TECHNICAL REPORT

Third external quality assessment on species identification and antimicrobial susceptibility testing of *Campylobacter*

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Suggested citation: European Centre for Disease Prevention and Control. Third external quality assessment on species identification and antimicrobial susceptibility testing of *Campylobacter* – 2017. Stockholm: ECDC; 2019.

Stockholm, May 2019

ISBN: 978-92-9498-340-4
DOI: 10.2900/936462
Catalogue number: TQ-02-19-383-EN-N

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CBP	Clinical breakpoint
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
I	Intermediate
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, it has been possible for European Union/European Economic Area (EU/EEA) countries to report antimicrobial resistance (AMR) data to the European Surveillance System (TESSy) as part of 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 (updated in 2016). In addition, ECDC launched an external quality assessment (EQA) scheme for antimicrobial susceptibility testing (AST) for *Salmonella* and *Campylobacter* with the purpose of supporting the implementation of the EU protocol in EU/EEA countries and to get an overview of the quality of the AMR data reported to ECDC.

This report presents the results of the third round of the EQA on AST for national public health laboratories for *Campylobacter* (*Campylobacter* EQA3-AST) within the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net). The objectives of this EQA3-AST 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 (NPHRLs) across Europe.

The *Campylobacter* third EQA-AST covered species identification and AST in *Campylobacter* spp. Twenty-three NPHRLs in EU/EEA countries participated in the EQA that took place from February 2017–January 2018. In addition, six EU candidate/potential candidate countries participated in the EQA. This report focuses only on the results and evaluation of results from EU/EEA countries.

Bacterial strains for the EQA were selected according to their current relevance to public health in Europe and shipped to participating laboratories. Testing and reporting of three mandatory antimicrobials (ciprofloxacin, erythromycin and tetracycline) were required for participation in the EQA and one additional optional antimicrobial, gentamicin, could also be reported. The test results from all participants were evaluated and individual feedback provided.

The test results for antimicrobial susceptibility were analysed by two different approaches. Laboratories reported the results as values from either disk diffusion (DD) or, when using dilution or gradient strip, the minimum inhibitory concentration (MIC). These values were compared to the values established by the EQA provider and the mm difference for DD values or number of dilution differences for MIC values were calculated. Reported quantitative results were further interpreted as wild type (WT) or non-wild type (NWT) based on the available epidemiological cut-off values (ECOFFs) from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) and the interpretation compared to the expected result established by the EQA provider. It was mandatory to report the species identification (*C. jejuni* or *C. coli*) as this is a requirement for the correct interpretation using EUCAST ECOFFs.

All laboratories performed species identification and submitted results for the mandatory antimicrobials ciprofloxacin, erythromycin and tetracycline for a total of seven bacterial strains. Thirteen laboratories also reported results for gentamicin.

Overall, there was good correspondence between the expected results established by the EQA provider and those reported by participating laboratories.

All participating laboratories except one were able to correctly identify the *Campylobacter* species of the seven test strains. One laboratory attributed the wrong species for two strains. For the mandatory antimicrobials, the relative accuracy, i.e. the percentage of DD and MIC results that were within the accepted range from the expected result, was 79% for both disk diffusion and MIC methods. With the exception of one tetracycline result, all reported DD results were correct when interpreted with the EUCAST ECOFFs. For MIC, 97% of the interpreted results were correct. This indicates that it is reasonable to compare routinely collected AST results from NPHRLs across Europe when interpreted with the EUCAST ECOFFs.

The performance of the individual laboratories varied substantially compared with the expected values. For the mandatory antimicrobials, the percentage of correct quantitative results varied from 52–100% for both DD and MIC results. This implies that it is feasible to improve the quality of AST data generated in some of the FWD laboratories. No common laboratory problems related to the guidance in the harmonised EU AST protocol were identified, but certain laboratories did not comply entirely with the protocol and it is of concern that some reported DD results for the reference strains that did not comply with the recommended EUCAST range.

The surveillance system that has been implemented as part of TESSy relies on the capacity of FWD-Net laboratories to produce comparable AST results. The overall results from the *Campylobacter* EQA3-AST indicate that it is feasible to compare AST results from FWD-Net laboratories.

1 Introduction

1.1 Background

ECDC is an EU agency with the mandate to operate infectious disease networks and identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC fosters the development of sufficient capacity within the EU for the diagnosis, detection, identification and characterisation of infectious agents that may threaten public health. ECDC maintains and extends such cooperation and supports the implementation of quality assurance schemes [1].

External quality assessments (EQA) are a part of quality management systems and evaluate the performance of laboratories by an external evaluator on material that is supplied specifically 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 the surveillance of diseases listed in Decision No 2000/96/EC [2] (repealed in June 2018 by Commission Implementing Decision (EU) 2018/945) and ensure the reliability and comparability of results in laboratories from all EU/EEA countries.

In June 2014, a framework service contract covering two lots on 'External quality assessment on antimicrobial susceptibility testing (AST) for national public health laboratories for *Salmonella* and *Campylobacter'* for 2014–2018 was put out to tender by ECDC. 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 for testing antimicrobial susceptibility and detecting extended spectrum beta lactamases (ESBL), acquired AmpC and carbapenemase-producers in *Salmonella* and species identification and testing of antimicrobial susceptibility in *Campylobacter* species. The current report presents the *Campylobacter* spp. results of the third EQA exercise of this contract (*Campylobacter* EQA3-AST).

1.2 Surveillance of *Campylobacter* antimicrobial resistance

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 the 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 constitute an essential source of information on the magnitude and trends of resistance.

Campylobacteriosis, followed by salmonellosis, is the leading cause of zoonotic foodborne diseases in the EU/EEA, with approximately 250 000 laboratory-confirmed cases reported in 2016 [3].

