The 1st EURL-AR Proficiency Test on selective isolation of E. coli with presumptive ESBL or AmpC phenotypes from meat or caecal samples - 2015

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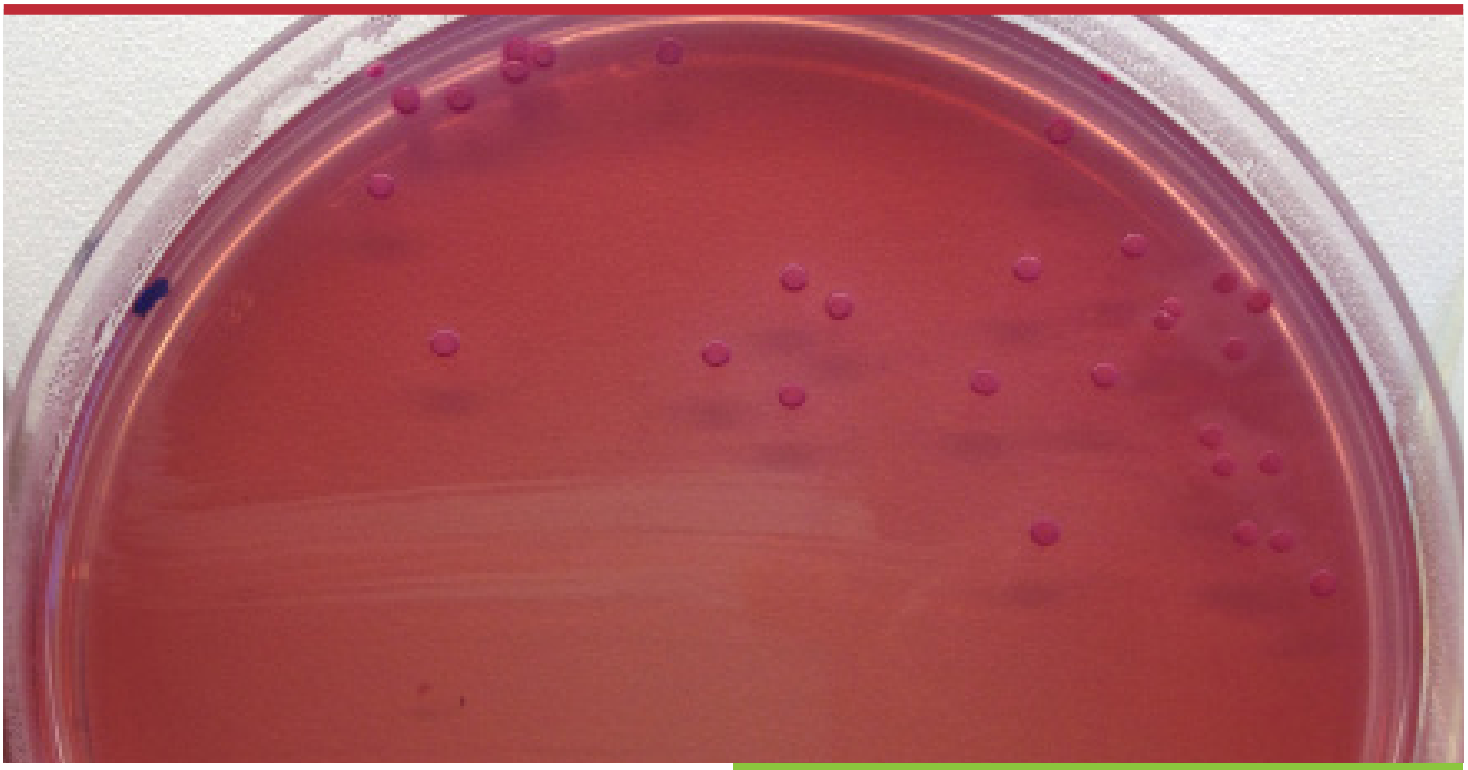

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THE 1st EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF *E. COLI* WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CAECAL SAMPLES – 2015

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1. Introduction

ESBL and AmpC producing *E. coli* have emerged and spread in in food producing animals. The emergence of this potential zoonotic pathogen in animals has raised the concerns of potential transmission to humans from the animal reservoirs with consequences on Public Health. In 2013, the European Commission (EC) took the decision of including the isolation of ESBL and AmpC producing *E. coli* as mandatory parts of the EU monitoring to be started during 2015 and focusing firstly on cattle and swine samples, including meat as representative of food samples and caecal content as representative of samples taken from the animal population. Thus, this screening has been started in the beginning of 2015 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 before the specific monitoring was implemented at MS (EURL-AR, 2014).

The EURL-AR took part in the development and support of the laboratories for this task by providing the protocol for testing and assistance in setting up the methods for isolation, identification, and typing of ESBL/AmpC (and carbapenemase) producing *E. coli* isolates. Additionally, the EURL-AR provided training in the specific methods at a training course organized in Lyngby in the autumn of 2014.

In October 2015, the EURL-AR launched, in addition to the External Quality Assurance System (EQAS) already performed, a matrix based specific EQAS to enhance the capacity of the laboratories in ESBL/AmpC producing *E. coli* isolation, identification and antimicrobial susceptibility testing (AST). This EQAS aimed towards identification of potential problems and identification of focus areas for future training/education that might improve data quality in future monitoring.

