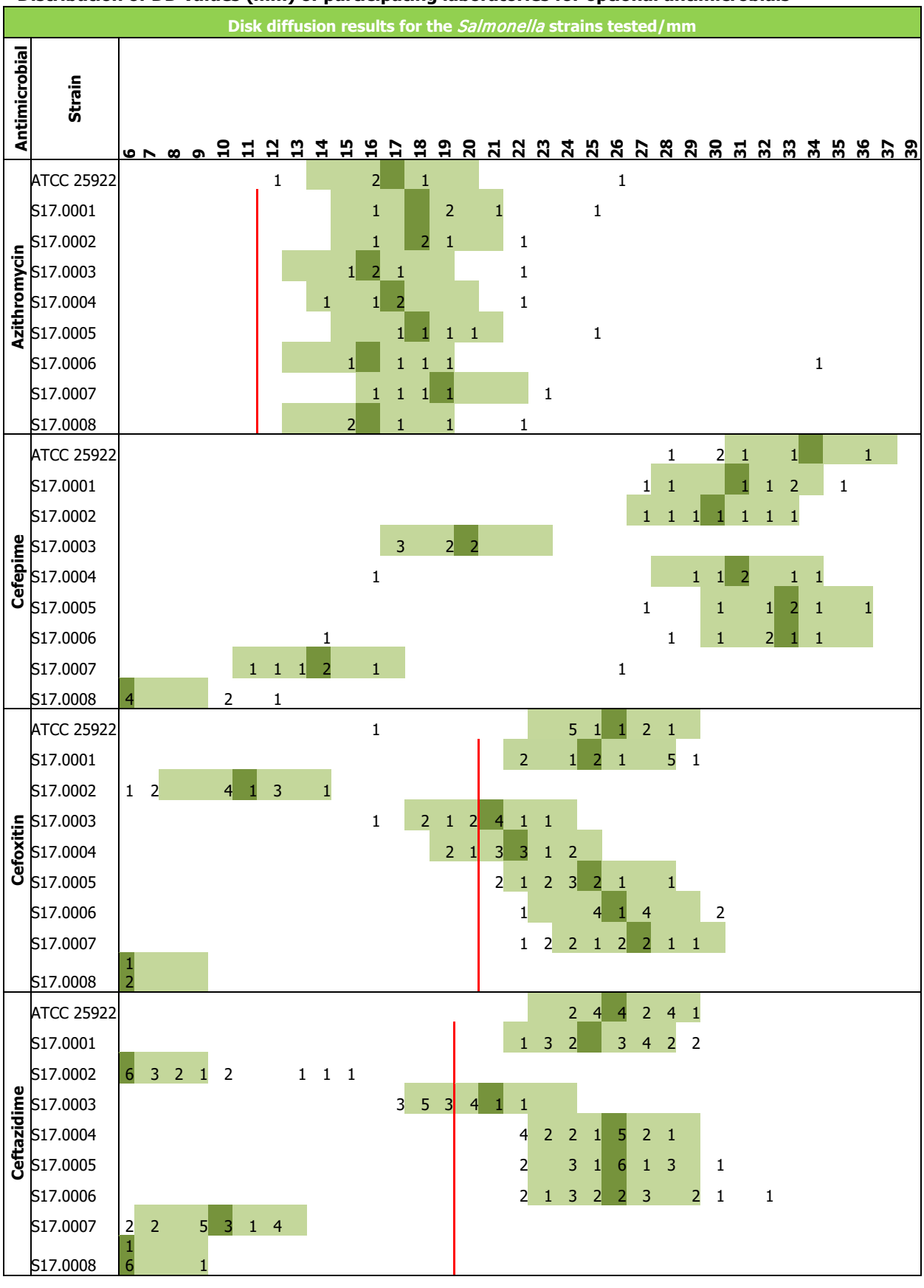
