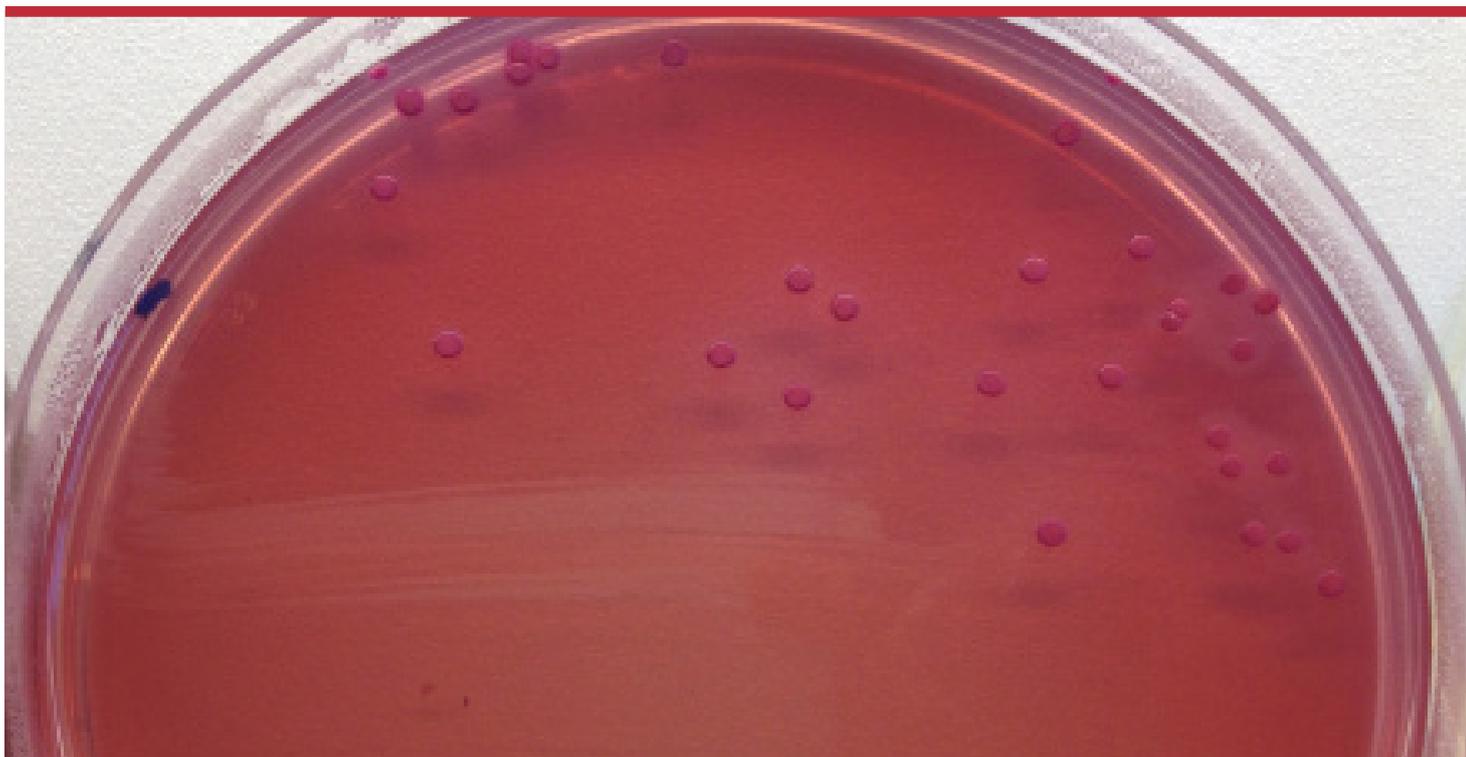


# THE 2<sup>nd</sup> EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF E. COLI WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CAECAL SAMPLES - 2016



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THE 2nd EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF *E. COLI*  
WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CAECAL  
SAMPLES – 2016

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# 1. Introduction

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Extended spectrum beta-lactamase (ESBL) and AmpC-producing *E. coli* continue to spread in food producing animals. In 2013, the European Commission (EC) has taken the decision of including the isolation of ESBL and AmpC-producing *E. coli* as mandatory parts of the EU monitoring which was started during 2015 and continued in 2016. The screening was performed during 2016 including both meat and caecal samples from poultry origin in the EU Member States and affiliated countries according to a common protocol defined by the EC and validated by the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR, 2014). Training on these protocols was provided in 2014 for all the involved National Reference laboratories (NRLs) and additional training was provided to two small groups of laboratories in 2015 and 2016.

In 2016 the EQAS was extended also to include carbapenemase and/or OXA-48-producing *E. coli*, thereby including the optional isolation of these using the EURL-AR selective isolation protocol on agar plates suitable for isolation of carbapenemase-producing *E. coli*.

Similarly to the previous EURL-AR matrix based EQAS, the aim of this specific EQAS was to enhance the capacity of the laboratories in isolation, identification and antimicrobial susceptibility testing (AST) of ESBL/AmpC or carbapenemase-producing *E. coli*. Furthermore the results may be used to identify potential problems and/or focus areas for future training/education.

Before October 2016 a preliminary study was conducted to prepare for the launch of this EQAS, using meat samples and caecal content matrices of poultry origin spiked with strains containing the resistance genes of interest.

We launched this second EQAS matrix in October 2016. As mentioned above, this trial included isolation steps, confirmation of the ID as *E. coli*, MIC testing and confirmation of ESBL/AmpC and/or carbapenemase phenotypes by antimicrobial susceptibility testing on the second panel of antimicrobials.

From January 2016, the laboratories should have implemented the methods and have started the monitoring on meat and caecal samples of poultry origin. Therefore the methods should have been implemented and validated at the NRL's before this EQAS. The participation in this EQAS may be used to assess retrospectively the quality of data provided to the European Food Safety Agency (EFSA).

No thresholds have been set in advance to evaluate the acceptance of the performance of the participating laboratories and therefore the results will not be classified as above or below a threshold, but evaluated case by case.

The EQAS was organized by the EURL-AR at the National Food Institute (DTU Food), Kgs. Lyngby, Denmark.

The data in this report are presented with laboratory codes. A laboratory code is known only by the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only by relevant representatives of the EURL-AR and the EU Commission. All conclusions are public.

The technical advisory group of the EURL-AR EQAS scheme consists of competent representatives from all National Reference Laboratories for Antimicrobial Resistance (NRLs-AR), who meet annually at the EURL-AR workshop.

## 2. Materials and Methods

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### 2.1 Participants in EQAS 2016

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A pre-notification (App. 1), inviting the participants to the matrix EQAS 2016 was issued by e-mail to the EURL-AR network on the 23<sup>rd</sup> of August 2016.

All participants were included in a participant list (App. 2) before the preparation and shipping of the samples. Participation was free of charge but each laboratory was expected to cover expenses associated with the analysis.

### 2.2 Preparation of samples

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Eight samples were prepared and dispatched for isolation of ESBL, AmpC or carbapenemase-producing *E. coli*, including identification, and antimicrobial susceptibility testing (AST) of the obtained isolates. The samples included were both meat and caecal samples from poultry origin and were either artificially prepared to contain the test strains or unmodified.

The meat used to prepare the samples was minced chicken meat acquired in local supermarkets (at least three batches were bought in sufficient amount for covering both the pre-tests and preparation of the samples). The meat was pretested using the official method for selective isolation of *E. coli* producing ESBL, AmpC or carbapenemase to ensure the batch used was negative for those and contained some background flora. A batch fulfilling these criteria was chosen for preparation of aliquots of 25 g of meat that were either directly used as blank samples or spiked as follows.

The test isolates used in the spiking of meat samples within the EQAS matrix 2016 were prepared in advance and sub-cultured the day before sample preparation. For the sample preparation and standardization of the spiking,

suspensions equal to McFarland 0.5 were prepared in saline tubes with the relevant isolates to contain about  $10^8$  CFU/mL, as confirmed by viable counts of serial dilutions on Luria Bertrani (LB) agar plates. The standardized suspensions were further diluted in tenfold dilutions and the meat samples (25 g) were spiked with 25  $\mu$ l of the chosen dilutions. The spiking dilutions were chosen based on the stability of the results obtained in a pilot test performed before the EQAS sample preparation. This pilot was performed with samples spiked with the intended test strains and aimed at testing the stability of the inoculum in the samples and defining the concentrations to use in the EQAS to maximise the sensitivity of detection but without compromising sample stability. The final inoculum found in the samples in this EQAS was expected to be circa  $10^3$  CFU/g of meat, for the samples M-2.1, M-2.2, M-2.4 and M-2.5, whereas the sample M-2.3 was spiked with a lower amount of about 100 CFU/g of the test strain. The sample M-2.1 was spiked as mentioned above, however with a susceptible *E. coli* strain (ATCC 25922) and therefore expected to be negative.

