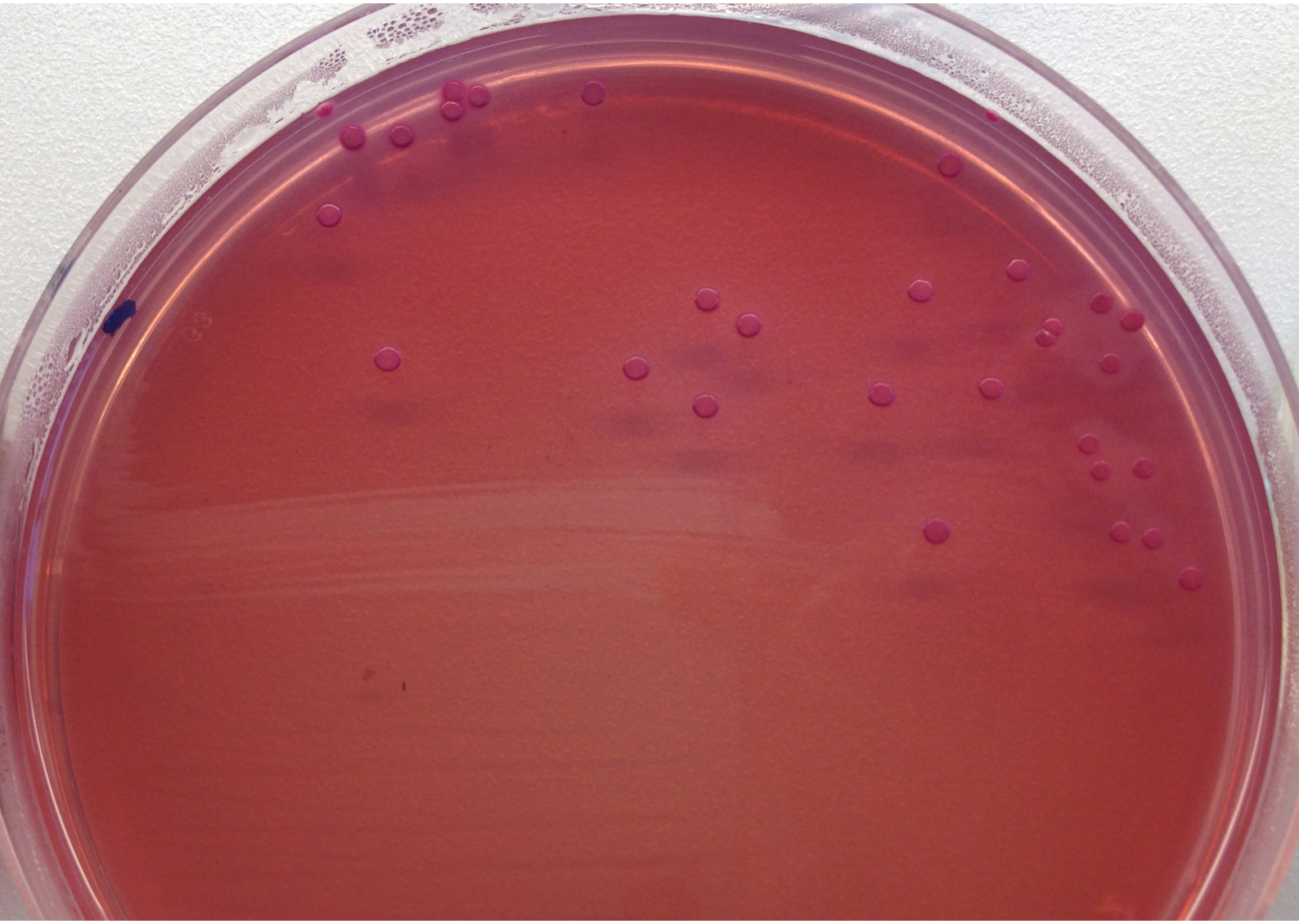
National Food Institute
Technical University of Denmark

The 5th EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF E. COLI WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CAECAL SAMPLES - 2019



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European Union Reference Laboratory -
Antimicrobial Resistance

THE 5th EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF *E. COLI* WITH PRESUMPTIVE ESBL OR AMPC PHENOTYPES FROM MEAT OR CAECAL SAMPLES – 2019

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Index

1. Introduction

This report describes and summarises results from the fifth matrix-based proficiency test conducted by The National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) as an External Quality Assurance System (EQAS). This proficiency test focuses on selective isolation of extended spectrum beta-lactamase (ESBL) and AmpC-producing *E. coli* from meat and caecal samples of animal origin and antimicrobial susceptibility testing (AST) of the isolated *E. coli*. In addition, the proficiency test includes optional isolation of carbapenemases and OXA-48-producing *E. coli*.

Extended spectrum beta-lactamase (ESBL) and AmpC-producing *E. coli* continue to spread in food producing animals. In 2013, the European Commission (EC) decided to include the isolation of ESBL and AmpC-producing *E. coli* as mandatory parts of the EU monitoring and this started during 2015. The screening includes matrix samples consisting of either meat or caecal samples of animal origin in the EU Member States (MS) and affiliated countries according to a common protocol defined by the EC and validated by the EURL-AR (EURL-AR, 2018).

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* (EURL-AR, 2017).