EU surveillance of AMR in foodborne human infections 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 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 according to Directive 2003/99/EC [4] and Implementing Decision 2013/652/EU [5]. Since 2012, both EFSA and ECDC have strived to harmonise AMR monitoring in zoonoses and zoonotic agents within and between their respective areas in order to achieve data that can be compared over the sectors. This work has also been requested by the European Commission in its Commission Action Plan on AMR. In this respect, ECDC published an EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates in 2014 [6] and further updated it in 2016 (hereafter harmonised EU AST protocol) [7]. The harmonised EU AST protocol primarily targets NPHRLs 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 AMR in zoonotic bacteria, specifically *Salmonella* spp. and *Campylobacter* spp., are to [6,7]:

- monitor in human clinical isolates, trends in the occurrence of resistance to antimicrobial agents relevant for the treatment of human *Salmonella* and *Campylobacter* infection, including comparison with food/animal isolates
- monitor in human clinical isolates trends in the occurrence of resistance to other antimicrobial agents of public and animal health importance, including comparisons with food/animal isolates
- monitor in human clinical isolates the prevalence of ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC) and carbapenemase phenotypes
- use antimicrobial resistance patterns to characterise human clinical isolates, i.e. as an epidemiological marker, to support identification of outbreaks and related cases

- dentify 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 multidrug-resistant *Salmonella* strains; and
- monitor in human clinical isolates trends in the occurrence of resistance to antimicrobial agents that may be needed for future therapeutic use.

1.3 Objectives of EQA3-AST scheme

The aim of EQA3-AST was to support the implementation of the harmonised EU AST protocol for monitoring AMR in human *Salmonella* and *Campylobacter* isolates and assess the quality of AST data obtained using MIC and/or DD in NPHRLs across Europe.

The *Campylobacter* EQA3-AST covered laboratory procedures when producing AST data including species identification as this is a prerequisite for interpreting quantitative data following the EUCAST ECOFFs. The objectives of the *Campylobacter* EQA3-AST scheme were to:

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

2 Study design

2.1 Organisation

The EQA was conducted from February 2017–January 2018 and included species determination and AST of eight *Campylobacter* spp strains.

On 17 March 2017, SSI emailed invitations to the 27 laboratories that had been nominated as contact points for the EQA by FWD national focal points in FWD-Net.

Twenty-three NPHRLs in EU/EEA countries accepted the invitation to participate (Figure 1, Annex). EQA test strains were sent to the participating laboratories on 10 May 2017. Participants were asked to submit their results using an electronic submission form by 7 July 2017. All laboratories were assigned an arbitrary laboratory number by the EQA provider and these numbers are used throughout the report to ensure the anonymity of the participating laboratories.

2.2 Selection of EQA strains

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

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

The EQA provider tested 16 *Campylobacter* spp. strains and selected eight (three *C. coli* and five *C. jejuni*) with different resistance profiles (Table 1). In addition to the eight test strains, laboratories could request a subculture of the reference strain used for susceptibility testing of *Campylobacter* (*C. jejuni* ATCC 33560).

In order to determine the accuracy of the reported results, the EQA provider established expected results for MIC and DD values for the eight test strains. The expected values were established following the harmonised EU AST protocol [4]. DD values were determined using disks from Oxoid and the MIC values were determined using the microbroth dilution-based MIC system from Thermo Scientific's TREK Diagnostic Systems.

Table 1. Campylobacter EQA3-AST test strains by species and resistance profile

Strain	Species	Resistance profile[1] (NWT)
EQA_AST.C17.0001	*Campylobacter jejuni*	Ciprofloxacin
EQA_AST.C17.0002	*Campylobacter jejuni* [2]	Ciprofloxacin, erythromycin, tetracycline
EQA_AST.C17.0003	*Campylobacter coli*	Ciprofloxacin, erythromycin, tetracycline
EQA_AST.C17.0004	*Campylobacter jejuni*	
EQA_AST.C17.0005	*Campylobacter jejuni*	Ciprofloxacin, tetracycline
EQA_AST.C17.0006	*Campylobacter coli*	
EQA_AST.C17.0007	*Campylobacter jejuni*	Ciprofloxacin, tetracycline
EQA_AST.C17.0008	*Campylobacter coli*	Ciprofloxacin, erythromycin, gentamicin, tetracycline

[1]: *based on MIC values and according to EUCAST ECOFFs*
[2]: *mixed culture of* Campylobacter *jejuni and coli.*

2.3 Preparation and shipment of strains

Cultures of the test stains were grown on blood agar and transferred to Stuart's transport medium using charcoal swabs. The parcels with the strains were shipped on 10 May 2017 from SSI and labelled following the IATA regulations (UN 3373 Biological Substance, Category B).

2.4 Testing and reporting

The *Campylobacter* EQA3-AST included ASTs of four first priority antimicrobials listed in the harmonised EU AST protocol [7] and species identification. Three first priority antimicrobials (ciprofloxacin, erythromycin and tetracycline) were mandatory for testing and it was additionally possible to report results for gentamicin.

Instructions for AMR testing were given in the invitation letter, an email following the shipment of strains and the reporting forms. Participants were asked to follow the harmonised EU AST protocol and could submit results using broth dilution, gradient strip (MIC results) and DD results. It was emphasised that participants should report the test result as a value (mg/L or mm). The harmonised EU AST protocol to a large extent refers to the methods

recommended by EUCAST available on the EUCAST website [8]. No instructions were given with regard to species identification and it was anticipated that participating laboratories would use their own standard methods.

Participating laboratories received an email with a link to an electronic submission form constructed with Enalyzer software (http://www.enalyzer.com) and were able to report their results in a fixed format by 28 July 2017. The deadline for submitting results was 7 July 2017. This deadline was later extended to 28 July 2017 due to delays in delivering strains to certain countries. Data reporting included *Campylobacter* species, quantitative DD or MIC results, information about the used methods, growth media, brand of disks for DD and brand of strips or panels for MIC determination.

2.5 Data analysis

Participating laboratories provided test results, i.e. inhibition zones measured as diameters in mm for DD methods and MIC values for broth dilution and gradient strip methods. It was mandatory to report the species identification (*C. jejuni* or *C. coli*) as this information was needed for the correct interpretation using EUCAST ECOFFs.

The test results were analysed by different approaches:

- Laboratories reported their results and these values were compared with the expected results established by the EQA provider either by calculating the mm difference for DD values or the number of dilution differences for MIC values.
- All reported DD values were included in the analysis.
- MIC dilution differences between the reported and expected results were calculated considering several situations:
  - 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 EQA provider's range was wider than that of participant's and the expected result was within this wider range, the dilution difference could not be calculated.

MIC values generated by the use of gradient strips for MIC determination were transformed on a log2 base scale, rounded to the nearest twofold dilution, then retransformed in order to enable comparison with the results from dilution methods.

Quantitative results were categorised into three groups. The first group designated correct included DD results that were within the accepted 4-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 in the relevant range for comparison with the expected results (ND).