For the caecal samples, one slaughterhouse provided us with four batches of caeca from poultry (named as A-D) and the amount per batch was ranging between 140-300g (so all samples could be prepared from one single batch of matrix). The batches were tested individually using the official selective isolation protocol for ESBL, AmpC and carbapenemase-producing *E. coli*. One negative caecal batch (batch C) was chosen to prepare all the caecal samples for the EQAS. Thereby 1g of caecal content was spiked with 10  $\mu$ l of a dilution containing  $10^6$  CFU/ml and therefore the expected spiking level was  $10^4$  CFU/g for samples M-2.6 and M-2.7, while sample M-2.8

was kept as blank.

The minimal inhibitory concentrations (MIC) of selected antimicrobials were determined using broth microdilution method at both for the strains used for spiking during the preparation work and for the isolates obtained in the homogeneity testing after sample preparation to generate expected results (App 3).and the genes identified in the spiking isolates were detected using PCR for the expected ESBL, AmpC and carbapenemase genes on the isolates obtained from the isolation procedure performed on the samples.

For following up on the stability of the samples after shipping, repeated testing was performed of one set including the eight samples in four timepoints after shipment (during two weeks). In this period the meat samples were kept frozen at -80°C and the caecal samples were kept at 4°C.

### **2.3 Isolation and identification of ESBL, AmpC or carbapenemase producing *E. coli* from meat and caecal samples**

The official protocols for selective isolation and identification of the ESBL, AmpC and/or carbapenemase-producing *E. coli* isolates contained in the samples were available on the EURL website, <http://www.eurl-ar.eu> (App. 4a and 4b). For the identification of *E. coli* species different methods were allowed as these are not specified in the EU Commission implementing decision on the monitoring and reporting Antimicrobial resistance in zoonotic and commensal bacteria 2013/652/EU). The description of the method used for selective isolation of presumptive ESBL, AmpC or carbapenemase-producing *E. coli* as well as species identification was requested as part of the methods sheet to be completed in the database upload system.

### **2.4 Antimicrobial susceptibility testing**

The panels of antimicrobials recommended for AST in this trial are those included in the EU Commission implementing decision on the monitoring and reporting Antimicrobial resistance in zoonotic and commensal bacteria 2013/652/EU) (Table 1).

Guidelines for performing the antimicrobial susceptibility testing using dilution methods were set according to the Clinical and Laboratory Standards Institute (CLSI) document – M7-A10 (2015) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - tenth Edition” and whenever commercial methods were used, the guidelines of the manufacturer were followed.

MIC results were interpreted by using EUCAST epidemiological cut-off values ([www.eucast.org](http://www.eucast.org)), as included in the regulation referred above or as recommended by EFSA and described in the EQAS protocol (App. 4). Results of the ESBL confirmatory testing were interpreted according to the recommendations by EFSA and as referred in the regulation, using MIC testing on the second panel of antimicrobials, which is intended to be used every time a strain is found resistant to either cefotaxime, ceftazidime or meropenem.

### **2.5 Distribution**

The meat samples were frozen at -80°C and kept at this temperature after preparation and until shipment. The caecal samples were sent short after preparation, and therefore kept at 4°C until shipment. At the day of shipment, the samples were tightly packed in thermoboxes with cooling elements frozen at -80°C. The parcels contained the eight samples in tubes, and an additional tube contained a thermologger to register the temperature at 15 min intervals during transport. Furthermore, the parcel contained a welcome letter with the login



**Table 1.** Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2016 component

<i>Escherichia coli</i>	<i>Escherichia coli</i> 2 <sup>nd</sup> panel
Ampicillin, AMP	Cefepime, FEP
Azithromycin, AZI	Cefotaxime + clavulanic acid (F/C)
Cefotaxime, FOT	Cefotaxime, FOT
Ceftazidime, TAZ	Cefoxitin, FOX
Chloramphenicol, CHL	Ceftazidime, TAZ
Ciprofloxacin, CIP	Ceftazidime+ clavulanic acid (T/C)
Colistin, COL	Ertapenem, ETP
Gentamicin, GEN	Imipenem, IMI
Meropenem, MERO	Meropenem, MERO
Nalidixic acid, NAL	Temocillin, TRM
Sulfamethoxazole, SMX	
Tetracycline, TET	
Tigecycline, TGC	

and password to the web based database for the data upload and a labelled envelope for returning the thermologger to the EURL-AR.

The protocol for the EQAS and the test forms were made available online on the EURL-AR website, <http://www.eurl-ar.eu>, before launching this EQAS.

The thermoboxes used for the shipment of samples were enclosed in double pack containers and sent to the selected laboratories according to the [International Air Transport Association](#) (IATA) regulations as “Biological Substance category B” classified UN3373. The parcels were dispatched from DTU-Food October 10<sup>th</sup>, 2016.

The laboratories were instructed to download the protocol and test forms (App. 4 and 5), from <http://www.eurl-ar.eu> and to process the samples following the EU protocol for selective isolation of presumptive ESBL, AmpC and carbapenemase producing *E. coli* from either meat or caecal samples, precisely as they would normally do for the EFSA monitoring. For the results collection the NRLS were instructed to upload of the data in the web based

database, which was designed and prepared for this EQAS and opened shortly after sample shipment and until the deadline.

After completion of the tests, the laboratories were requested to enter the obtained results into the electronic sheet in the EURL-AR web based database through a secured individual login (App 5). The database was activated on the 3<sup>rd</sup> November 2016, and closed December 15<sup>th</sup> 2016.

For the first part of the results of the selective isolation procedure for ESBL /AmpC and for carbapenemases, the results obtained from the isolation procedures samples were evaluated separately by defining the samples as positive if an isolate was obtained and positively identified as *E. coli*. Additionally, the results of susceptibility testing of the obtained isolates using both MIC panels were analysed separately in similar way as to the similarly to the *E. coli* AST EQAS, including the read values of MIC and their interpretations. As a conclusion of the susceptibility testing, the participants were asked to classify the isolates obtained according to the defined EFSA criteria



for interpretation of ESBL/Ampc and/or carbapenemase producing isolates.

After the deadline, the qualitative results indicating if the samples were positive or negative for ESBL/AmpC, or carbapenemase-producing *E. coli* (OXA-48 and other), as well as the interpretations of the susceptibility tests

### 3. Results

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A total of 33 parcels were sent to the 34 laboratories that participated in the EQAS; including 31 participant laboratories representing the NRLs from all 28 MS (please note that for three MS the samples were divided by type between two laboratories), and laboratories from Norway, Switzerland and Iceland responded to the pre-notification and were additionally enrolled in the EQAS. Two additional non-NRL laboratories from EU were enrolled and provided results but were not included further in the report. For more information on the countries participating consult the map below (Figure 1).

When the deadline was reached, 33 sets of data representing the 34 enrolled laboratories were uploaded for the samples tested, thus all participating countries had one complete set of data.

As requested, the participating laboratories have, returned the thermologgers to the EURL. Here the registration of the temperature was extracted and read to provide the temperature ranges along the shipment and at sample reception/opening. Furthermore, the participants were requested to provide more information in a small introductory questionnaire on the database, including details on sample reception (measured temperature and date/time), the monitoring activities, and the methods used in their laboratory.

The temperature data were analysed at the EURL and showed a range of temperatures between -10°C and 10°C for measures taken by

results, and the conclusion on the observed *E. coli* phenotypes were evaluated against the expected results and scored as correct or incorrect. As no threshold is agreed the performance was evaluated case by case and not classified into acceptable or unacceptable based on the deviation percentage.

the participants, and -14.5°C and 7°C, when inferred from the temperature at opening time from the thermologger registration (Figure 2). This showed that most laboratories measured temperatures above those registered in the logger, however when looking at the logger temperatures most samples were expected to be in good conditions for testing at opening of the samples, and all samples were tested, independently of the temperature measured by the participants. In a few cases (Labs #29, #37, #41 and #58) it was not possible to correctly estimate the temperature at sample processing as the sample was probably not processed immediately, but kept in refrigeration or frozen from reception to the start of processing of samples as the thermologger data indicates there was a storage period.