Before November 2015 several preliminary studies were conducted to prepare for the launch of this EQAS, using meat samples and caecal content matrices spiked with strains of interest or naturally positive.

In November 2015, we launched the first EQAS performed on ESBL/AmpC producing *E. coli* isolation, identification and AST in matrices of meat and caecal samples (EQAS matrix). This trial included isolation steps, confirmation of the ID as *E. coli*, MIC testing and confirmation of ESBL/AmpC phenotypes and as these are part of the mandatory tasks for confirmation of ESBL /AmpC strains the conclusions drawn were included in the trials. At the present time the detection of carbapenemases is not compulsory, and the additional isolation procedures for carbapenemase isolation were not included in the EQAS trial.

Starting in January 2015, the laboratories should have implemented the methods and have started the monitoring on meat and caecal samples from porcine and bovine 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 the quality of data provided to EFSA, retrospectively.

No thresholds have been set in advance to evaluate the performance of the participating laboratories, nor classify the results of this EQAS, as there were issues related to stability which have caused deviations.

The EQAS was organized by 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 for the EURL-AR EQAS scheme consists of competent representatives from all National Reference Laboratories for Antimicrobial Resistance (NRLs-AR), who meets annually at the EURL-AR workshop.

2. Materials and Methods

2.1 Participants in EQAS 2015

A pre-notification (App. 1), inviting the participants to the matrix EQAS 2015 was issued by e-mail to the EURL-AR network on the 11th of September 2015 to the NRL-AR's.

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 their own analysis.

2.2 Preparation of samples

Eight samples were prepared and dispatched for isolation of ESBL or AmpC *E. coli*, including identification, and AST of the obtained isolates. The samples included were both meat and caecal samples and were either artificially prepared to contain the test strains or unmodified.

The meat used to prepare the samples was minced beef with 8-15% fat 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 or AmpC to make sure the batch used was negative for ESBL or AmpC producing *E. coli* but contained background flora. The chosen batch should contain average *E. coli* background but not ESBL or AmpC suspected colonies. 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 2015 were prepared in advance and subcultured 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 and counted using viable counts of serial dilutions on agar. The standardized suspensions were further diluted in tenfold dilution row and the meat samples (25 g) were spiked with 25 µl of the chosen dilutions. The final inoculum was expected to be about 10^3 CFU/g of meat, for samples M-1.1, M-1.2, and M-1.5, where the sample M-1.4 was spiked with a lower amount of about 10 CFU/g of the test strain, and the sample M-1.3 was left as blank, containing only the background flora present in the meat. The sample M-1.5 was spiked as mentioned above, however with a susceptible strain (ATCC 25922) and therefore expected to be negative.

For the caecal samples, the tests performed in the preparation phase showed challenges in keeping the strains viable and stable along time, therefore we opted for natural samples. One slaughterhouse provided us with 10-15 caecal samples from pig origin, each batch in sufficient amount so that the samples could be prepared from one of these single batches of matrix. The samples were tested individually using the official selective isolation protocol for ESBL and AmpC testing. Furthermore, the samples were tested to approximate *E. coli* counts using spot method plated on Mac

Conkey agar with and without cefotaxime 1 mg /L. The one negative caecal sample was chosen to prepare the blank sample (EURL-M-1.6) and two positive samples were chosen to prepare positive samples (EURL-M-1.7 and 1.8). However, during the last part of the preparation one of the isolates obtained from one of the positive samples (EURL-M-1.7) failed to grow on the MIC panels. We re-evaluated the possibilities and prepared a spiked caecal sample using negative caecal material and spiked with one isolate which was recently obtained from a pig caecal sample and this solution seemed to be more stable. However to avoid issues with stability the spiking was performed with a larger inoculum at 10^5 CFU/g. This sample was prepared and coded as EURL-M-1.7. The latter sample (EURL M 1.8) was a naturally positive sample showing an AmpC AST profile that had shown good stability up to 2 weeks after shipment. The minimal inhibitory concentrations (MIC) of the selected antimicrobial panels were determined using microdilution methods at two different occasions (firstly, on the strains used for spiking during the preparation work and again from 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 on the isolates obtained from the isolation procedure performed on the samples.

Stability testing was performed for following up on the stability of the samples after shipping. The test was performed on one set of the eight samples and in four occasions after shipment (during two weeks). In this period the meat samples were kept frozen and the caecal samples were kept at cooling temperature at 4°C.

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

The official protocols for selective isolation and identification of the ESBL or AmpC *E. coli* isolates contained in swab 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 regulation. The description of the method used for selective isolation and species identification were requested as part of the methods sheet under 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 regulation for Antimicrobial susceptibility monitoring at EU level - EC 652/2013 (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), 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 by the second panel of antimicrobials, which is intended to be tested every time a strain was found resistant to either

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2015 component

Escherichia coli	*Escherichia coli* 2nd 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	

cefotaxime, ceftazidime or meropenem in the first *E. coli* panel and interpreted according to the protocol, enabling to conclude on the strain's presumptive ESBL/AmpC or carbapenemase phenotype.

2.5 Distribution

For keeping the stability 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 reception and pre testing, and therefore kept at refrigerator temperature 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 additional one tube containing a thermologger to register the temperature at 15 min intervals during transit. Furthermore, the parcel contained a welcome letter containing the login 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 with detailed instructions and respective 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 November 2nd , 2015.