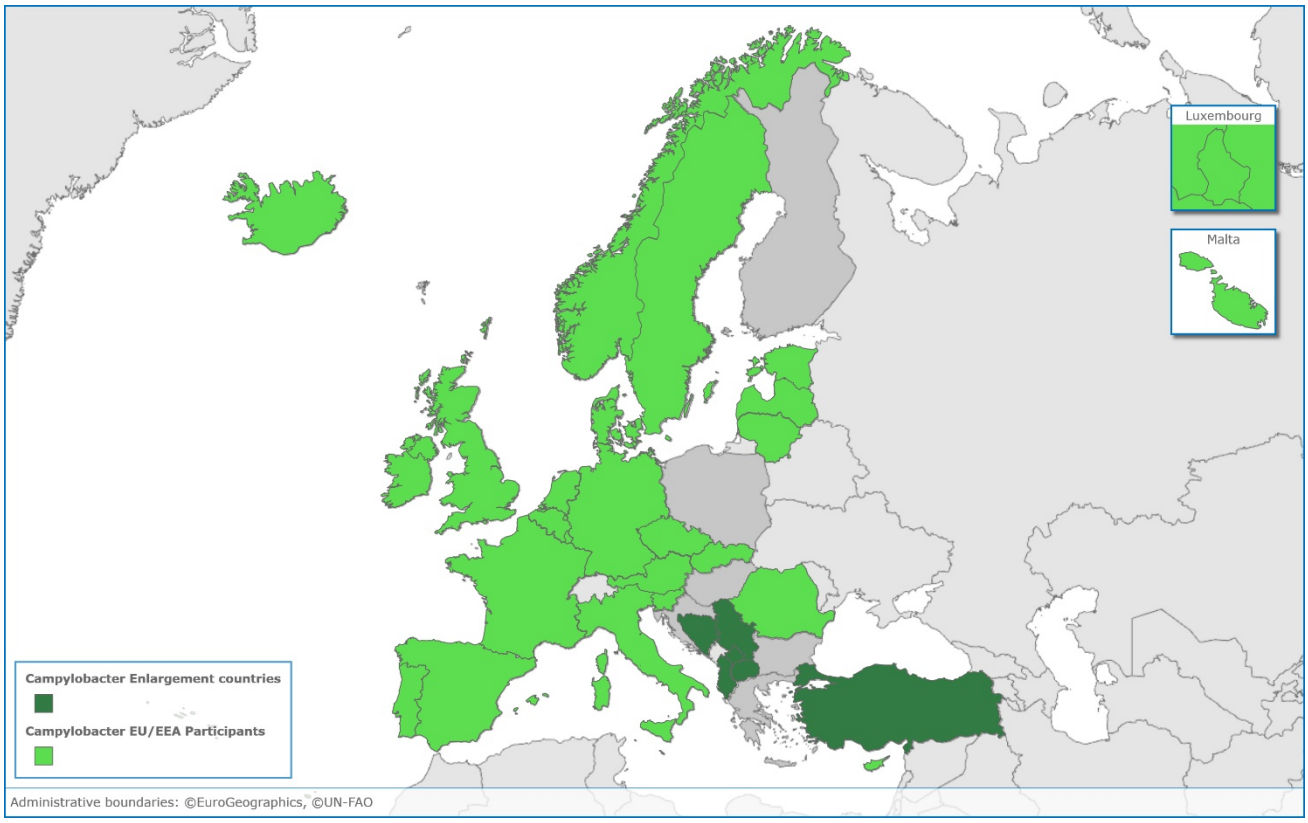
Reported results were further used to make an interpretation based on the available EUCAST ECOFFs. This interpretation (WT or NWT) was compared with the expected result as established by the EQA provider. These qualitative results where categorised into three groups. The first group included results that were in compliance with the expected interpretation (correct), the second included interpreted results not in compliance with the expected (incorrect) and the third included results where comparison was impossible due to the lack of EUCAST ECOFFs for the antimicrobial (NA).

3 Results

3.1 Participation

Twenty-three laboratories from EU/EEA countries participated in the *Campylobacter* scheme (Figure 1). In addition, six EU candidate/potential candidate countries participated in the EQA. Test results from all participants were evaluated and feedback provided individually. The report focuses solely on the results and evaluation of data from EU/EEA countries. The 23 participating countries tested all test strains for susceptibility to the mandatory antimicrobials ciprofloxacin, erythromycin and tetracycline and 13 also tested for gentamicin.

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



3.2 Applied methods in participating laboratories

Sixteen laboratories reported DD results. All used the EUCAST recommended disk concentration for the mandatory antimicrobials. For gentamicin, three laboratories used a disk concentration that deviated from the recommendation, i.e. 10 µg. Two used disks with 15 µg and one with 30 µg.

All laboratories except one used Mueller Hinton supplemented with 5% blood and six additionally supplemented the blood agar with L ß-NAD at a concentration of 20 mg/L. The one laboratory that did not use Mueller Hinton agar used Colombia blood agar.

Disks from Oxoid were widely used as 43% of the DD results were generated with this brand. Bio-Rad and Becton Dickinson disks were used for 27% and 20% of the results respectively and disks from i2A Diagnostics and Rosco Diagnostica were used to generate 7% and 4% of the results respectively.

For AST DD testing of *Campylobacter,* EUCAST recommends an incubation temperature of 41±1°C for 24 hours in a microaerobic environment. Two laboratories deviated from this recommendation and used an incubation temperature of 35°C.

Twelve laboratories reported MIC results. Seven used gradient strips, six used 'Etest' from bioMérieux and two used strips from Liofilchem. All gradient strip results were generated using Mueller Hinton agar supplemented with blood and four laboratories further reported that they supplemented the agar with L ß-NAD. Four laboratories used an incubation temperature of 41–42°C and three used an incubation temperature of 35–37°C.

Five laboratories reported MIC data based on (micro) broth dilution, four used the TREK Sensititre microdilution system (also used by the EQA provider) and one used an in-house assay. Four laboratories used Mueller Hinton broth supplemented with 5% blood and two reported that their media were supplemented with L ß-NAD. One laboratory, L12, used *Brucella* broth. Two laboratories used an incubation temperature of 41–42°C and three used 35–36.5°C as incubation temperature. One laboratory, L12, used an in-house assay that applied 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 qualitative MIC results for gentamicin (7 strains) and erythromycin (2) when applying the principles described in section 2.5 and consequently these results were classified as ND. The laboratory used oxytetracycline instead of tetracycline for MIC determinations.