#### 3.1 Methods used by EQAS-participants

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All 33 participating laboratories which have submitted results participated in the ESBL and AmpC isolation and performed the identification and susceptibility of the respective isolates. The number of qualitative isolation tests results reported was variable including results for three to eight samples, depending on how many samples were tested (a few participants only tested meat or caecal sample while others tested both), for the antimicrobial susceptibility test it depended on how many isolates were found and further tested in the MIC panels. The number of AST tests performed in ranged from 44 to 132 tests per participant.

Information on the methods used for isolation, identification and typing was collected from the participants through the database.

Most laboratories (n=32) reported that isolation has been performed following the exact procedures described in the protocol provided. One participant reported that the incubation was processed at 41.5°C instead of 44° and

20h.

The species identification testing was performed using MALDI TOF in the majority of the laboratories (n=12), followed by biochemical tests (n=11), chromogenic agar plating (n=7), and PCR using specific targets to confirm the ID (n=3). Additionally many laboratories reported using second and third identification

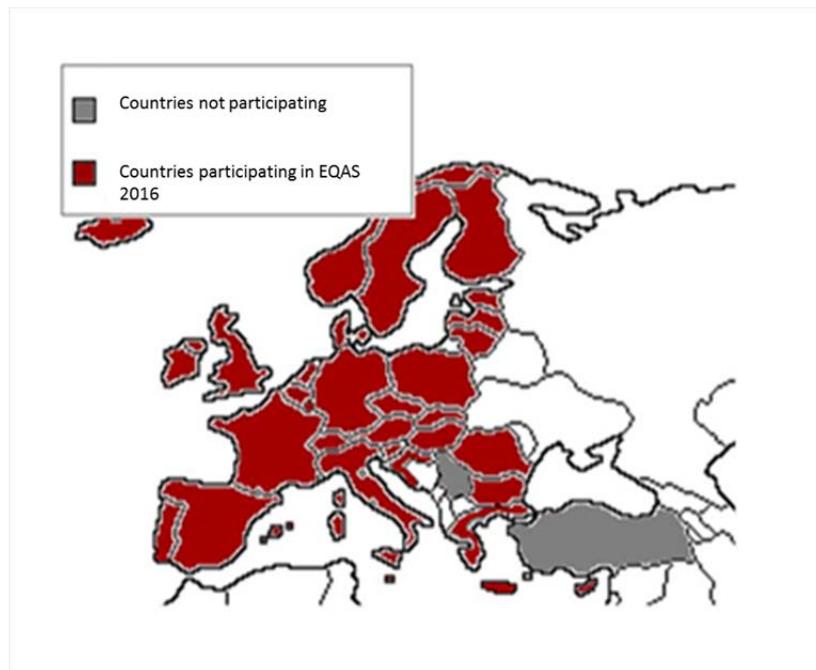


Figure 1. Countries participating in EQAS matrix 2016.

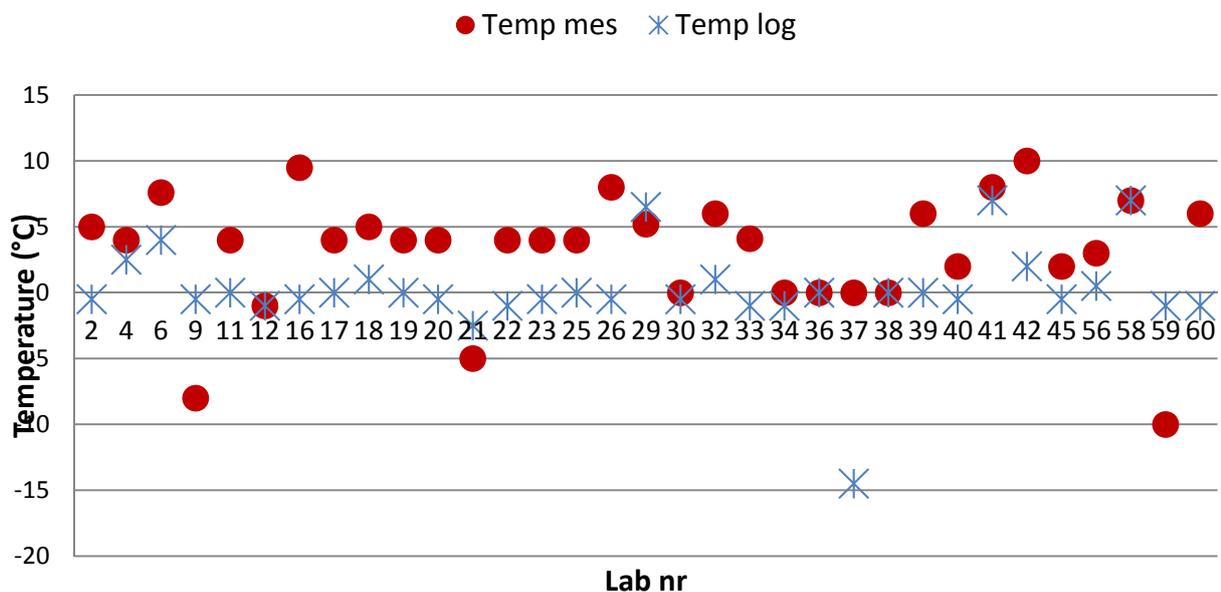


Figure 2. Temperatures measured at reception/opening measured by own measurement (Temp mes) and obtained from temperature logger (Temp log) in parcels for EQAS matrix 2016.

**Table 2.** The overall performance of ESBL/AmpC isolation and identification, 2016.

Isolation of ESBL /AMPC from samples		Correctly classified samples	
Number of performed tests		Number of correct tests N(%)	
N	%	N	%
248	100	247	99.6
Number of expected negative tests		Number of correctly identified negative tests	
N	%	N	%
62	25	62	100
Number of expected positive tests		Number of correctly identified positive tests	
N	%	N	%
186	75	185	99.5

methods as supplementary.

The broth microdilution testing was performed using the antimicrobials and ranges defined under the EU Commission regulation 652/2013 for testing the isolated and identified *E. coli* isolates using panel 1 (EUVSEC) additional AST of the presumptive ESBL/AmpC and/or carbapenemase isolates was performed using panel 2 (EUVSEC2) if relevant and interpretation of the results according to the EFSA criteria for ESBL/AmpC and carbapenemase phenotypic classification. One participant (Lab #37) performed the testing using agar dilution testing of the same range of antimicrobials and concentrations.

### 3.2 ESBL /AmpC and carbapenemase producing *E. coli* isolation and identification

#### ESBL/AmpC

The total amount of test results was 248 tests for the ESBL/AmpC isolation qualitative results. In this trial, twenty six participating NRL's reported results for all the eight samples sent. Two laboratories reported only results for the

meat samples (Labs, #38, and #41) and two laboratories reported only results for the caecal samples (Labs #32 and #58). Lab #4 uploaded results for all eight samples tested in the country, but tests of meat and caecal samples were performed in two different locations. All in all 247 tests were correct, corresponding to 99.6% of correct results (and 0.4% deviations). From the 62 samples expected to be negative all were correctly assigned. Regarding the 186 samples expected to be found positive, all but one were correctly found positive. Only one deviation was observed by Lab #37 that considered the caecal sample M-2.7 as negative (Table 2).

#### Other carbapenemases and OXA-48

The specific isolation of presumptive carbapenemase producing *E. coli* was performed by extending the protocol by addition of isolation on CARBA selective agar plates as described in the EURL-AR protocols. In total 233 tests results were submitted. Please note that even though 33 sets of results were submitted, eight of the laboratories did not

actually perform the specific isolation procedure for carbapenemase –producing *E. coli*, but defined results based on the findings with the in the ESBL/ampC selective method and thereby reported results for one to eight samples while the remaining 25 laboratories used the exact protocol as defined by the EURL-AR. The plates used for this purpose were chosen by the laboratories as the protocol defines that any suitable plates for selective isolation of carbapenemase- and OXA-48-producing *E. coli* may be used. Most participants declared the use of the chromogenic agar Chrom ID CARBA and Chrom ID OXA or CARBA smart combination plates (as reported by eight and five participants, respectively) only one participant used Liofilchem plates (Lab #23). A total of 11 participants did not report the brand of plates being used for this purpose, even though they report the EURL-AR protocol was followed.

A total of 224 results were considered correct, meaning that nine deviating results were observed for the presumptive carbapenemase-producing *E. coli* isolation. Eight of the deviations were due to false negative result of isolation from sample M-2.2 which contained a VIM-1 positive *E.coli* that should grow on the CARBA plate but was not detected by some of the participants (Labs #4, #11, #19, #25, #26, #40, #45, #56). The single remaining deviation was due to one false positive result of sample M-2.1 (lab #21) which is likely a transcription mistake as the comments refer that an isolate that was not identified as *E. coli* was isolated from this sample (Figure 3).