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 and the analysis following the EU protocol for selective isolation of ESBL and AmpC presumptive *E. coli* from either meat or caecal samples, exactly as they would normally proceed in the EFSA monitoring, and to follow the instructions given for upload of the data in the web based database. Furthermore, the test forms contained the schemes to collect the data to be uploaded

on the database, which was designed and prepared for this EQAS and opened shortly after sample shipment and until the deadline (App 4 and 5).

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 19th November, 2015 and closed December 9th 2015.

For the first part of the results of the selective isolation procedure, the samples were categorised as positive or negative and this qualitative result was registered in the database. Additionally, the results of susceptibility testing using both MIC panels were uploaded for the isolates obtained, 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 regarding the phenotypes observed.

After the deadline, the qualitative results indicating if the samples were positive or negative, as well as the interpretations of the susceptibility tests performed, and the conclusion on the observed *E. coli* phenotypes were evaluated against the expected results and scored as correct or incorrect, without further classification of the deviation.

3. Results

A total of 35 laboratories participated in the EQAS; including 31 labs representing 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. One additional non-NRL from EU was enrolled and provided results but was not included further in the report. For more information on the countries participating consult the map below (Figure 1).

Two laboratories (#40 and #34) received their set of samples at a later date (17th November 2015) due to issues with the shipment of samples and therefore their results were not included in the report as the stability could be affected, and the samples had been kept for a longer time in storage compared to the EURL-AR premises.

When the deadline for submitting results was reached, all enrolled laboratories had uploaded data for the respective samples tested, thus all represented countries had one complete set of data.

The participating laboratories have, as requested, returned the termologgers 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 webpage, including details on sample reception (measured temperature and time), the monitoring activities (samples tested and amounts), and the methods used in their laboratory.

The temperature data was analysed at the EURL and showed a range of temperatures between -5°C and 8°C for measures taken by the participants, and -1°C and 4°C, when

inferred from the temperature at opening time from the thermologger registration (Figure 2). This showed that the samples were expected to be in good conditions for testing at opening of the samples. In only one case (#Lab 39) it was not possible to correctly estimate the temperature at sample processing as the participants reported that the sample was not processed immediately, but kept in refrigeration from reception (5th November) to the start of processing of samples 15th November.

3.1 Methods used by EQAS-participants

Of the 32 laboratories submitting results all (100%) of the laboratories participated in the ESBL and AmpC isolation and identification parts as well as the susceptibility testing parts. The number of tests uploaded was rather diverse, depending on both how many samples were tested in the laboratory in question (as some only tested meat or caecal samples and some tested both (three to eight samples)), and how many of these were found positive. The number of tests performed ranged from 20 to

Figure 1. Participating countries in EQAS matrix 2015.

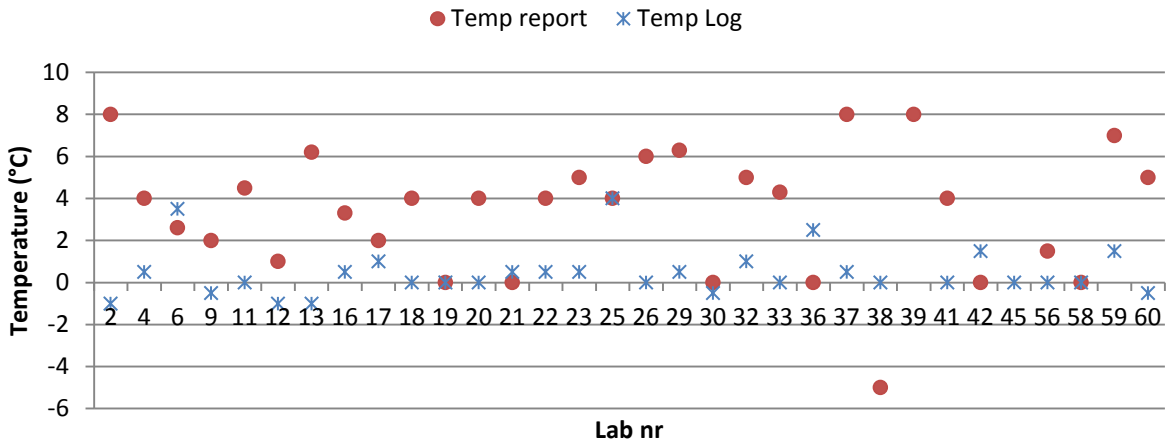

Figure 2. Temperatures measured at reception/opening measured by own measurement and temperature logger in parcels EQAS matrix 2015.

105 tests per participant.

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

Most laboratories (n=30) reported that isolation has been performed exactly according to the protocol provided by the EURL-AR for this EQAS and any changes in media, concentrations of antibiotics, etc. were referred. The differences reported by some laboratories from the method described in the original protocol were: incubation at 41.5°C and 20h incubation for the selective plates (Lab #36); and the use of 0.8% cefotaxime for supplementing the selective plates (Lab #58). One participant did not provide data (Lab #60)

The species confirmation testing was performed in the majority of the laboratories using biochemical tests (n=13) followed by chromogenic agar plating (n=8), MALDI TOF (n=8), and PCR (n=3).

The MIC was as for the AST EQAS only done using the antimicrobials and ranges regulated under the EU Commission regulation 652/2013.