3.3 Campylobacter species identification

Overall, species identification was done 100% correctly for five strains (Table 2). For two strains (C17.0005 and C17.0006), 22/23 laboratories designated the species correctly and the same laboratory was responsible for both incorrect results. The C.17.0002 strain was identified as *C. jejuni* by the EQA provider, but since 11 of the participating laboratories identified the strain as *C. coli*, it was concluded that the strain shipped by the EQA provider was most likely a mixed culture. Consequently, all results for this strain have been excluded from this report.

Table 2. Results of *Campylobacter* species identification and laboratories causing deviations

Strain	Species	Correct	Laboratory
EQA_AST.C17.0001	*C. jejuni*	23/23 (100%)	-
EQA_AST.C17.0003	*C. coli*	23/23 (100%)	-
EQA_AST.C17.0004	*C. jejuni*	23/23 (100%)	-
EQA_AST.C17.0005	*C. jejuni*	22/23 (96%)	L032
EQA_AST.C17.0006	*C. coli*	22/23 (96%)	L032
EQA_AST.C17.0007	*C. jejuni*	23/23 (100%)	-
EQA_AST.C17.0008	*C. coli*	23/23 (100%)	-

3.4 Antimicrobial susceptibility testing of *Campylobacter*

The laboratories' participation with DD and MIC results, as well as the percentage of correct qualitative and quantitative results that were reported for the seven test strains, are presented in Table 3. Eleven of 23 laboratories tested all mandatory antimicrobials using DD only, seven used MIC determinations only and five tested all mandatory antimicrobials with both DD and MIC methods or a combination of the two (3).

For DD, the total number of correct results within the accepted ±4 mm difference from the expected value was 79% for the mandatory antimicrobials ciprofloxacin, erythromycin and tetracycline. Six laboratories reported more than 90% correct quantitative DD results and three reported less than 65% correct results (Table 3). The percentage of correct quantitative DD results for the optional antimicrobial gentamicin was 54% and varied from 14–100% by laboratory (Table 3).

For MIC, the total number of correct results within the accepted one dilution difference was 79% for the mandatory antimicrobials and 88% for gentamicin (Table 3). The percentage of correct quantitative MIC results for gentamicin varied from 86–100% among the laboratories that applied the recommended concentration range. Three laboratories, all using microdilution, reported 100% correct quantitative data for both the mandatory and optional antimicrobial (Table 3). All three laboratories used TREK Sensititre microdilution plates; two used the EUCAMP2 plate (also used by the EQA provider) and one used a customised plate. The other laboratories, two using microdilution plates and seven using gradient strips, reported from 52–79% correct results for the mandatory antimicrobials (Table 3).

After interpreting results using EUCAST ECOFFs, the overall proportions of correct qualitative DD results were 99.7% for the mandatory antimicrobials and 100% for gentamicin. Only one laboratory reported any incorrect results. The proportions of correct qualitative MIC results after interpretation using EUCAST ECOFFs were 97% for the mandatory antimicrobials and 100% for gentamicin. Nine laboratories reported 100% correct qualitative results for the mandatory antimicrobials and the remaining laboratories reported from 81–95% correct results for the mandatory antimicrobials (Table 3).

Table 3. Laboratories participating (represented by arbitrary number) from EU/EEA countries in *Campylobacter* EQA3-AST, participation of mandatory antimicrobials by method and percentage correct results of test strains (C17.0001, C17.0003-0008)

	DD								MIC							
	Mandatory					Optional			Mandatory					Optional		
Laboratory ID	Ciprofloxacin	Erythromycin	Tetracycline	Correct quantitative results	Correct qualitative results	Gentamicin	Correct quantitative results	Correct qualitative results	Ciprofloxacin	Erythromycin	Tetracycline	Correct quantitative results	Correct qualitative results	Gentamicin	Correct quantitative results	Correct qualitative results
L003									B	B	B	100%	100%	B	100%	100%
L006				71%	100%											
L007				86%	100%				G	G	G	76%	100%			
L008				81%	100%		43%	100%	B	B	B	76%	95%	B	100%	100%
L011				76%	100%		29%	100%								
L012									B	B	B	71%	90%	B	14%	100%
L015				95%	100%											
L016				100%	100%											
L017				52%	100%		29%	100%								
L020				52%	100%											
L021				95%	100%		14%	100%	G	G		79%	100%			
L022									G	G	G	71%	100%	G	86%	100%
L024				67%	100%		86%	100%								
L028				76%	100%											
L029				62%	95%		29%	100%								
L030				90%	100%											
L032									G	G	G	52%	81%	G	100%	100%
L033				90%	100%											
L034									B	B	B	100%	100%	B	100%	100%
L037									G	G	G	71%	100%			
L038				76%	100%		100%	100%	G	G	G	71%	100%	G	100%	100%
L039									G	G	G	76%	100%			
L040				95%	100%		100%	100%	B	B	B	100%	100%	B	100%	100%
Total				79%	99.7%		54%	100%				79%	97%		88%	100%

All results categorised as NA excluded from total number of results.
B: broth microdilution
G: gradient strip
: all results for 7 test strains reported.

3.4.1 Results by antimicrobial and strain

Table 4 provides an overview of the DD and MIC results from all participating laboratories by antimicrobial.

Sixteen laboratories reported DD results for all mandatory antimicrobials and eight also tested for gentamicin susceptibility (Table 4). The highest proportion of correct quantitative DD results was reported for ciprofloxacin (86%) and the lowest for gentamicin (55%). With the exception of one tetracycline result, all DD results were correct when interpreted using EUCAST ECOFFs (Table 4).

Twelve laboratories reported MIC results for the mandatory antimicrobials ciprofloxacin and erythromycin and 11 reported MIC results for tetracycline. Eight laboratories reported MIC results for gentamicin (Table 4). For erythromycin, tetracycline and gentamicin, the proportion of correct quantitative MIC results was 86–88%, while for ciprofloxacin, it was 63%. The proportion of correct quantitative MIC results for ciprofloxacin was significantly lower (Chi2 test, $p<0.01$) than the corresponding ciprofloxacin results for DD (86%). However, MIC results for ciprofloxacin differed greatly by method, with 97% correct results from microbroth dilution and only 39% of results from gradient strips. When applying the EUCAST ECOFFs, the proportion of correct qualitative results varied from 95% for tetracycline to 100% for gentamicin (Table 4).