All 233 OXA-48 results reported were correctly interpreted as negative.

### 3.3 Antimicrobial susceptibility testing

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A total of 4049 tests results were uploaded and 3976 (98.2%) of these were correct. All 33 labs uploaded a variable number of results,

depending on the samples found positive and isolates tested in one or both panels, ranging from 44 to 132 test results per participant.

The analysis per laboratory identified seven laboratories with no deviations while the others had deviation percentages ranging from 0.8% to 5.3% (Figure 4). As mentioned before, as the performance on the AST depends on the isolation and identification procedures no threshold was set for acceptance as the capacity for performing AST of *E. coli* is analysed more accurately in the *E. coli* AST EQAS.

In the analysis of the results per antimicrobial we observed that the results showed the highest deviation percentage for cefoxitin (12.4%) followed by temocillin (6.2%) and cefepime (5.9%). This might be related to expected results lying close to the breakpoint. The remaining results per antimicrobial substance showed deviation levels below 5% (Figure 5).

The analysis performed per sample indicates that the highest deviation level (4.0%, 27 deviations) was observed for sample M-2.2, followed by sample 2.3 (2.8% deviations). All other samples had deviation levels below 2% (Figure 6).

### 3.4 ESBL/AmpC phenotypic testing conclusions

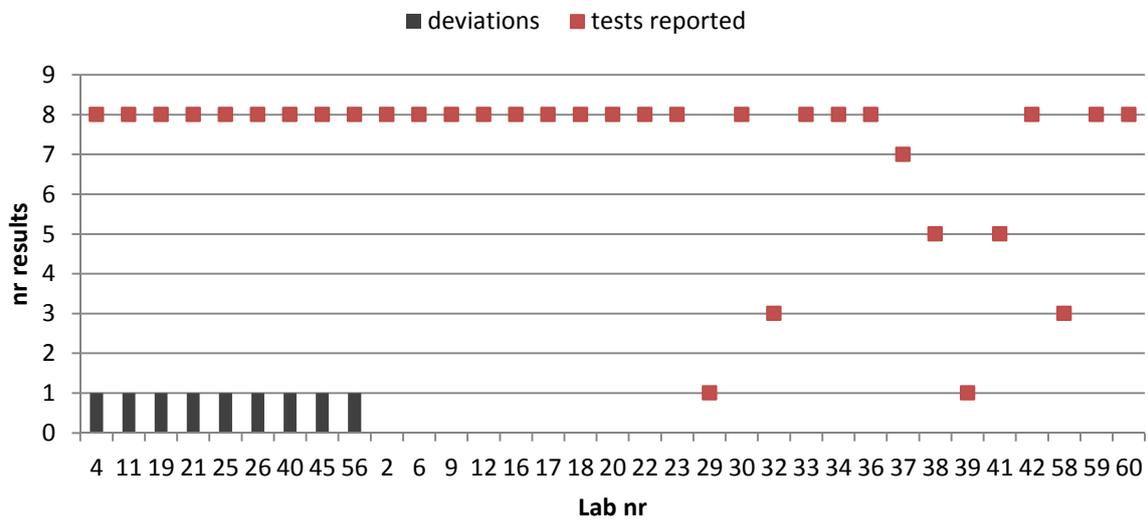
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The sample M-2.2 contained a carbapenemase -producing (VIM-1) *E. coli* while samples M-2.3 and M-2.6 contained ESBL producing strains carrying the *bla*<sub>TEM52</sub> and *bla*<sub>SHV-12</sub> genes, respectively. The samples M-2.4 and M-2.7 contained isolates with AmpC phenotypes mediated by *bla*<sub>CMY-2</sub>. The sample M-2.5 contained a strain which was both ESBL and AmpC (*bla*<sub>CTX-M-1</sub> and *bla*<sub>CMY-2</sub>) and the remaining samples (M-2.1 and M-2.8) did not contain ESBL or AmpC presumptive isolates and were expected to be negative in the first part of the EQAS for the selective isolation.

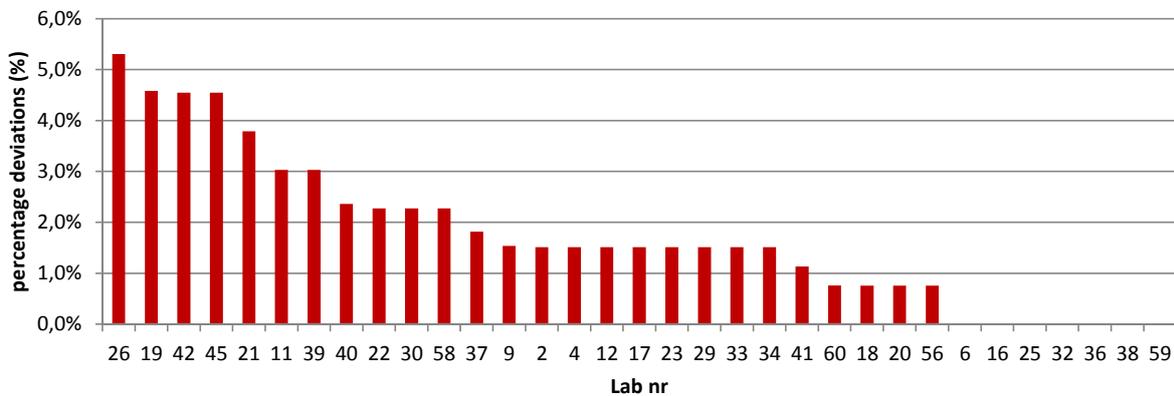
Due to the mixed phenotypes obtained (because of the ceftaxime values close to the breakpoint) we decided that the option ESBL+AmpC would be accepted as correct as well as the option ESBL for the strains isolated from sample M 2.3 and 2.6 (Appendix 3).

Of 185 results uploaded, 177 were correct (95.7%) and 8 (4.3%) were deviating. The most frequent deviation was related to sample 2.5 where the isolated strain had both ESBL and AmpC encoding genes and four participants

only identified the AmpC phenotype (Labs #11, #20, #25, #45). Two deviations were caused by lack of identification of the carbapenemase in sample 2.2 (Labs #19 and #26) and the remaining were related to sample 2.3 identified as presumptive AmpC and thereby missing the ESBL phenotype which should have been noticed by the detection of synergy in the tests with clavulanic acid (Lab #42) and sample 2.4 which contained an AmpC gene was reported as ESBL+AmpC in one instance (Lab #41).



**Figure 3.** Results of carbapenemase isolation per participant laboratory in the EQAS matrix 2016.



**Figure 4.** Results of antimicrobial susceptibility testing per participating laboratory in the EQAS matrix 2016.

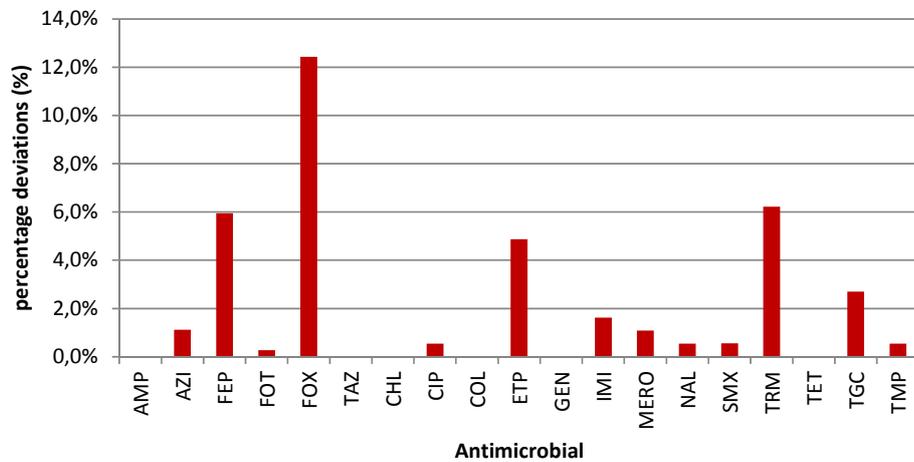


Figure 5- Number of deviations per antimicrobial in EQAS matrix 2016 (AST results).