3.2 ESBL /AmpC producing E. coli isolation and identification

A total of 232 tests results were uploaded. Twenty six of the participating laboratories have reported results for all the eight samples sent. Three laboratories reported only the five results for the meat samples (Labs #13, #38, and #41) and three laboratories reported the results for the three caecal samples (Labs #16, #32, and #58).

All in all 204 tests were correct, corresponding to 87.9% of correct results (and 12.1% deviations). From the 87 samples expected to be negative 97.7% (n=85) were correctly assigned, and the remaining two were found false positive. Regarding the samples expected to be found positive, 82.1% were correctly found positive (119 out of 145). When looking at the analysis per sample type we observe that among the meat samples 93.1% (135 out of 145) samples were correctly tested whereas among the caecal samples 79.3% (69 out of 87) samples were found correctly assigned qualitatively (Table 2).

Table2**.** The overall performance of ESBL/AmpC isolation and identification, 2015.

Isolation of ESBL /AMPC from samples		Correctly classified samples	
Number of performed tests		Number of correct tests N(%)	
N	%	N	%
232	100	204	87.9
Number of expected negative tests		Number of correctly identified negative tests	
N	%	N	%
87	37.5	85	97.7
Number of expected positive tests		Number of correctly identified positive tests	
N	%	N	%
145	62.5	119	82.1

As described above, most deviations were caused by a lack of sensitivity of the methods used, leading to false negative results in 29 samples in total, whereas only four false positives were observed.

Also from the analysis above we can observe that the results were better for meat samples than for the caecal samples which might be due to issues with stability, especially regarding sample M-1.8.

The results per sample show that most deviations were observed for sample EURL-M-1.8 which caused 18 deviations and therefore a total of 62.1% deviating results. This was probably due to stability or eventual uneven homogeneity of this particular caecal sample which might have caused some of the negative results in the isolation of the resistant strain which was present naturally in this sample. The remaining samples had deviation percentages around or below 10%, with sample M-1.1 and M-1.4 highest at 10.3% (3 deviations each), 1.2, at 6.9% (2 deviations), and the remaining samples had less than 5% deviations. As observed above the highest deviation percentages were observed for the positive samples, especially for samples M-1.1 and M-1.4 which were expected to be positive as and ESBL producing strain had been included in these samples, however in different amounts. M-1.1 was inoculated with a high amount at around 10^3 CFU/g whereas 1.4 had been spiked with circa 10 CFU/g of the same strain. This strain had the particularity of growing as white colonies on TBX agar even though it was an *E. coli* strain and therefore using this type of chromogenic agar as identification method was not appropriate for this particular strain, which might have influenced the result for some of the participants, showing the need to use other ID methods or supplement with additional tests, for identifying this type of strains (Figure 3).

When analysing the results per laboratory three participants (Labs #6, #18, and #20) had three deviations, out of the eight samples tested, accounting for 37.5% deviations (all three labs had a deviation in the M-1.8 sample and two additional samples). One participant among the three laboratories only analysing the caecal samples (#16) had 33.3% deviation due to one deviation on the sample M-1.8 while the other two laboratories analysing only the ceacal samples had no deviations. Two participants (Lab #30 and #39) had two deviations each (one on sample 1.8 and one additional) obtaining 25% deviations. Fourteen participants had one deviation only and the remaining 12 laboratories did not have any deviation in this qualitative exercise (Figure 4). Further communication with Labs #6 and #18 showed that they both experienced the same issue, as they actually had isolated a suspected isolate from samples M-1.1 and M.1.4 but did not confirm the ID as *E. coli* due to the use of chromogenic agar (TBX), which did not show the expected blue colour as this particular strain included in both of the samples appeared to have white colonies on this type of agar. Therefore, these labs have been advised to use different ID tests as alternative or complement to the use of chromogenic agar for species ID.

3.3 Antimicrobial susceptibility testing

A total of 2473 tests results were uploaded and 2371 (95.9%) of these were correct. All 32 labs have uploaded a variable number of results, depending on the samples found positive and isolates tested in one or both panels, ranging from 20 to 105 test results per participant.

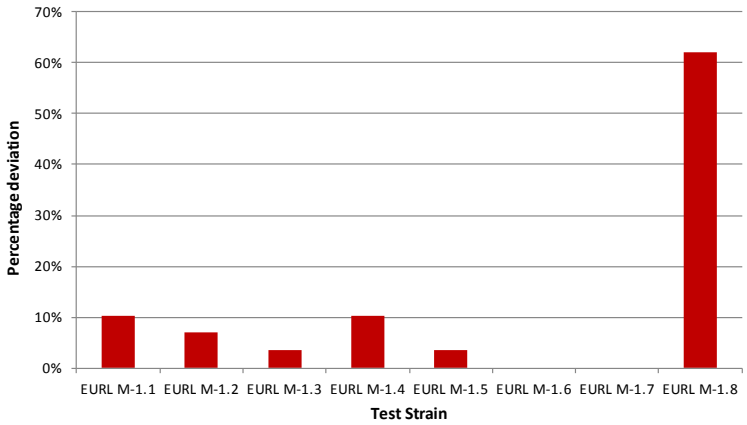

Figure 3- Number of deviations per sample for isolation and identification of ESBL and or AmpC producing *E. coli*