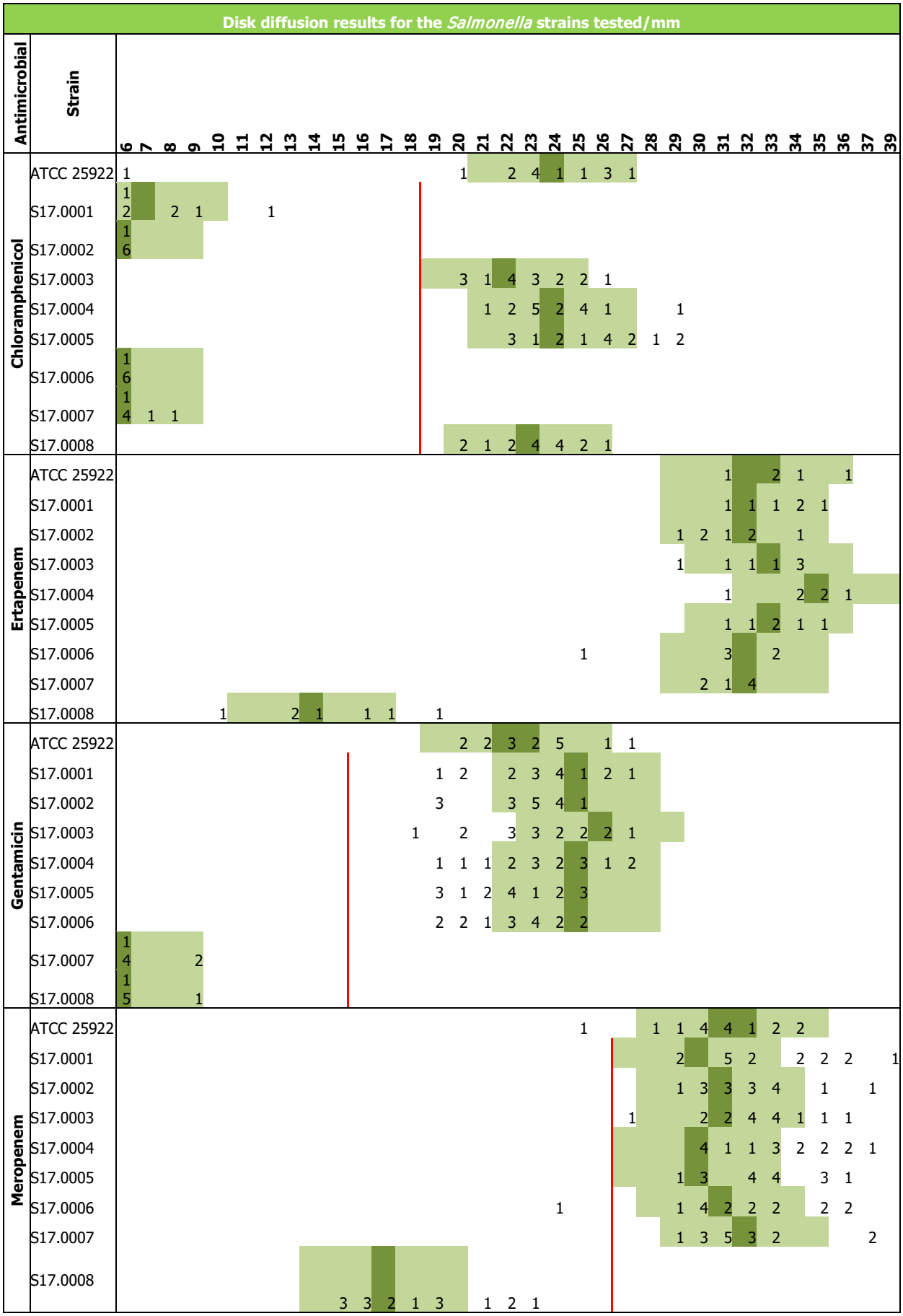
Annex 1. List of participants