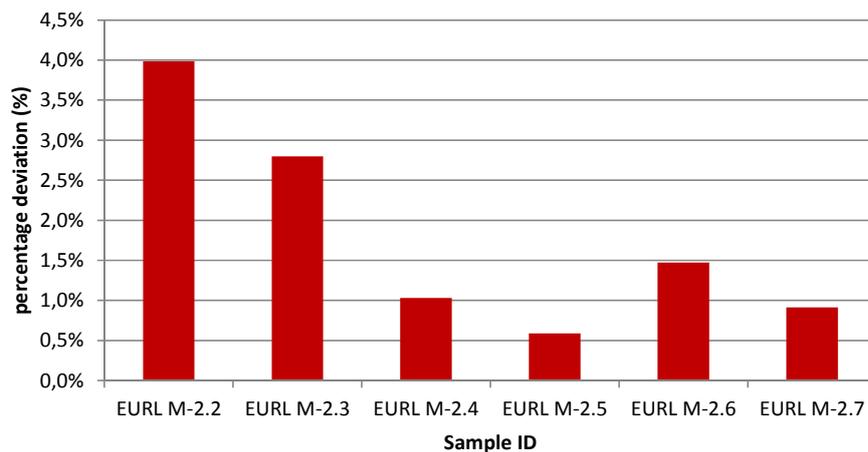


Figure 6- Number of deviations per sample in EQAS matrix 2016 (AST results).

## 4. Discussion

### 4.1 ESBL and AmpC and carbapenemase-producing *E. coli* isolation and identification

The 2016 EURL-AR matrix EQAS trial was the second of this kind and as it was run with samples of different origin compared to the first

one. It was still challenging in the setting up and planning the sample preparation, however the results were overall mostly correct according to expected, both regarding the isolation and AST parts, with all except one of the ESBL/AmpC isolation results correct.

Most of the carbapenemase isolation results



were found correct, however still eight labs were not able to isolate the sample 2.2 VIM-1 positive isolate and one additional and found one false positive isolate in sample 2.1.

## 4.2 Antimicrobial susceptibility testing

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The results uploaded were in general according to expected even though in such a trial challenges could have occurred in retrieving the right isolates from the samples, or changes could have occurred in the isolate composition in the samples or the isolate characteristics (conjugation, or plasmid losses). Some of the deviating results were caused by results lying close to breakpoint.

## 5. Conclusion

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In general, the results of this matrix EQAS demonstrate that most participating labs have set up the methods and are able to isolate ESBL and AmpC carrying strains from meat or caecal samples in a reliable fashion. However the results were less good for the selective isolation of carbapenemase -producing isolates using selective media. There is some need to strictly control the media and the procedures of the selective isolation procedure to select ESBL and AmpC or carbapenemase -producing *E. coli* and to do species identification with reliable methods to allow detection of all relevant

## 4.3 ESBL /AmpC phenotypic testing conclusions

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As what regards to the final conclusions for the AST testing and phenotypic confirmation, the conclusions depends heavily on the isolation process, thus some of the deviations might be related to the isolation of isolates that have different characteristics. However, we noticed that most deviations in this part were related to the lack of identification of the carbapenem resistance and problems with detection of the mixed ESBL+AmpC phenotypes in distinction of the simple ESBL or AmpC phenotypes.

isolates with increased sensitivity.

The susceptibility testing results were in general according to expected, however a relatively number of deviations were observed than in other AST EQAS and this might be related with the fact that the results of AST being dependent on the performance in the isolation, but also due to few tests results lying very close to the breakpoint and therefore leading to differences in the interpretations.

## 6. References

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EC 652/2013- COMMISSION IMPLEMENTING DECISION of 12 November 2013 on the

monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

## Appendix 1- EURL-AR EQAS pre-notification

G00-06-001/01.12.2014

### **EQAS 2016 FOR SELECTIVE ISOLATION OF *E. COLI* WITH PRESUMPTIVE ESBL, AMPC PHENOTYPES OR CARBAPENEMASES FROM MEAT OR CAECAL SAMPLES**

The EURL-AR announces the launch of the second EQAS on matrix samples, providing the opportunity for proficiency testing which is considered an essential tool for the generation of reliable laboratory results of consistently good quality.

This EQAS consists of testing of eight samples for selective isolation of ESBL, AmpC or carbapenemase-presumptive *E. coli*.

This EQAS is specifically for NRL's on antimicrobial resistance involved in the monitoring according to the EU Commission legislation 652/2013 and specifically processing meat and caecal samples in the specific monitoring for ESBL implemented in 2015. Previously to this prenotification, the laboratories designated to be NRL-AR have been contacted to confirm the addresses for the shipment of these samples. Participation is free of charge for all above-mentioned designated laboratories.

### **TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY**

The content of the parcel is categorized as "UN3373, Biological Substance Category B". Eight samples which might contain ESBL, AmpC or carbapenemase-producing *E. coli* included in a matrix of meat and/or caecal will be shipped. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g. specific text that should be written on the proforma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

### **TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE**

Shipment of isolates and protocol: The isolates are expected to be shipped in the second week of October. The protocol for this proficiency test will be available for download from the website ([www.eurl-ar.eu](http://www.eurl-ar.eu) ).

Submission of results: Results must be submitted to the National Food Institute **no later than December, 9<sup>th</sup>, 2016** via a password-protected website. Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

EQAS report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

Next EQAS: The next EURL-AR EQAS that we will have is on antimicrobial susceptibility testing of *E. coli*, staphylococci and enterococci which will be carried out in *June, 2017*.

**Please contact me if you have comments or questions regarding the EQAS.**

Sincerely,

Lina Cavaco

## List of participants

Institute	Country	Meat	Caecal
Austrian Agency for Health and Food Safety	Austria	X	X
ARSIA (reported by the Institute of Public Health)	Belgium		X
Institute of Public Health	Belgium	X	
National Centre of Food Safety	Bulgaria	X	X
Croatian Veterinary Institut	Croatia	X	X
Veterinary Services	Cyprus	X	X
State Veterinary Institute Praha	Czech Republic	X	X
Danish Veterinary and Food Administration, DVFA	Denmark	X	X
Estonian Veterinary and Food Laboratory	Estonia	X	X
Finnish Food Safety Authority EVIRA	Finland	X	X
ANSES - Laboratoire de Fougères LERMVD	France	X	X
Federal Institute for Risk Assessment	Germany	X	X
Veterinary Laboratory of Chalkis	Greece	X	X
Central Agricultural Office Veterinary Diagnostic Directorate	Hungary	X	X
Institute For Experimental Pathology, University of Iceland, KELDUR	Iceland	X	X
Central Veterinary Research Laboratory	Ireland	X	X
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy	X	X
Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia	X	X
National Food and Veterinary Risk Assessment Institute	Lithuania	X	X
Laboratoire de Medecine Vétérinaire	Luxembourg	X	X
Public Health Laboratory	Malta	X	X
Central Veterinary Institute of Wageningen UR	Netherlands	X	X
Food and Consumer Product Safety Authority (VWA)	Netherlands	X	X
Veterinærinstituttet	Norway	X	X
National Veterinary Research Institute	Poland	X	X
Laboratorio Nacional de Investigação Veterinaria	Portugal	X	X
Institute for Diagnosis and Animal Health	Romania		X
Institute for Hygiene and Veterinary Public Health	Romania	X	
State Veterinary and Food Institute (SVFI)	Slovakia	X	X
National Veterinary Institute	Slovenia	X	X
Centro Nacional de Alimentación (AECOSAN)	Spain	X	
Laboratorio Central de Sanidad, Animal de Algete	Spain		X
VISAVET Health Surveillance Center, Complutense University	Spain	X	X
National Veterinary Institute, SVA	Sweden	X	X
Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland	X	X
Animal and Plant Health Agency (APHA)	United Kingdom	X	X

NRL's	
non- NRL enrolled for EQAS	
not EU-member state	

Appendix 3 - Expected results for the matrix trial 2016

Qualitative results

Meat

Sample	Gene background	Expected results selection ESBL /AmpC	Expected results selection CARBA	Expected results selection OXA-48	CFU per 25 g	CFU/g meat
EURL-M-2.1	None (ATCC strain)	Negative	Negative	Negative	$2,5 \times 10^4$	$1 \times 10^3$
EURL-M-2.2	VIM-1	Positive	Positive	Negative	$2,5 \times 10^4$	$1 \times 10^3$
EURL-M-2.3	TEM 52	Positive	Negative	Negative	$2,5 \times 10^3$	100
EURL-M-2.4	CMY-2	Positive	Negative	Negative	$2,5 \times 10^4$	$1 \times 10^3$
EURL-M-2.5	CTX-M-1+CMY-2	Positive	Negative	Negative	$2,5 \times 10^4$	$1 \times 10^3$

Caecal

Sample	Sample origin	Expected results selection ESBL /AmpC	Expected results selection CARBA	Expected results selection OXA-48	CFU/g caecal sample
EURL-M-2.6	SHV-12	Positive	Negative	Negative	$1 \times 10^4$
EURL-M-2.7	CMY-2	Positive	Negative	Negative	$1 \times 10^4$
EURL-M-2.8	None	Negative	Negative	Negative	0