Table 4. Performance per antimicrobial for DD and MIC for three mandatory and one optional antimicrobial

Mandatory antimicrobials	Number of laboratories performing DD	Numbers of DD results within accepted 4 mm difference out of total tested	Number of correct results when using EUCAST ECOFF	Number of laboratories performing MIC	Numbers of MIC results within accepted 1-dilution difference out of total tested	Number of correct results when using EUCAST ECOFF
Ciprofloxacin	16	96/112 (86%)	112/112 (100%)	12	53/84 (63%)	82/84 (98%)
Erythromycin	16	82/112 (73%)	112/112 (100%)	12	74/84 (88%)	83/84 (99%)
Tetracycline	16	88/112 (79%)	111/112 (99%)	11	66/77 (86%)	873/77 (95%)
Gentamicin	8	35/64 (55%)	40/40* (100%)	8	49/56 (88%)	56/56 (100%)

All results categorised as NA excluded from total number of results due to lack of EUCAST ECOFFs for C. coli.

Disk diffusion

The distributions of reported *Campylobacter* DD values (mm) from all laboratories for each test strain and the control strains *C. jejuni* ATCC 33560 are presented in Table 5.

EUCAST has defined acceptance criteria for the size of the inhibition zone for the control strain *C. jejuni* ATCC 33560. The targets are 38 mm, 31 mm and 34 mm inhibition zones for ciprofloxacin, erythromycin and tetracycline respectively and diameter zones within ±4 mm are considered acceptable [9]. Eight laboratories reported correct values for the control strains for these three antimicrobials. For erythromycin, all laboratories reported results within the acceptable range, while for ciprofloxacin, five reported inhibition values that were either too low (2) or high (3) and for tetracycline, seven reported values that were too high. EUCAST has not defined acceptance criteria for gentamicin, but three of the reported gentamicin DD results for the control strain were outside the accepted range compared with the expected value established by the EQA provider.

Ciprofloxacin DD results for three of the NWT strains (C17.0003, C17.0007 and C17.0008) were all within the accepted range and for the remaining WT (C17.0004 and C17.0006) and NWT (C17.0001 and C17.0005) strains, three to five results were up to 6 mm outside the accepted range (Table 5).

For erythromycin DD results, only one NWT strain, C17.0003, was within the accepted DD range of 4 mm. For the remaining six strains, three to nine of the reported results were outside the accepted range by up to 12 mm and mostly above the accepted range (Table 5). A similar pattern was seen for tetracycline DD results, where only the NWT strain C17.0003 was within the accepted DD range. For the remaining six strains, one to seven of the reported results were outside the accepted range.

There was also a considerable span observed in the range of reported DD values for gentamicin for six of the test strains, all WT. The reported values were mostly above the expected zone diameter, some up to 17 mm above (Table 5). The exception was strain C17.0008, the only gentamicin NWT strain in the test panel, where all gentamicin DD results were within the accepted range (Table 5).

Table 5. Distribution of *Campylobacter* DD values (mm) of participating laboratories

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	41	42	43	44	45	46	>46
Ciprofloxacin	ATCC 33560																											1	1	1		1		1	2	2	3	1	1	1	1		
Ciprofloxacin	C17.0001	9			1	2	1		2	1																																
Ciprofloxacin	C17.0003	13		2	1																																					
Ciprofloxacin	C17.0004																							1							3	1		2		2	4	1			1			1
Ciprofloxacin	C17.0005	10		1	1	1	1	1		1																																
Ciprofloxacin	C17.0006																											1		1			1	2		1	1	4	1		1	1	2
Ciprofloxacin	C17.0007	11		3	1	1																																				
Ciprofloxacin	C17.0008	14			1	1																																				
Erythromycin	ATCC 33560																						2	2		2	2	4		4													
Erythromycin	C17.0001																										2	1	3	1	3	1	2	1	1				1				
Erythromycin	C17.0003	15		1																																						
Erythromycin	C17.0004																						1	2		4	1	2		1	2	1								1			1
Erythromycin	C17.0005																							1		2	2	1	2	1	1	1	1	1		2		1				
Erythromycin	C17.0006																			1	1	1	1	3	2	2	1	2	1									1				
Erythromycin	C17.0007																			1		1	1		1	3	1	2	1	1		1	1			1	1					
Erythromycin	C17.0008	11		2				1			1		1																												
Tetracycline	ATCC 33560																											1	1			3	2	1	1		2	1	1	1	1	1		
Tetracycline	C17.0001																																	1	2	1		3	3	1	1	1	3
Tetracycline	C17.0003	9		1	1	1	2	2																																		
Tetracycline	C17.0004	1																										1				1	2	1	1	1			2	2	1	1	2
Tetracycline	C17.0005	6		2		4	1	2			1																															
Tetracycline	C17.0006																												1	1	1	2		3	2		1	2		1		1	1
Tetracycline	C17.0007	7				1	1	1	2	2				1								1																				
Tetracycline	C17.0008	13				1	2																																			
Gentamicin	ATCC 33560																			1	1	1			1					2						1					
Gentamicin	C17.0001																							2			1		1	1		2				1					
Gentamicin	C17.0003																				2	1					1		1	2						1					
Gentamicin	C17.0004																				1		1		1	1			1		1		1						1		
Gentamicin	C17.0005																								1	2		2		1	1					1					
Gentamicin	C17.0006																						1		1	1	1	1			2									1		
Gentamicin	C17.0007																								1			1	1	2		1			1	1					
Gentamicin	C17.0008	7			1																																					

Column header: DD results for strains tested (mm)

(dark green): *expected value*
(light green): *accepted range.*
Red line indicates ECOFF according to EUCAST for respective antimicrobial, WT right of red line.

Dilution and gradient strip

Table 6 shows the distribution of reported MIC results for all antimicrobials and each strain individually, including the reference strain *C. jejuni* ATCC 33560.

EUCAST has not defined acceptance criteria for MIC values for the reference strain C. jejuni ATCC 33560, so the reported MIC values were compared with the values established by the EQA provider. For erythromycin, all reported results (12) were within ± one dilution range and in accordance with the expected value. For ciprofloxacin, one laboratory reported a result that was one dilution step out of range (too low) and the remaining results were within ± one dilution range. For tetracycline, eight laboratories reported results within ± one dilution range, two one dilution step too high and one two dilution steps too low. For gentamicin, five laboratories reported results that were within the acceptable ± one dilution range and two reported results that were one dilution too high.