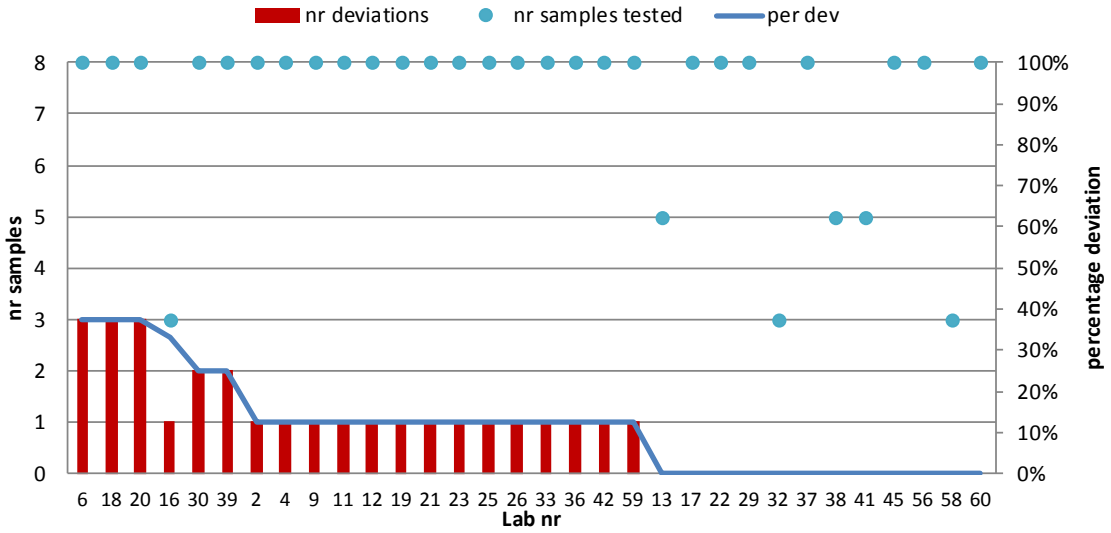

Figure 4- Number of deviations per laboratory for isolation and identification of ESBL and or AmpC producing *E. coli*

When analysing the results per antimicrobial we observed that the results for ertapenem showed the highest deviation percentage, at around 39.8% followed by ceftazidime and cefoxitin with 8.4% and 7.7%, respectively. The remaining antimicrobials showed deviation levels below 5% (Figure 5). This might be related to results lying close to the breakpoint for these particular drugs, and issues related to permeability causing relatively high MIC to ertapenem.

The analysis performed per sample indicates that the highest deviation level (6.8%) was observed for sample M-1.8, caused by 15 deviations as this sample was not tested by a number of participants that did not obtain a presumptive resistant strain from this sample. All remaining samples had deviation levels below 5% (Figure 6).

We did not perform analysis per laboratory as the performance on the AST depends on the isolation and on the number of strains tested and is therefore not suitable to compare different participants nor would give a realistic image on the participant's capacity to perform the susceptibility testing which is analysed in the *E. coli* AST EQAS.

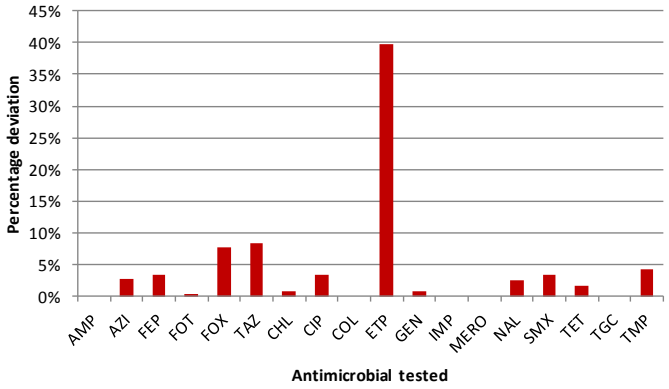

Figure 5- Number of deviations per antimicrobial for AST ESBL and or AmpC producing *E. coli*

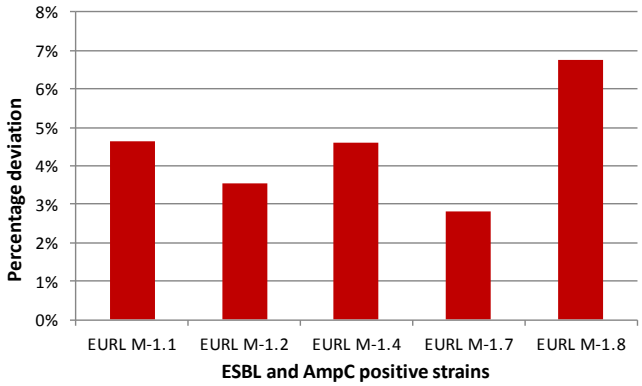

Figure 6- Number of deviations per sample for AST of ESBL and or AmpC producing *E. coli*

3.3 ESBL /AmpC phenotypic testing conclusions

Among the samples tested, the samples M-1.1, M-1.4 and M-1.7 contained ESBL producing strains carrying CTX-M genes (CTX-M-1, CTX-M1 and CTX-M-9, respectively), whereas the samples M-1.2 and M-1.8 contained isolates with AmpC phenotypes (CMY-2 and chromosomal AmpC) and the remaining samples (M-1.3, M-1.5 and M-1.6) 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 phenotype obtained (essentially because of the cefoxitin resistance observed for the strain isolated from sample M 1.1 and 1.4 and the variable cefepime result around the breakpoint for strain M-1.2), we have also determined that the option ESBL+pAmpC would be accepted as correct for the samples M-1.1, M-1.2 and M-1.4.