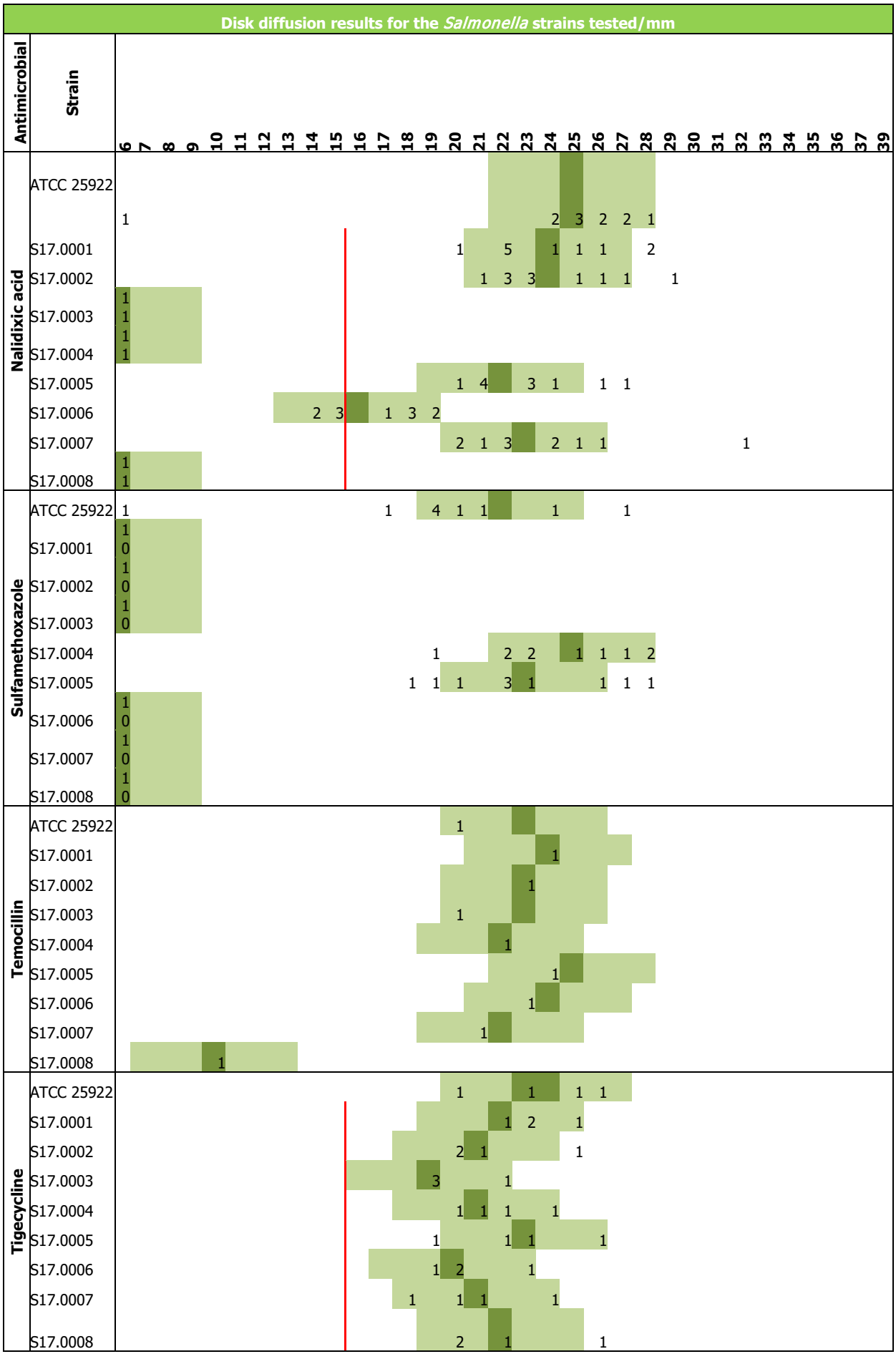
Country	EU status	Laboratory	Institute
Albania	Enlargement	Laboratory of Enterobacteriology	Institute of Public Health
Austria	EU/EEA	NRC *Salmonella* Austria	Institute for Medical Microbiology and Hygiene Graz
Belgium	EU/EEA	Bacterial Diseases	Scientific Institute of Public Health
Bosnia-Herzegovina	Enlargement	Department of Microbiology	Public Health Institute Republic of Srpska
Cyprus	EU/EEA	Reference Laboratory for *Salmonella* and other Enteric Pathogens	Nicosia General Hospital
Czech Republic	EU/EEA	National reference laboratory for antibiotics	National Institute of Public Health
Denmark	EU/EEA	Foodborne Infections	Statens Serum Institut
Estonia	EU/EEA	Laboratory of Communicable Diseases	Health Board
Finland	EU/EEA	Unit of Bacterial Infections	National Institute for Health and Welfare
France	EU/EEA	NRC for *Salmonella*	Institut Pasteur
Germany	EU/EEA	NRC *Salmonella*	Robert Koch Institute
Greece	EU/EEA	NRC for *Salmonella*	National School of Public Health
Hungary	EU/EEA	Department of Phage-typing and molecular epidemiology	National Public Health Institute (before National Center for Epidemiology)
Iceland	EU/EEA	Department of Clinical Microbiology	Landspítali University Hospital
Ireland	EU/EEA	NSSLRL	Medical Microbiology Department
Italy	EU/EEA	Department of Infectious diseases	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, Latvian Centre of Infectious Diseases
Lithuania	EU/EEA	National Public Health Surveillance Laboratory	Bacteriology Section
Luxembourg	EU/EEA	Laboratoire MycoBac-ARH	Laboratoire National de Santé
Malta	EU/EEA	Bacteriology Laboratory	Pathology Department
Norway	EU/EEA	Norwegian Reference Laboratory of Enteropathogenic Bacteria	Norwegian Institute of Public Health
Portugal	EU/EEA	LNR Infeções Gastrintestinais	INSA
Republic of Macedonia	Enlargement	Laboratory of bacteriology and AMR	Institute of Public Health of Macedonia
Republic of Serbia	Enlargement	Reference Laboratory for *Salmonella, Shigella, V. cholerae, Y. enterocolitica*	Institute of Public Health of Serbia
Romania	EU/EEA	Bacterial Enteric Infections Laboratory	Cantacuzino National Institute of Research
Slovak Republic	EU/EEA	NRC for Salmonelloses, NRC for ATB	Public Health Authority of the Slovak Republic
Slovenia	EU/EEA	Oddelek za medicinsko mikrobiologijo, Celje	Nacionalni laboratorij za zdravje okolje in hrano - NLZOH
Spain	EU/EEA	Unidad de Enterobacterias	Centro Nacional de Microbiología
Sweden	EU/EEA	Clinical Microbiology	Central Hospital
The Netherlands	EU/EEA	National Reference Laboratory for Antimicrobial Resistance in Animals	Wageningen Bioveterinary Research (WBVR)
Turkey	Enlargement	National Reference Laboratory for Enteric Pathogens	Public Health Institution of Turkey
United Kingdom	EU/EEA	Gastrointestinal Bacteria Reference Unit	National Infection Service