**Susceptibility testing (MIC- values and interpretations)**

**Panel 1**

Strain nr	Species	MERO	COL	AMP	AZI	TAZ	CHL	CIP	FOT	GEN	NAL	SMX	TET	TMP	TGC
EURL-M-2.2	<i>E. coli</i>	0.5	<=1	>64	8	>8	<=8	<=0.015	>4	1	<=4	>1024	<=2	<=0.25	<=0.25
EURL-M-2.3	<i>E. coli</i>	<=0.03	<=1	>64	8	>8	16	>8	>4	1	>128	>1024	>64	>32	<=0.25
EURL-M-2.4	<i>E. coli</i>	<=0.03	<=1	>64	8	8	>128	0.5	>4	<=0.5	>128	>1024	64	>32	<=0.25
EURL-M-2.5	<i>E. coli</i>	0.06	<=1	>64	8	>8	<=8	>8	>4	1	>128	>1024	>64	<=0.25	<=0.25
EURL-M-2.6	<i>E. coli</i>	<=0.03	<=1	>64	8	>8	<=8	1	>4	<=0.5	8	>1024	4	0.5	<=0.25
EURL-M-2.7	<i>E. coli</i>	<=0.03	<=1	>64	8	8	<=8	<=0.015	>4	1	<=4	16	<=2	0.5	<=0.25

Strain nr	<i>E. coli</i>	MERO	COL	AMP	AZI	TAZ	CHL	CIP	FOT	GEN	NAL	SMX	TET	TMP	TGC
EURL-M-2.2	<i>E. coli</i>	R	S	R	S	R	S	S	R	S	S	R	S	S	S
EURL-M-2.3	<i>E. coli</i>	S	S	R	S	R	S	R	R	S	R	R	R	R	S
EURL-M-2.4	<i>E. coli</i>	S	S	R	S	R	R	R	R	S	R	R	R	R	S
EURL-M-2.5	<i>E. coli</i>	S	S	R	S	R	S	R	R	S	R	R	R	S	S
EURL-M-2.6	<i>E. coli</i>	S	S	R	S	R	S	R	R	S	S	R	S	S	S
EURL-M-2.7	<i>E. coli</i>	S	S	R	S	R	S	S	R	S	S	S	S	S	S

**Susceptibility testing (MIC- values and interpretations), ctd.**

**Panel 2**

Strain nr	Species	MERO	FEP	FOX	TAZ	FOT	T/C	F/C	IMI	ETP	TRM	ESBL conclusion
EURL-M-2.2	<i>E. coli</i>	0.5	16	64	128	>64	128	>64	2	0.25	64	CARBA
EURL-M-2.3	<i>E. coli</i>	<=0.03	2	16	16	8	0.25	0.25	0.12	<=0.015	16	ESBL (or ESBL+AmpC)*
EURL-M-2.4	<i>E. coli</i>	<=0.03	0.25	32	8	8	8	8	0.25	0.03	4	AmpC
EURL-M-2.5	<i>E. coli</i>	<=0.03	8	32	32	>64	8	8	0.25	0.03	8	ESBL+AmpC
EURL-M-2.6	<i>E. coli</i>	<=0.03	1	8	32	8	0.25	0.12	0.25	<=0.015	8	ESBL (or ESBL+AmpC)*
EURL-M-2.7	<i>E. coli</i>	<=0.03	0.12	32	8	8	4	4	0.25	0.03	8	AmpC

Strain nr	Species	MERO	FEP	FOX	TAZ	FOT	T/C	F/C	IMI	ETP	TRM
EURL-M-2.2	<i>E. coli</i>	R	R	R	R	R	NA	NA	R	R	R
EURL-M-2.3	<i>E. coli</i>	S	R	R	R	R	NA	NA	S	S	S
EURL-M-2.4	<i>E. coli</i>	S	R	R	R	R	NA	NA	S	S	S
EURL-M-2.5	<i>E. coli</i>	S	R	R	R	R	NA	NA	S	S	S
EURL-M-2.6	<i>E. coli</i>	S	R	S	R	R	NA	NA	S	S	S
EURL-M-2.7	<i>E. coli</i>	S	S	R	R	R	NA	NA	S	S	S

Resistant

NA

Not applicable or not tested

**Abbreviations:** AMP - ampicillin, AZI - Azithromycin, CHL - chloramphenicol, CIP - ciprofloxacin, COL - colistin, ETP - ertapenem, FEP - cefepime, FOT - cefotaxime, FOT/CLA - cefotaxime/clavulanic acid, GEN- gentamicin, IMI - imipenem, MER- meropenem, NAL - nalidixic acid, SMX - sulfamethoxazole, TAZ - ceftazidime, TAZ/CLA - ceftazidime/clavulanic acid, TET - tetracycline, TMP - trimethoprim, TGC - tigecycline, TRM - temocillin.

\*Regarding the interpretations, see page 14 in the report

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# PROTOCOL

for selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from meat and caecal samples (matrix EQAS)

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## 1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on selective isolation of presumptive extended spectrum beta-lactamase (ESBL)-, AmpC- or carbapenemase-producing *E. coli* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), and will include the selective isolation procedures and antimicrobials susceptibility testing (AST) of obtained isolates of eight samples of either meat or caecal content. In 2016, these eight samples will include five samples of 25g meat and three

samples of 1g of caecal content, both of poultry origin. These samples may or may not contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes.

It is expected that the participating laboratories for the analyses apply the same procedures used in the monitoring described by the regulation EC/652/2013, and perform the selective isolation following the EU recommended methods published on the EURL-AR website [www.eurl-ar.eu](http://www.eurl-ar.eu).

## 2 OBJECTIVES

This EQAS aims to assess and, if necessary, to improve the quality of results obtained in the selective isolation of presumptive ESBL-, AmpC- or carbapenemase-producing isolates from meat and caecal samples. Further objectives are to evaluate and improve the comparability of surveillance data on ESBL-, AmpC- or carbapenemase -producing *E. coli* reported to EFSA by different laboratories.

## 3 OUTLINE OF THE EQAS

### 3.1 Shipping, receipt and storage of samples

In October 2016, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight samples from the National Food Institute. All strains used in the spiking of samples belong to UN3373, Biological substance, category B. Participants should expect that ESBL-, AmpC- and/or carbapenemase-enzymes producing strains will be included in some of the sample matrix.

The samples will be either 25g of meat (spiked matrix) or 1g of caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 2.1 to 2.8 (2.1 to 2.5 being samples with 25g meat and 1.6 to 1.8 being samples containing 1g each of caecal content)

The samples will be shipped in frozen state in tubes and contained in cooling boxes with temperature control devices and cooling elements.

Upon reception it is very important to open the parcel as soon as possible and proceed to the analysis (following the normal procedures for sample testing in the monitoring).

#### **It is required that participants**

- **when opening the parcel, note the date and exact time at opening (this data is very important to follow the temperature data checks)**
- **proceed to sample analysis immediately after opening the parcel**
- **register the date for analysis start for each sample**
- **collect the temperature control device (small discoid device located in a bag inserted in a labelled tube, located inside the parcel);** open the tube and take out the bag with the device inside. Place this bag with the device in the labelled bubble envelope provided and send it back to the EURL-AR as soon as possible. Please note that you will have to arrange for stamps/postage (the post systems differ from country to country, why this cannot be arranged and paid from the EURL-AR in advance).

### 3.2 Selective isolation of ESBL, AmpC or carbapenemase producing *E. coli* from the samples

The samples provided in each parcel are weighed beforehand and therefore no further weighing is required. Proceed immediately to the first enrichment step by adding the sample to the necessary amount of media (225 ml of Buffered Peptone water for the meat samples and 9 ml for the caecal samples as referred in the official EURL-AR protocols. All the following procedures should follow the methods used in the monitoring for ESBL and AmpC *E. coli* according to the EC/652/2013 regulation. If any changes are introduced to the official protocols, these changes should be described with details in the online database on the methods upload page. The participants are responsible for assuring the validity of the plates and therefore the protocol for “Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC producing *E. coli* in meat and animals” should be run beforehand, as stated on the EURL-AR webpage (see <http://eurl-ar.eu/233-protocols.htm>).

Optionally, the participants may perform the additional plating for isolation of carbapenemase-producing *E. coli* from the samples, following the official protocols and plating on suitable agar plates. Similarly, the agar plates used for the carbapenemase isolation should be validated using the protocol for “Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*”.