In all, in 122 results uploaded, 113 were found correct (92.6%) and 9 (7.3%) were deviating. These deviations were mainly due to considering carbapenemase as a presumptive phenotype, based on finding the MIC to ertapenem above the breakpoint. This was the case for three deviations for lab #60, and one deviation each for lab# 42 and #56.

Two deviations regarding the isolate obtained from sample M-1.8 were due to not classifying it as ESBL or AmpC (Lab #29 and #33), and one for the same sample was due to classifying it as presumptive ESBL (Lab #32). An additional deviation was related to the isolate from sample M-1.2 which was found as unusual phenotype by the participant from lab #26 as both cefepime and cefoxitin were found resistant.

4. Discussion

4.1 ESBL and AmpC isolation and identification

The 2015 EURL-AR matrix EQAS trial was the first of this kind and therefore it was a challenging exercise. The samples were prepared with strains selected beforehand for meat samples, but the caecal samples were not as straightforward and different approaches were used both with naturally positive samples and one spiked sample. The results were overall quite good, both regarding the isolation and AST parts, with expected negative and expected positive samples mostly well classified correct. However, there were issues related to the logistics with shipment that impeded two laboratories of having their results included in this report and among the samples sent, there were issues related with stability or possibly homogeneity of the contents regarding sample M-1.8 that might be deviations for a number of participants.

4.2 Antimicrobial susceptibility testing

The results uploaded were in general good, especially taking in account that they needed to retrieve the same isolate from the samples, were changes could have occurred (stability issues, conjugation into other strains, or plasmid losses). The range of uploaded results by the participating laboratories was very broad and some deviating results were caused by the different strains being obtained in the isolation procedures, than actual issues regarding the AST method. Therefore the analysis was not performed by laboratory.

4.3 ESBL /AmpC phenotypic testing conclusions

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 interpretation of carbapenemase resistance, based on ertapenem results, which are very sensitive, but not very specific, leading to false classification as carbapenemase producers. Also some deviations were observed due to the difficulty in fitting in some of the isolates, due to results that lead to unusual phenotypes as cefepime might not fit into the classification scheme in some cases.

5. Conclusion

In general, the results of the first 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. There are, however still some MS that have issues with the methods, as all steps are relevant for the final result. One of the issues is the need to strictly control the conditions of the selective isolation procedure to select ESBL and AmpC *E. coli* and to do species identification with reliable methods to allow to detect all relevant isolates and assure that the species is correctly identified.

The susceptibility testing results were in general

good, however a larger 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 first part with isolation of the expected isolates (spiked or natural), which will be highly affected by testing different isolates or isolates that could have been modified in the sample (plasmid transfers or losses, etc).

6. References

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 2015 FOR SELECTIVE ISOLATION OF *E. COLI* WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CEACAL SAMPLES

The EURL-AR announces the launch of another EQAS, thus 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 or AmpC 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 faecal 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 "UN3373, Biological Substance Category B": Eight samples which might contain *ESBL* or *AmpC producing E. coli* included in a matrix of meat and/or ceacal sample 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 first week of November. The protocol for this proficiency test will be available for download from the website (www.eurl-ar.eu).

Submission of results: Results must be submitted to the National Food Institute **no later than** December, 4, 2015 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, 2015*.

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

Sincerely,

Lina Cavaco

Appendix 2- List of participants

Institute	Country	Meat	Caecal
Austrian Agency for Health and Food Safety	Austria	x	x
Institute of Public Health	Belgium	x	x
Veterinary Agrochemical Research center	Belgium		x
National Centre of Food Safety, NRL "Salmonella, Campylobacter, Staphylococci and Antimicrobial Resistance"	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 (CAECAL)		x
ANSES - Laboratoire de sécurité alimentaire (LSAL) Maisons Alfort LERQAP	France (MEAT)	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
Veterinærinstituttet	Norway	x	x
National Veterinary Research Institute	Poland	x	x
Laboratorio National de Investigacáo Veterinaria	Portugal	x	x
Institute for Diagnosis and Animal Health	Romania	x	x
State Veterinary and Food Institute (SVFI)	Slovakia	x	x
National Veterinary Institute	Slovenia	x	x
Laboratorio Central de Sanidad, Animal de Algete	Spain		x
VISAVET Health Surveillance Center, Complutense University	Spain	x	x
Centro Nacional de Alimentación (AECOSAN)	Spain	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

Qualitative results

Meat

Sample	Gene background	Expected results selection isolation	CFU per 25 g	CFU/g meat
EURL-M-1.1	CTX-M-1	Positive	2.5×10^4	1×10^3
EURL-M-1.2	CMY-2	Positive	2.5×10^4	1×10^3
EURL-M-1.3	None	Negative	0	0
EURL-M-1.4	CTX-M-1	Positive	2.5×10^2	10
EURL-M-1.5	ATCC	Negative	2.5×10^4	1×10^3

Caecal

Sample	Sample origin	Expected results selection isolation	CFU/g caecal sample
EURL-M-1.6	Negative caecal sample	Negative	0
EURL-M-1.7	Spiked high level with pig strain	Positive	1×10^5
EURL-M-1.8	Naturally positive	Positive	1.5×10^2