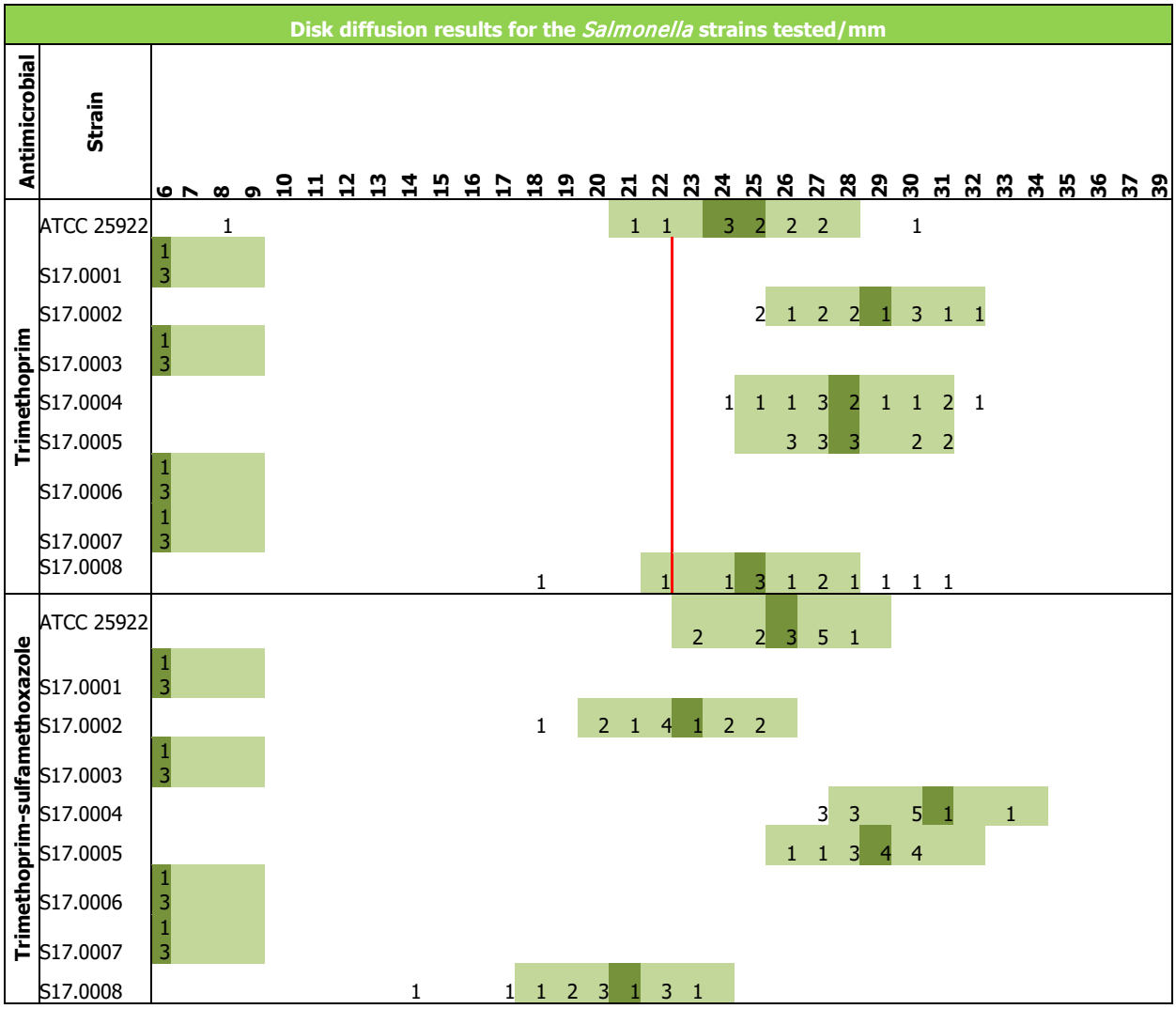
Annex 2. Distribution of zone values, optional antimicrobials