The officially recommended protocols are found on the EURL-AR webpage (<http://eurl-ar.eu/233-protocols.htm>):

- Follow the protocol for meat when testing samples 2.1 to 2.5
- Follow the protocol for caecal content when testing samples 2.6 to 2.8

As referred in these protocols, the isolates obtained from isolation procedure should be identified as *E. coli* using the procedures for *E. coli* species identification applied at the participant’s laboratory for the specific monitoring of ESBL- and AmpC-producing *E. coli*.

Please store the isolates obtained in the isolation procedure and document the whole process as well as all the findings in each step.

As part of the results submission, you will be requested to describe the findings along the enrichment process and selective isolation including growth in the media, isolation of suspected colonies, species identification results and finally regarding the finding (or not) of presumptive *E. coli* isolates harbouring one of the selected resistances (this result will be evaluated in relation to the expected result as a qualitative result) (see details in the Test Form).

### 3.3 Antimicrobial susceptibility testing

If the sample is deemed positive for ESBL-, AmpC- or carbapenemase -producing *E. coli*, one *E. coli* isolate per sample should be taken further and tested for susceptibility to the antimicrobials as stated in the EU regulation (antimicrobials listed in Tables 1 and 2 in this document).

Only one *E. coli* isolate is expected to be tested for AST and these results will be evaluated in the database against the expected results.

AST results to be reported should be from:

- A presumptive carbapenemase positive isolate (from the CARBA or OXA-48 selective plates), if this optional part was performed and a presumptive carbapenemase positive *E. coli* isolate was detected.
- An ESBL- or AmpC-presumptive isolate (if you do not have a carbapenemase positive isolate or if you did not perform the optional plating) if an ESBL- or AmpC-presumptive isolate was detected.

The testing should be performed using the same method as implemented in your laboratory for performing AST when monitoring for EFSA according to the regulation EC/652/2013 (using the two-step approach, i.e. both testing panels) and applying the interpretative criteria listed below.

**Table 1.** Antimicrobials recommended for AST of *Escherichia coli* and interpretative criteria according to table 1 in Commission Implementing Decision 2013/652/EU

Antimicrobials for <i>E. coli</i>	MIC (µg/mL) R is >
Ampicillin, AMP	8
Azithromycin, AZI	16*
Cefotaxime, FOT	0.25
Ceftazidime, TAZ	0.5
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.064
Colistin, COL	2
Gentamicin, GEN	2
Meropenem, MERO	0.125
Nalidixic acid, NAL	16
Sulfamethoxazole, SMX	64
Tetracycline, TET	8
Tigecycline, TGC	0.5**
Trimethoprim, TMP	2

\* Tentative ECOFF

\*\* EUCAST.org

Plasmid-mediated quinolone resistance

When performing antimicrobial susceptibility testing of *E. coli*, the interpretative criteria listed in Table 1 for results obtained by MIC-determination should allow detection of plasmid-mediated quinolone-resistant test strains.

Beta-lactam resistance

**Confirmatory testing for ESBL production is mandatory** on all strains resistant to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) and should be performed by testing the second panel of antimicrobials (Table 2).

**Table 2.** Antimicrobials recommended for additional AST of *Escherichia coli* resistant to cefotaxime, ceftazidime or meropenem and interpretative criteria according to Table 4 in Commission Implementing Decision 2013/652/EU.

Antimicrobials for <i>E. coli</i>	MIC ( $\mu\text{g/mL}$ ) <b>R is &gt;</b>
Cefepime, FEP	0.125
Cefotaxime, FOT	0.25
Cefotaxime + clavulanic acid (F/C)	Not applicable
Cefoxitin, FOX	8
Ceftazidime, TAZ	0.5
Ceftazidime+ clavulanic acid (T/C)	Not applicable
Ertapenem, ETP	0.064
Imipenem, IMI	0.5
Meropenem, MERO	0.125
Temocillin, TRM	>32*

\*Tentative ECOFF

Confirmatory test for ESBL production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a  $\beta$ -lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a  $\geq 3$  twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. the MIC of the agent when tested alone (MIC FOT : FOT/CL or TAZ : TAZ/CL ratio  $\geq 8$ ) (CLSI M100 Table 3A, Tests for ESBLs). The presence of synergy indicates ESBL production.

Confirmatory test for carbapenemase production requires the testing of meropenem (MERO).

Detection of AmpC-type beta-lactamases can be performed by testing the bacterium for susceptibility to cefoxitin (FOX). Resistance to FOX could indicate the presence of an AmpC-type beta-lactamase.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (EURL-AR Workshop 2016, [http://www.crl-ar.eu/data/images/ws\\_april-2016/f11\\_efs\\_a\\_criteria.pdf](http://www.crl-ar.eu/data/images/ws_april-2016/f11_efs_a_criteria.pdf) and in the appendix to this protocol).

## 4 REPORTING OF RESULTS AND EVALUATION

Please write your results in the test forms, and enter your results into the interactive web database.

### 4.1 General recommendations for data upload

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than 9th, December, 2015.** After the deadline when all participants have uploaded results, you will be able to login to the database once again, and to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as ‘correct’, while results deviating from the expected interpretation are categorised as ‘incorrect’.

If you experience difficulties in entering your results, please contact us directly.

All results will be summarized in a report which will be publicly available. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the complete list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions will be public.

If you have questions, please do not hesitate to contact the EQAS Coordinator:

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National Food Institute  
Technical University of Denmark  
Søltofts Plads, Building 221, DK-2800 Lyngby

Denmark

Tel: +45 3588 6269

Fax: +45 3588 6341

E-mail: [licav@food.dtu.dk](mailto:licav@food.dtu.dk)

## 5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read carefully this paragraph before entering the web page.

Remember that you need by your side the completed test forms.

Enter the EURL-AR EQAS 2016 start web page (<http://eurl.food.dtu.dk/matrix>), write your username and password in lower-cases and press enter. Your username and password are indicated in the Welcome letter following the samples. Do not hesitate to contact us if you experience problems with the login.

You can browse back and forth by using the Home or back keys, but please remember to save your inputs before.

### 5.1 Sample reception/testing

Please fill in with information in relation to date and time (please note the exact time) and temperature at arrival of the parcel contents as measured by you (we will also check on the thermologgers data after you send back the device).

## **5.2 Selective enrichment methods**

Please fill in with the details of the methods use and insert any changes made to the official method

## **5.3 Test results**

### **5.3.1 Selective enrichment of presumptive ESBL- , AmpC- or carbapenemase -producing *E. coli***

Fill in the answers for the questions regarding the selective enrichment results along the process

### **5.3.2 Species identification enrichment of presumptive ESBL- or AmpC-producing *E. coli***

Please confirm the results and conclude if you found an *E. coli* presumptive of producing an ESBL or AmpC gene in the sample (this conclusion will be evaluated).

Please confirm the results and conclude also if you found an *E. coli* presumptive of producing a carbapenemase or OXA-type enzyme in the sample (these conclusions will be evaluated separately).

If you respond to the above questions indicating that you did not find a presumptive isolate to go further you are not expected to fill in the remaining questions.

If your answer is 'yes' for both or one of the above, you are expected to fill in the MIC tables and final conclusion of the AST and confirmatory testing.

### **5.3.3 AST of *E. coli***

Based on the first MIC panel results, indicate if the isolate fulfils the criteria to be tested on the second panel (confirmatory phenotypic testing) or not, and fill in the results for the second panel in case you decide to do the confirmatory testing.

Complete the fields in the result tables related to the results obtained.

Click on "save" and then go back using the tab "home" and enter another test page to upload results

In the data entry pages, enter the obtained values and the interpretation (R, resistant or S, susceptible) for each *E. coli* isolate.

Remember to report also the conclusion of the phenotypic testing on the second panel (will be evaluated separately).

If you did not test for susceptibility to a given antimicrobial, please leave the field empty.

Click on "save" and then go back using the tab "home" and enter another test page to upload results.

Click on "save".

## **5.4 Finalizing data input, EQAS evaluation and approval of result upload**

Review the input pages by browsing through the pages and make corrections if necessary. Remember to save a page if you make corrections. If you press home a page without saving changes, you will see an error screen. In this case, click on “save“ to save your results, browse back to the page and then continue.

Please complete the evaluation form for the EQAS when you finalize the data input. You can find the tab on the Home page, on the tab “Evaluation”

Before approving your input, please be sure that you have filled in all the relevant fields for the sample sheet, the methods and the test results for all samples tested because **YOU CAN ONLY APPROVE ONCE!** The approval blocks your data entry in the interactive database.