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-1.1	*E. coli*	<=0.03	<=1	>64	16	4	16	0,5	>4	2	128	>1024	>64	>32	1
EURL-M-1.2	*E. coli*	<=0.03	<=1	>64	4	8	<=8	0.03	>4	1	<=4	<=8	<=2	<=0.25	<=0.25
EURL-M-1.4	*E. coli*	<=0.03	<=1	>64	16	4	16	0.5	>4	2	128	>1024	>64	>32	1
EURL-M-1.7	*E. coli*	<=0,03	<=1	>64	>64	0.5	>128	>8	>4	1	>128	>1024	>64	>32	<=0.25
EURL-M-1.8	*E. coli*	<=0.03	<=1	>64	8	8	<=8	0.03	4	2	<=4	16	<=2	<=0.25	<=0.25

Strain nr	Species	MERO	COL	AMP	AZI	TAZ	CHL	CIP	FOT	GEN	NAL	SMX	TET	TMP	TGC
EURL-M-1.1	*E. coli*	S	S	R	S	R	S	R	R	S	R	R	R	R	S
EURL-M-1.2	*E. coli*	S	S	R	S	R	S	S	R	S	S	S	S	S	S
EURL-M-1.4	*E. coli*	S	S	R	S	R	S	R	R	S	R	R	R	R	S
EURL-M-1.7	*E. coli*	S	S	R	R	S	R	R	R	S	R	R	R	R	S
EURL-M-1.8	*E. coli*	S	S	R	S	R	S	S	R	S	S	S	S	S	S

Panel 2

Strain nr	Species	MERO	FEP	FOX	TAZ	FOT	T/C	F/C	IMI	ETP	TRM	ESBL conclusion
EURL-M-1.1	*E. coli*	<=0.03	16	16	4	64	0.25	0.12	<=0.12	0.06	16	ESBL or ESBL+pAmpC
EURL-M-1.2	*E. coli*	<=0.03	0.25	64	8	8	4	8	0.25	0.12	16	AmpC or ESBL or ESBL+pAmpC
EURL-M-1.4	*E. coli*	<=0.03	16	16	4	64	0.25	0.12	<=0.12	0.06	16	ESBL or ESBL+pAmpC
EURL-M-1.7	*E. coli*	<=0.03	4	8	0,5	32	0.12	0.06	0.25	<=0.015	8	ESBL
EURL-M-1.8	*E. coli*	<=0.03	0.12	>64	8	4	8	2	0.25	0.03	16	AmpC

Strain nr	Species	MERO	FEP	FOX	TAZ	FOT	T/C	F/C	IMI	ETP	TRM
EURL-M-1.1	*E. coli*	S	R	R	R	R	NA	NA	S	S	NA
EURL-M-1.2	*E. coli*	S	R	R	R	R	NA	NA	S	R	NA
EURL-M-1.4	*E. coli*	S	R	R	R	R	NA	NA	S	S	NA
EURL-M-1.7	*E. coli*	S	R	S	S	R	NA	NA	S	S	NA
EURL-M-1.8	*E. coli*	S	S	R	R	R	NA	NA	S	S	NA

	Resistant
NA	Not applicable or not testet

Abbreviations: AMP - ampicillin, AZI - Azithromycicn, 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 - sulphametoxazole, TAZ - ceftazidime, TAZ/CLA - ceftazidime/clavulanic acid, TET - tetracycline, TMP - trimethoprim, TGC - tigecycline, TRM - temocillin.

PROTOCOL

For selective isolation of presumptive ESBL/AmpC 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 ESBL/AmpC *E. coli* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), and will include the selective isolation procedures and AST of obtained isolates of eight samples of either meat or caecal content. In 2015, these eight samples will include five samples of 25g meat from bovine origin and three samples of 1g of caecal content of porcine origin. These samples may contain (or not) *E. coli* presumptive of producing either ESBL or AmpC enzymes.

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

2 OBJECTIVES

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

3 OUTLINE OF THE EQAS ON SELECTIVE ISOLATION OF PRESUMPTIVE ESBL AND AMPC ISOLATES FROM MEAT AND CAECAL SAMPLES (MATRIX EQAS) 2015

Shipping, receipt and storage of samples

In November 2015, 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 extended spectrum beta-lactamase (ESBL)-producing strains as well as AmpC-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 1.1 to 1.8 (1.1 to 1.5 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 (this could not be paid from the EURL-AR beforehand as you will have to dispatch it from your country and the post systems differ).

Selective isolation of ESBL or AmpC *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 http://eurl-ar.eu/data/images/protocols/protocol_for_validation_of_macconkey_and_ctx_agarplates_nov2014.pdf.

This EQAS does NOT focus on the specific isolation of *E. coli* producing carbapenemase enzymes, and therefore it is not needed to plate on the additional carbapenemase selective plates.

Links to the officially recommended protocols:

- For meat (to follow when testing samples 1.1 to 1.5): http://eurl-ar.eu/data/images/protocols/esbl_ampc_cpeprotocol_version_meat_dec2014_version2.pdf
- For caecal content (to follow when testing samples 1.6 to 1.8): http://eurl-ar.eu/data/images/protocols/esbl_ampc_cpeprotocol_version_caecal_dec2014_version2.pdf

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 and all the findings in each step. As part of the submission, you will be requested to describe the findings along the enrichment process and selective isolation including to 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 Testform).