For ciprofloxacin, five NWT strains (C17.0001, C17.0003, C17.0005, C17.0007 and C17.0008) were included in the test panel. Twenty-eight of the reported ciprofloxacin MIC values for these NWT strains were out of range. These 28 MIC values, reported as >32, were all generated by the use of gradient strips. With the exception of one result, all broth dilution MIC values were within the accepted range and thus in accordance with the expected values for the five NWT strains. One laboratory, L32, reported ciprofloxacin MIC values for C17.0005 and C17.0006 that

deviated several dilution steps from the expected value and also deviated from the expected result when interpreted by the EUCAST ECOFFs. Ciprofloxacin WT strains were all within the expected range (Table 6).

For erythromycin, MIC results reported for strains C17.0003, C17.0005 and C17.0007 were all within the accepted range. For strains C17.0001, C17.0004 and C17.0006, two, two and one result respectively were lower that the accepted range. For strain C17.0008, two results deviated more than three dilution steps from the expected value and one, reported by L008 and generated using microbroth dilution also gave a deviating result when interpreted by the EUCAST ECOFF. Two erythromycin MIC results were classified as ND because the results were not in the relevant range for comparison with the EQA provider's results (Table 6).

In general, several of the reported tetracycline MIC results were outside the accepted range for the test strains (Table 6). Two of the reported MIC values for strain C17.005, one for C17.006 and one for C17.007, were several dilution steps out of range and when interpreted with the EUCAST ECOFF value, they were classified incorrectly (Table 6).

The reported gentamicin MIC results for the six WT strains (C17.0001 and C17.0003–7) were all within the accepted range except one for C17.004 that was one dilution higher the accepted range. Strain C17.0008 was a NWT strain and the gentamicin MIC results were reported as >8 or >16 mg/L using microdilution methods and 512 mg/L using gradient strips. These results were all assigned as correct. Seven of the gentamicin MIC results were classified as ND because the results were outside the relevant range for comparison with the EQA provider's results (Table 6).

Table 6. Distribution of MIC values (mg/L) of participating laboratories

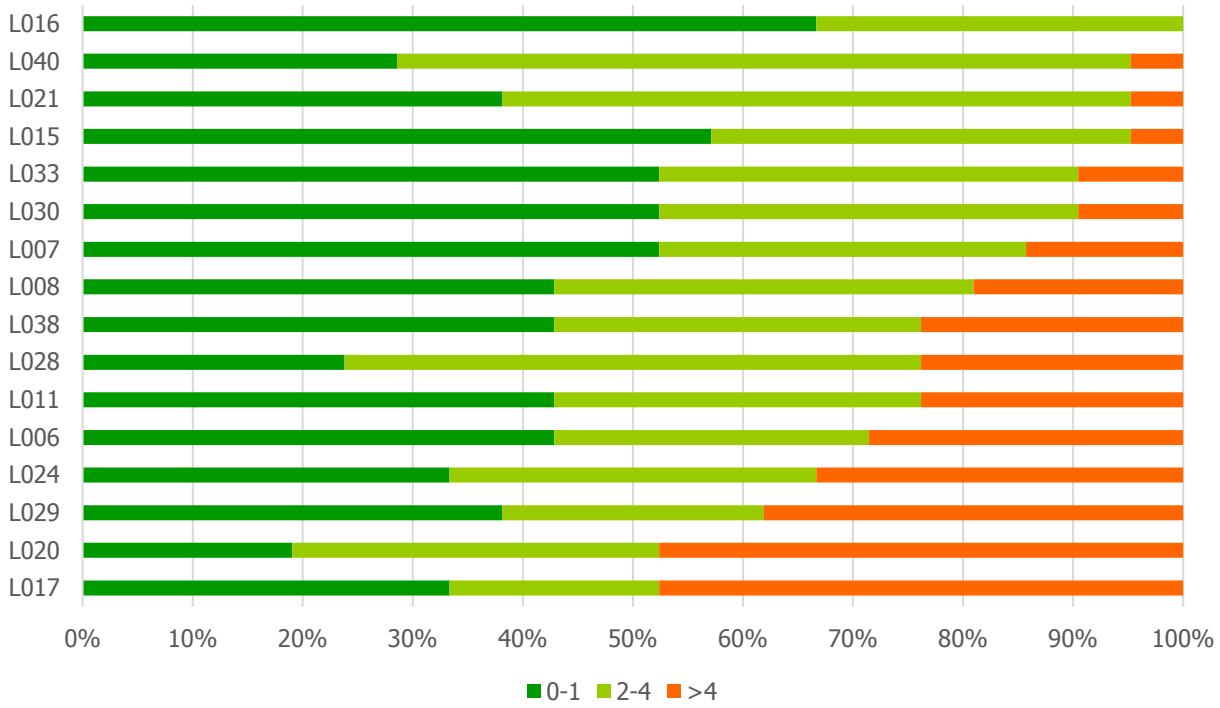
Antimicrobial	Strain	MIC results (mg/L)																	
		ND	<0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>512
Ciprofloxacin	ATCC 33560			1	5	5	1											
	C17.0001											4	4		4			
	C17.0003											1	3	1	7			
	C17.0004				5	5	2											
	C17.0005					1						4	3		4			
	C17.0006			4	2	5								1				
	C17.0007											3	3		6			
	C17.0008										1		2	2	7			
Erythromycin	ATCC 33560							1	9	2								
	C17.0001				1		1	4	6									
	C17.0003	1															11	
	C17.0004					1	1	4	5	1								
	C17.0005							6	5	1								
	C17.0006						1		4	7								
	C17.0007							4	3	5								
	C17.0008	1							1				1				4	5
Tetracycline	ATCC 33560				1		2	5	1	2								
	C17.0001		1	1		3	2	3	1									
	C17.0003											1		1		5		4
	C17.0004			1	1	1	3	5										
	C17.0005						1	1							4	5		
	C17.0006							4	6						1			
	C17.0007								1					3	2	2	1	2
	C17.0008											1		1	1	3	1	4
Gentamicin	ATCC 33560	1				2	1	2	2									
	C17.0001	1				3	2	2										
	C17.0003	1					2	4	1									
	C17.0004	1				3	3	1										
	C17.0005	1				1	3	3										
	C17.0006						1	4	3									
	C17.0007	1				2	2	3										
	C17.0008	1												4				3

ND: not determined due to reported MIC result not in relevant range for comparison with EQA provider's result
: expected value
: accepted range.
Red line indicates ECOFF according to EUCAST for respective antimicrobial, WT above red line.