## APPENDIX

### Criteria for interpretation of *Escherichia coli*, panel 2 results

**efsa**  
European Food Safety Authority

## CRITERIA

**ESBL-Phenotype**

- FOT or TAZ > 1 mg/L AND
- MERO ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND
- SYN FOT/CLV and/or TAZ/CLV

**AmpC-Phenotype**

- FOT or TAZ > 1 mg/L AND
- MERO ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- No SYN FOT/CLV nor TAZ/CLV
- (Not excluded presence of ESBLs)

**ESBL + AmpC-Phenotype**

- FOT or TAZ > 1 mg/L AND
- MERO ≤ 0.12 mg/L AND
- FOX >8 mg/L AND
- SYN FOT/CLV and/or TAZ/CLV

**Carbapenemase-Phenotype**

- MEROM > 0.12 mg/L
- Needs confirmation
- (Not excluded presence of ESBLs or AmpC)

**Susceptible**

FOT-TAZ-FOX-MEM  
≤ ECOFF

**Other phenotypes**

1) If FOT or TAZ > 1 mg/ml AND

- MEM ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND
- NO SYN FOT/CLV nor TAZ/CLV
- Not excluded CPs (consult EURL)

2) If FOT and/or TAZ ≤ 1 mg/L AND > ECOFF AND

- MERO ≤ 0.12 mg/L
- FOX ≤ 8 mg/L

3) If FOT and/or TAZ ≤ 1 mg/L

- MERO ≤ 0.12 mg/L
- FOX > 8 mg/L.
- \*cAmpCs could be included here

4) If MERO ≤ 0.12 mg/L BUT

- ETP > ECOFF AND/OR
- IMI > ECOFF
- Not excluded CPs, needs confirmation (consult EURL)

5) Any other combinations not described in previous boxes (contact EURL)

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Please refer to the full presentation at [http://www.crl-ar.eu/data/images/ws\\_april-2016/f11\\_efs\\_criteria.pdf](http://www.crl-ar.eu/data/images/ws_april-2016/f11_efs_criteria.pdf)



## Test forms, Isolation of ESBL/AmpC- and carbapenemase-producers from matrices

Username:  
Contact person:  
  
Country:  
Date for filling in test forms:

### SAMPLES

Reception date and exact time of opening the parcel of the proficiency test samples at the laboratory: (date and time is required)

Temperature of the contents of the parcel at arrival: °C

How many samples did your laboratory process in 2016 for monitoring of ESBL/AmpC-detection in relation to 2013/652/EU? (choose only one option)

- less than 100
- 101-200
- 201-300
- 301-400
- 401- 1000
- more than 1000

Which kind of samples did your laboratory process in 2016 for monitoring of ESBL/AmpC-detection in relation to 2013/652/EU? (you may choose more than one option)

- caecal, poultry (chicken)
- caecal, poultry (turkey)
- meat, poultry (chicken)
- other matrices, please specify:



Did you process samples for carbapenemase-selective isolation?

- Yes  
 No

How many samples did your laboratory process in 2016 for monitoring of carbapenemases in relation to 2013/652/EU? (Choose only one option)

- less than 100  
 101-200  
 201-300  
 301-400  
 401- 1000  
 more than 1000

Which kind of samples did your laboratory process in 2016 for monitoring of carbapenemase-production in relation to 2013/652/EU? (you may choose more than one option)

- caecal, poultry (chicken)  
 caecal, poultry (turkey)  
 meat, poultry (chicken)  
 other matrices, please specify:

Any other comments:



## METHODS

1- Method used for selective isolation of ESBL/AmpC in this EQAS:

Selective isolation procedure using the EURL recommended protocols that refer to the EU regulation 652/2013/EU

- The protocol was used without modifications (please jump to question 2)
- The protocol was used, however, the pre-enrichment was modified (please respond question 1.1)
- The protocol was used, however, the selective isolation procedures were modified (please respond question 1.2)
- The protocol was used, however, the incubation conditions in the selective plating were modified (please respond question 1.3)

1.1- If you modified the pre-enrichment, please indicate the differences introduced:

Different sample amount (weight) used for the enrichment procedure:

g in meat samples

g for caecal samples

Different volume of enrichment in the isolation step:

ml for meat samples

ml for caecal samples

Different pre-enrichment medium:

Different incubation conditions in pre-enrichment                      °C/                      h;

Please justify these changes:

1.2- If you made changes in the selective isolation procedure:

Different sample amount (weight) used for the enrichment procedure:

g in meat samples

g for caecal samples

Different concentration of cefotaxime:                      mg/l

Different antimicrobial

Different medium

Please justify these changes:



1.3- If you used different incubation conditions in the selective plating, please indicate the conditions used:                    °C/                    h;

Please justify these changes:

2- Method used for selective isolation of carbapenemase-producers (in case you run this method) in this EQAS:

Selective isolation procedure using the EURL recommended protocols for isolation of carbapenemase-producers:

- We did not perform carbapenemase selective isolation
- The protocol was used without modifications
- The protocol was modified

Plates used (brand/type)

Please justify these changes:

3- Method used for confirmation of *E. coli* species identification. Please indicate the primary *E. coli* identification method used (choose only one option; if you used more than one method, please explain in the comments field)

- PCR using published methods
- PCR using in-house method
- Biochemical tests
- MALDI-TOF
- DNA Sequencing
- Chromogenic media

Comments:

4- Method used for general antimicrobial susceptibility testing of the strains (choose only one option)

- Microbroth dilution test on EUVSEC panel
- Microbroth dilution test on another panel
- Agar dilution method
- E-test
- Disk diffusion test

5- Method used for phenotypic confirmatory testing of ESBL/AmpC (choose only one option)



- Microbroth dilution test on EUVSEC2 panel
- Microbroth dilution test on another panel
- Agar dilution method
- E-test
- Disk diffusion test

6- Additional comments. Please include here description and justification of your choice if you modified something in relation to the method defined in the EU regulation 2013/652/EU:



## TEST FORM – SAMPLE ‘EURL M-2.X’

Date the isolation procedure was started:

Please describe the results you have observed regarding this sample:

Visible growth in pre-enrichment:

Yes  / No

Growth on ESBL/AmpC-selective plates:

Yes  / No

Please describe the growth observed on ESBL/AmpC-selective plates? (choose only one option)

- Mixed culture containing typical *E. coli* colonies
- Mixed culture without typical *E. coli* colonies
- Pure culture of typical *E. coli* colonies
- Pure culture without typical *E. coli* colonies
- No growth

Results of species identification: (choose only one option)

- No isolates tested (sample negative)
- Presumptive ESBL/AmpC isolate identified as *E. coli* (sample considered positive)

Comments:

Did you perform carbapenemase selective plating?

Yes  / No

Growth on CARBA-selective plates:

Yes  / No

Growth on OXA-48 selective plates:

Yes  / No

Results of species identification (isolates from carbapenemase selective plating): (choose only one option)

- No isolates tested (sample negative)
- Presumptive other carbapenemase isolate identified as *E. coli* (sample considered positive)
- Presumptive OXA-48 isolate identified as *E. coli* (sample considered positive)

Comments:

If you have found a presumptive carbapenemase positive isolate, please insert the results of antimicrobial susceptibility testing for the selected *E. coli* isolate, if you do not have a carbapenemase positive isolate and you have an ESBL presumptive isolate, please insert the results for this isolate (only one *E. coli* isolate is expected to be tested and these results will be evaluated in our database against the expected results).

Please confirm where the isolate tested for antimicrobial susceptibility originated from (compulsory):



- ESBL/ampC isolation on Mac Conkey with cefotaxime
- CARBA plate
- OXA-48 plate

**Based on the results from the first AST panel, was the isolate found resistant to cefotaxime, ceftazidime or meropenem so that the second panel was tested?**

Yes  / No



**AST results**

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (µg/ml)	S / R
<i>E. coli</i> EURL M-2.X	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

**Second *E. coli* AST panel** (confirmatory testing for ESBL/AmpC/carbapenemase-production)

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (µg/ml)	S / R
<i>E. coli</i> EURL M-2.X	Cefepime, FEP			
	Cefotaxime + clavulanic acid (F/C)			
	Cefotaxime, FOT			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid (T/C)			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			

**Conclusions of confirmatory phenotypic testing:** (choose only one option and please note that the final result will be evaluated by the database)

**Interpretation of PANEL 2 results:**

<input type="checkbox"/> Presumptive ESBL	<input type="checkbox"/> Presumptive AmpC	<input type="checkbox"/> Other phenotype
<input type="checkbox"/> Presumptive ESBL+ AmpC	<input type="checkbox"/> Presumptive carbapenemase	<input type="checkbox"/> Susceptible

Comments (include optional genotype or other results):

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