Distribution of DD values (mm) of participating laboratories 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 39
Chloramphenicol
ATCC 25922 1 1 2 4 1 1 3 1
S17.0001 1 2 2 1 1
S17.0002 1 6
S17.0003 3 1 4 3 2 2 1
S17.0004 1 2 5 2 4 1 1
S17.0005 3 1 2 1 4 2 1 2
S17.0006 1 6
S17.0007 1 4 1 1
S17.0008 2 1 2 4 4 2 1
Ertapenem
ATCC 25922 1 2 1 1
S17.0001 1 1 1 2 1
S17.0002 1 2 1 2 1
S17.0003 1 1 1 1 3
S17.0004 1 2 2 1
S17.0005 1 1 2 1 1
S17.0006 1 3 2
S17.0007 2 1 4
S17.0008 1 2 1 1 1 1
Gentamicin
ATCC 25922 2 2 3 2 5 1 1
S17.0001 1 2 2 3 4 1 2 1
S17.0002 3 3 5 4 1
S17.0003 1 2 3 3 2 2 2 1
S17.0004 1 1 1 2 3 2 3 1 2
S17.0005 3 1 2 4 1 2 3
S17.0006 2 2 1 3 4 2 2
S17.0007 1 4 2
S17.0008 1 5 1
Meropenem
ATCC 25922 1 1 1 4 4 1 2 2
S17.0001 2 5 2 2 2 2 1
S17.0002 1 3 3 3 4 1 1
S17.0003 1 2 2 4 4 1 1 1
S17.0004 4 1 1 3 2 2 2 1
S17.0005 1 3 4 4 3 1
S17.0006 1 1 4 2 2 2 2 2
S17.0007 1 3 5 3 2 2
S17.0008 3 3 2 1 3 1 2 1