3.4.2 Individual laboratory results

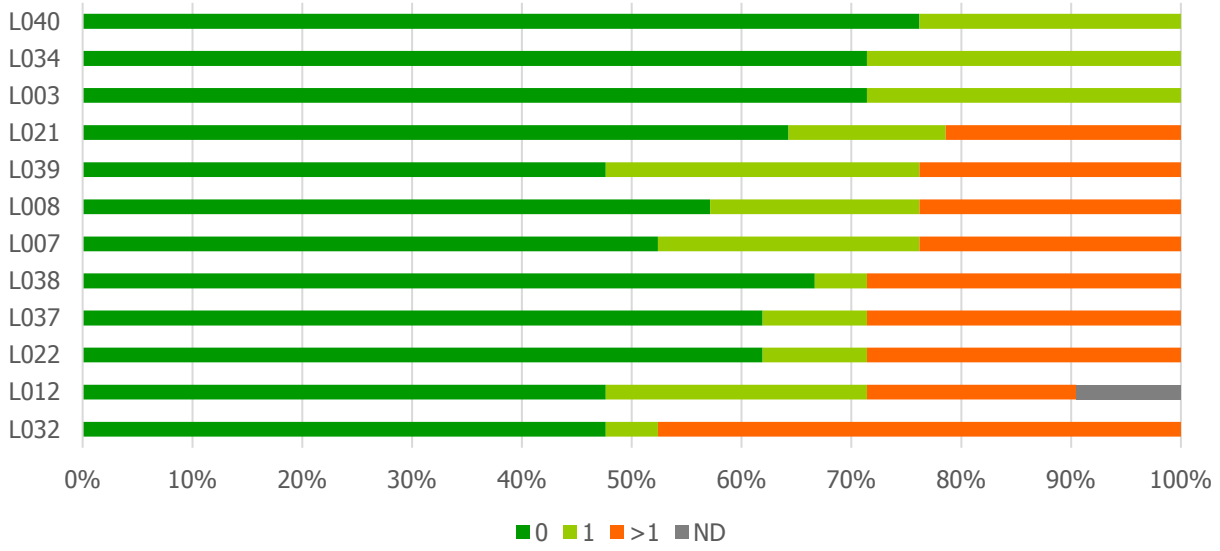
A comparison of the performance of individual laboratories for quantitative DD results of mandatory antimicrobials is presented in Figure 2. Data are shown as percentage of results within a 0–1, 2–4 (correct) or >4 mm difference (incorrect) from the value established by the EQA provider. DD results reported by the individual laboratories varied from 52–100% correct. One laboratory (L016) reported 100% correct DD results, three (L015, L021 and L040) 95% and two (L030 and L033) 90% correct results (Figure 2). Six laboratories (L006, L007, L008, L011, L028 and L038) reported 71–86% correct results, while four laboratories (L017, L020, L024 and L029) reported 52–67% correct results (Figure 2).

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



In Figure 3, a comparison of the performance of each laboratory for quantitative MIC results for the mandatory antimicrobials is shown. Data are presented as the percentage of results within 0, 1 (correct) or >1 (incorrect) dilution difference to the value established by the EQA provider. The proportions of correct MIC results reported by the individual laboratories varied from 52–100%. The three laboratories (L003, L034 and L040) that reported 100% correct quantitative MIC results all used the TREK Sensititre system for microdilution testing. The eight laboratories reporting from 71– 79% correct quantitative results used either other microdilution systems (L008 and L012) or gradient strip (L007, L021, L022, L037, L038 and L039; Figure 3). Laboratory L012 was the only one that reported MIC results not fulfilling the criteria for the recommended range and therefore this laboratory had certain results classified as ND (Figure 3).

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



ND: not determined due to reported MIC result not in relevant range for comparison with expected result.

4 Discussion

Since 2008, EU/ EEA countries can report AMR data to TESSy as part of routine surveillance data for campylobacteriosis. In 2014, ECDC published the harmonised EU AST protocol (updated in 2016) with guidance on laboratory procedures and the interpretation of data [6,7]. The purpose of the EQA3-AST on *Campylobacter* was to evaluate the quality of the AST data generated in the FWD laboratory network when they followed the harmonised EU AST protocol. The submitted data were used to determine the relative accuracy of quantitative and qualitative AST data and assess the overall comparability of AST data. An additional aim was to collect information on the methods used by each laboratory to produce data on antimicrobial susceptibility.

The quality of the reported results was generally in line with what have been seen in the previous EQAs in the FWD laboratory network. All quantitative DD results, with the exception of one tetracycline result, were correct when interpreted with the EUCAST ECOFFs and 95%–100% of the ECOFF-interpreted MIC results were correct by antimicrobial. The quantitate results were overall marginally better than the results obtained in EQA2-AST performed in 2016. The performance is also in line with the results obtained in the most recent proficiency test in the EU reference laboratory network for antimicrobial resistance [10], where the ECOFF-interpreted MIC results for the four antimicrobials tested in the present EQA all were above 98.7%.

Twenty-seven NPHRLs in EU/EEA countries were invited to participate in the EQA and 23 countries accepted the invitation. The number of participating laboratories was exactly the same as in EQA2-AST. The logistics of the EQA went well overall. All laboratories were able to recover the test strains and the submission of results on the Enalyzer platform went well. All participating laboratories submitted results for the mandatory antimicrobials for all test strains. Because of inconsistency in the species identification of strain C17.0002, it was suspected that the culture was a mixture of *C. jejuni* and *C. coli* and all results for this test strain were consequently excluded from data analysis. In order to improve the quality of the test strains in future EQAs, it was decided that test strains should be evaluated in a second laboratory before shipment to EQA participants. There was good agreement overall between the quantitative results reported by participants and the expected results established by the EQA provider.

Different criteria are applicable to interpret the AST values for *C. jejuni* and *C. coli* respectively. Correct species identification is therefore essential for the correct interpretation and comparability of surveillance results in general. The reported species of the *Campylobacter* strains in the EQA was in line with the expected and performance on this capability has improved compared to previous EQA exercises.

For the purpose of the present report, it was decided to analyse all MIC data together, i.e. both results generated by gradient strips and broth dilution methods. It could be argued that that gradient strip and DD results are more related than gradient strip and broth dilution methods as they rely on diffusion of the antimicrobial into agar-based media. Data for the three different methodologies are presented in Table 4. From this table, it is obvious that quantitative results for broth dilution and disk diffusion are superior to gradient disk results for ciprofloxacin, but apart from this, the data appear to be similar. The main reason for the discrepancy in the ciprofloxacin results were the five tested NWT strains. They were all attributed a ciprofloxacin MIC value of >32 with gradient strips, while the reported broth dilutions values were =8 or =16. The reason for the difference is not known. However, it should also be kept in mind that the difference in the MIC values results did not affect the qualitative results.