Antimicrobial susceptibility testing

If the sample is deemed positive for ESBL or AmpC 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), using a two-step approach using both testing panels.

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 and applying the interpretative criteria listed below.

Table 1: Antimicrobials recommended for AST of *Escherichia coli* and interpretative criteria according to table 1 in EC regulation 652/2013

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Ampicillin, AMP	8
Azithromycin, AZI	16*
Cefotaxime, FOT	0.25
Ceftazidime, TAZ	0.5
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.06
Colistin, COL	2
Gentamicin, GEN	2
Meropenem, MERO	0.125
Nalidixic acid, NAL	16
Sulfamethoxazole, SMX	64
Tetracycline, TET	8
Tigecycline, TGC	1
Trimethoprim, TMP	2

* Tentative ECOFF established from EFSA data.

Beta-lactam resistance

Confirmatory tests for ESBL production are mandatory on all strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem and should be performed by testing the second panel of antimicrobials (Table 2 in this document corresponding to Table 4 in EC regulation 652/2013).

When submitting results to the database, please confirm for each sample if the second panel was included in the test.

Table 2: Antimicrobials recommended for additional AST of *Escherichia coli* resistant to cefotaxime, ceftazidime or meropenem and interpretative criteria according to table 4 in EC regulation 652/2013

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
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	0.06
Imipenem, IMI	0.5
Meropenem, MERO	0.125
Temocillin, TRM	Not available*

*Where no interpretative criteria are available, we request the participants upload the MIC value obtained, and do not select an interpretation.

Confirmatory test for ESBL production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (MIC FOT : FOT/CL or TAZ : TAZ/CL ratio ≥ 8) (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production. Resistance to cefepime gives further indication of ESBL production, but is not essential.

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, that may be verified by PCR and sequencing.

The classification of the phenotypic results should be based on the most recent EFSA recommendations (EFSA 2012), indicating the strains as:

- Presumptive ESBL: strains with positive synergy test, susceptible to cefoxitin and resistant to cefepime
- Presumptive ESBL+pAmpC: strains with positive or negative synergy test, resistant to cefoxitin and resistant to cefepime
- Presumptive pAmpC phenotype: strains with negative synergy test, resistant to cefoxitin and susceptible to cefepime
- Presumptive carbapenemase phenotype: strain resistant to meropenem
- Unusual phenotype: any other combinations

(However we recommend that strains which show synergy with clavulanic acid for at least one of the third generation cephalosporins cefotaxime or ceftazidime should be considered ESBL, independently of the cefepime result)

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** *4th, 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
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Denmark

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Fax: +45 3588 6341

E-mail: 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 and the breakpoint values you used.

Enter the EURL-AR EQAS 2015 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 thermo-loggers 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 or AmpC 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 AmpC gene in the sample (this conclusion will be evaluated).

If you respond to this question 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, 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*

Please respond with basis on the first MIC panel results 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 related to the results obtained in the result tables.

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 ESBL phenotypic testing.
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.

DTU Food
National Food Institute

Test forms

Selective isolation of presumptive ESBL/AmpC producing *Escherichia coli* from meat and caecal samples (matrix EQAS)

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 2015 for monitoring of ESBL/AmpC-detection in relation to EC/2013/652? (choose only one option)

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

Which kind of samples did your laboratory process in 2015 for monitoring of ESBL/AmpC-detection in relation to EC/2013/652? (you may chose more than one option)

- ☐ caecal, cattle
- ☐ caecal, swine
- ☐ meat, beef
- ☐ meat, pork
- ☐ other matrices Specify:

Any other comments:

DTU Food
National Food Institute

TEST FORM – ESBL/AmpC methods

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

With reference to the selective isolation procedure using the EURL recommended protocols that refer to EC/652/2013;

☐ The protocol was used without modifications (please jump to question 2)

☐ The protocol was used, however, the pre-enrichment was modified (please respond to question 1.1)

☐ The protocol was used, however, the selective isolation procedures were modified (please respond to question 1.2)

☐ The protocol was used, however, the incubation conditions in the selective plating were modified (please respond to question 1.3)

Question 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:

Question 1.2
If you made changes in the selective isolation procedure, please indicate the differences introduced

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:

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Question 1.3
If you used different incubation conditions in the selective plating, please indicate the differences introduced

Please indicate the conditions used: °C/ h;

Please justify these changes:

Question 2
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

Comments:

Question 3
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

Question 4
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

Question 5
Additional comments.

Please describe and justify your choice if you modified something in relation to the method defined in EC/652/2013**:**

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TEST FORM
Sample EURL M-1.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*
Comments:

If you have found a presumptive ESBL/AmpC-positive isolate, please insert the results of antimicrobial susceptibility testing for the selected *E. coli* isolate (only one *E.coli* isolate is expected to be tested and these results will be evaluated in our database against the expected results).

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? (please note this question will be compared to the expected results and evaluated by the database)
Yes ☐ / No ☐

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AST results

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (µg/ml)	S / R
E. coli EURL M-1.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 (ESBL/AmpC confirmatory testing)

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (µg/ml)	S / R
E. coli EURL M-1.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)

☐ Presumptive ESBL	☐ Presumptive pAmpC	☐ Unusual phenotype
☐ Presumptive ESBL+ pAmpC	☐ Presumptive carbapenemase	☐ No ESBL, AmpC or carbapenemase

Comments (include optional genotype or other results):

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