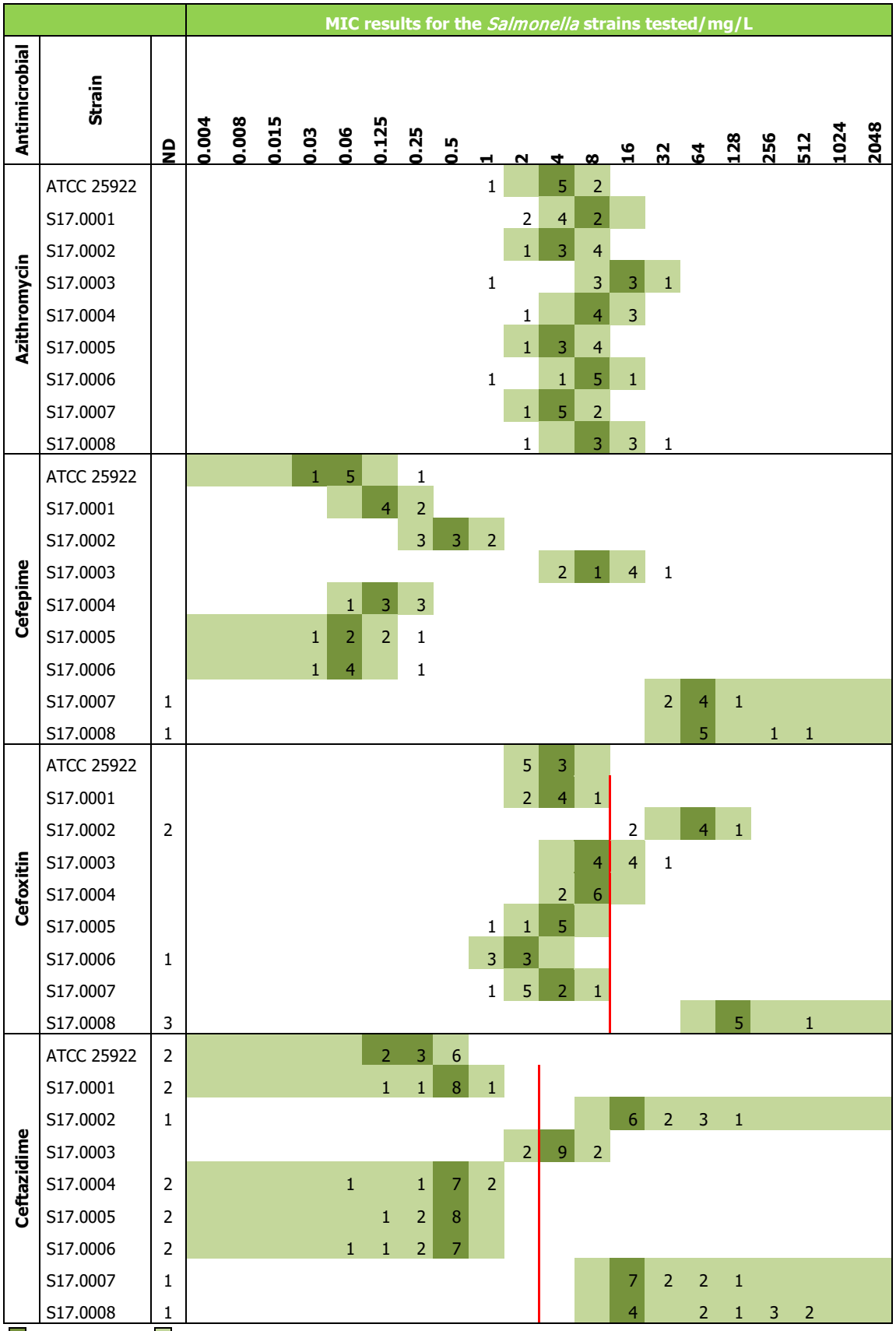

Disk diffusion results for the Salmonella strains tested/mm
Antimicrobial
Strain
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 39
Nalidixic acid
ATCC 25922
S17.0001
S17.0002
S17.0003
S17.0004
S17.0005
S17.0006
S17.0007
S17.0008
Sulfamethoxazole
ATCC 25922
S17.0001
S17.0002
S17.0003
S17.0004
S17.0005
S17.0006
S17.0007
S17.0008
Temocillin
ATCC 25922
S17.0001
S17.0002
S17.0003
S17.0004
S17.0005
S17.0006
S17.0007
S17.0008
Tigecycline
ATCC 25922
S17.0001
S17.0002
S17.0003
S17.0004
S17.0005
S17.0006
S17.0007
S17.0008



Reference value *Accepted range*

The red line indicates the ECOFF according to EUCAST for the respective antibiotic, WT strains above the line.

Annex 3. Distribution of MIC values, optional antimicrobials



Reference value *Accepted range*

The red line indicates the ECOFF according to EUCAST for the respective antibiotic, WT strains below the line.

European Centre for Disease Prevention and Control (ECDC)

Gustav III:s Boulevard 40, 16973 Solna, Sweden

Tel. +46 858601000
Fax +46 858601001
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