Participating laboratories were asked to follow the same protocol for AST testing. The protocol gives laboratories a large degree of freedom to choose between methods (DD, gradient strip and broth dilution) and this presents certain technical challenges when data are analysed and compared.

Expected MIC values were determined using microbroth dilution method applying the twofold dilution range recommended in the harmonised EU AST protocol. Certain laboratories reported data using a range that was narrower that the recommended, making it impossible to calculate the dilution difference for results that were meaningful when interpreted with the ECOFFs.

Certain gradient strips can determine MIC values on a finer scale than the recommended twofold dilution range and therefore certain MIC values from gradient strips are approximated to the nearest twofold dilution MIC value before comparison with the expected value. The effect of this intrinsic bias is hard to ascertain and it is likewise difficult to ascertain the potential effects of the use of different consumable suppliers.

Certain laboratories did not fully adhere to the recommendations given in the harmonised EU AST protocol and it is notable that some submitted results for the reference strain that were out of the quality control range. Generally, laboratories used disks with recommended concentration of antimicrobials and most used media prescribed by EUCAST.

The harmonised EU AST protocol recommends (micro) broth dilution as the preferred testing method for monitoring purposes. However, validated methods of gradient strip diffusion or DD according to EUCAST protocols are also accepted. In the present EQA, five laboratories used broth dilution methods. Three of these laboratories,

all using the TREK Sensititre system, reported 100% correct quantitative MIC results, supporting the EUCAST recommendation on choice of methods.

The performance of the individual laboratories varied substantially compared with the expected results established by the EQA provider. For both DD and MIC, the percentage of correct results for the mandatory antimicrobials varied from 52–100%. These figures are overall in line with the results seen in EQA2-AST and indicate that it is feasible to improve the quality of AST data generated by certain FWD laboratories.

5 Conclusion

Twenty-three of 27 invited laboratories from the FWD network participated in the EQA. All laboratories performed species identification and submitted results for the mandatory antimicrobials ciprofloxacin, erythromycin and tetracycline. Thirteen laboratories additionally reported results for gentamicin.

Overall, there was good correspondence between the expected results established by the EQA provider and the results reported by the participating laboratories. For the mandatory antimicrobials, relative accuracy, i.e. the percentage of DD and MIC results that were within the accepted range from the expected result, was 79% for both DD and MIC methods. With the exception of one tetracycline result, all reported DD results were correct when interpreted with the EUCAST ECOFFs. For MIC, 97% of the interpreted results were correct. This shows that it is possible to compare routinely collected AST results from NPHRLs across Europe when interpreted with the EUCAST ECOFFs.

For the mandatory antimicrobials, the percentage of correct quantitative results varied from 52–100% for both DD and MIC results. This indicates that it is feasible to improve the quality of AST data generated in certain FWD laboratories. No common laboratory problems were identified related to guidance in the harmonised EU AST protocol, but certain laboratories did not comply entirely with the protocol and it is of concern that some reported DD results for the reference strain that did not comply with the EUCAST target range for quality control.

6 Recommendations

6.1 Laboratories

The evaluation of the results obtained by the FWD-Net laboratories in this EQA identifies a number of issues. First and foremost, it is important that the laboratories follow the recommendations stipulated in the harmonised EU AST protocol, which specifies that the guidelines provided by EUCAST should be followed. These guidelines includes specifications for control strains, media, incubation temperature, disk concentrations for DD testing and range for MIC determination. Certain laboratories did not follow all these recommendations. For both DD and MIC testing, there were laboratories that submitted results that were 100% in accordance with the expected values established by the EQA provider, indicating that it is feasible to improve the quality of AST data in many FWD laboratories.

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 interpretation of data in Member States. To ensure a better understanding of the methods used for testing of antimicrobial susceptibility, one option could be to provide educational/consultancy facilities for FWD-Net laboratories or even provide FWD-Net courses.

6.3 EQA provider

In order to ensure the quality of the test strains and avoid mixed cultures, the strains will be shared with another laboratory for testing and validation of the expected values. To further help target the troubleshooting of laboratories, the current reporting scheme will be further developed for a more detailed and uniform collection of methods, manufacturers, growth media and incubation temperatures used by the participating laboratories.

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Annex. List of participants in the *Campylobacter* EQA3-AST

Country	EU status	ECDC laboratory	ECDC institute
Albania	Enlargement	Laboratory of Enterobacteriology	Institute of Public Health
Austria	EU/EEA	NRC Campylobacter Austria	Institute for Medical Microbiology and Hygiene Graz
Belgium	EU/EEA	LHUB-ULB Site Porte de Hal	CHU Saint-Pierre
Bosnia and 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
Former Yugoslav Republic of Macedonia	Enlargement	Laboratory of Bacteriology and Antimicrobial Resistance	Institute of Public Health of the Republic of Macedonia
France	EU/EEA	CNR des Campylobacters, Laboratoire de Bactériologie	Hopital Pellegrin
Germany	EU/EEA	NRC Salmonella	Robert Koch Institute
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 Insitute of Public Health of Kosovo
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
Netherlands	EU/EEA	National Reference Laboratory for Antimicrobial Resistance in Animals	Wageningen Bioveterinary Research (WBVR)
Norway	EU/EEA	Norwegian Reference Laboratory of Enteropathogenic Bacteria	Norwegian Institute of Public Health
Portugal	EU/EEA	LNR Infeções Gastrintestinais	INSA
Serbia	Enlargement	Reference Laboratory for Campylobacter and Helicobacter	Institute for Public Health Nis
Romania	EU/EEA	Enteric Bacterial Infections Laboratory	Cantacuzino National Institute of Research
Slovakia	EU/EEA	NRC for Salmonelloses, NRC for ATB	Public Health Authority of the Slovak Republic
Slovenia	EU/EEA	NLZOH – CMM – Oddelek za medicinsko mikrobiologijo Nova Gorica	Nacionalni laboratorij za zdravje, okolje in hrano
Spain	EU/EEA	Unidad de Enterobacterias	Centro Nacional de Microbiología
Sweden	EU/EEA	Clinical Microbiology	Central Hospital
Turkey	Enlargement	National Reference Laboratory for Enteric Pathogens	Public Health Institution of Turkey
United Kingdom	EU/EEA	Gastrointestinal Bacteria Reference Unit	National Infection Service

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