Similar to the previous EURL-AR matrix based EQAS', the aim of this specific EQAS was to i) monitor the capacity of the National Reference Laboratories (NRL-AR) for isolation, identification and AST of ESBL/AmpC or carbapenemase-producing *E. coli,* ii) identify laboratories which may need assistance to improve their performance in isolation and AST of *E. coli* from matrices, and iii) identify potential problems or focus areas for future training and research.

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

In reading this report, the following important considerations should be taken into account:

1) Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determination for all test strains prior to selection of strains and MIC's were confirmed upon selection of strains at the Technical University of Denmark, National Food Institute (DTU Food). The genetic basis for resistance was known, as all the selected test strains had been whole-genome sequenced (WGS). The MIC determination was repeated after preparation of the matrix samples of meat and caecal, which revealed a risk for deviating phenotypic results (See section 3.1).

2) 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 will be evaluated case by case.

3) Evaluation of a result as 'deviating from the expected interpretation' should be carefully analysed in a self-evaluation performed by the participant, including considerations of corrective actions in the laboratory. Note that since methods used for MIC determination has limitations, it is not considered a mistake to obtain a one-fold dilution difference in the MIC of a specific antimicrobial when testing the same strains. If, however, the expected MIC is close to the breakpoint value for categorising the strain

as susceptible or resistant, one two-fold dilution difference (which is acceptable) may result in two different interpretations, i.e. the same strain can be categorized as susceptible and resistant. This result will be evaluated as correct in one case, but incorrect when the evaluation is based on AST interpretations. In the organization of the EQAS, we try to avoid these situations by choosing test strains with MIC values distant from the cut-offs for resistance, which is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AR network unanimously established in 2008 that if there are less than 75% correct results for a specific strain/antimicrobial combination, the reasons for this situation must be further examined and, on selected occasions explained in details case by case, these results may subsequently be omitted from the evaluation report.

The data in this report is 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.

This fifht matrix EQAS was organized by the EURL-AR at the National Food Institute (DTU Food), Kgs. Lyngby, Denmark. The reporting of this 2019 version of the matrix EQAS has been delayed, among other factors, by the relatively high amount of deviations obtained in the reporting of both AST and ESBL phenotypes, which required additional analysis. The overall results of the EQAS were presented to the competent representatives from all NRL-ARs, who meets annually at the EURL-AR workshop, and hereafter the report was finalised.

2. Materials and Methods

2.1 Participants in EQAS 2019

A pre-notification (App. 1), announcing the matrix EQAS 2019, was distributed on the 13th of August 2019 by e-mail to the designated NRLs including all EU countries and Iceland, Norway and Switzerland. In total 36 laboratories participated in the matrix EQAS (App. 2) involving one NRL from each of the 28 MS (two from two countries, analysing meat and caecal sample in different laboratories), and from Iceland, Norway and Switzerland, plus additional laboratories. As results from only one laboratory per country are included in this report, 33 laboratory results from 31 countries are described. The exception was the two countries, who has different laboratories enrolled for handling meat and caecal samples, and therefore had two different NRLs enrolled.

Furthermore, one additional laboratory from each of the Netherlands, Spain and United Kingdom participated. These were invited based on their participation in previous EQAS iterations and/or affiliation to the EU network and provided results but were not included further in the report. Participants from non-EU MS were charged a fee for participation whereas participation was free of charge for EU MS, but each laboratory was expected to cover expenses associated with the analyses. The European countries participating are marked on map in Figure 1.

2.2 Preparation of samples

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 five

pork meat and three calf caecal samples and were either prepared by spiking with test strains or unmodified.

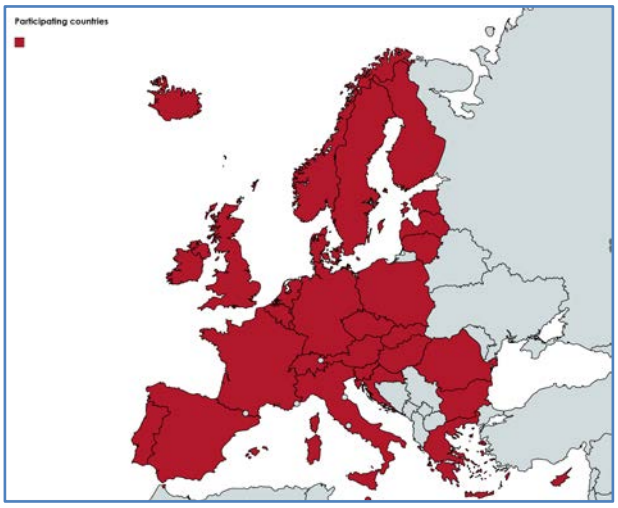


Figure 1 Countries participating in matrix EQAS 2019

The meat used to prepare the samples was minced pork meat of Danish origin (raised, slaughtered and packed in Denmark) acquired in local supermarkets (four batches were bought on August 26th 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 used directly as blank samples or spiked as follows.

The test isolates used in the spiking of meat samples within the EQAS matrix 2019 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 Bertani (LB) agar plates. The standardized suspensions were further diluted in ten-fold dilutions and the meat samples (25 g) were spiked with 25 µl of the chosen dilutions. The spiking dilutions were chosen based on the results obtained in the previous matrix EQAS rounds. The final inoculum found in the samples in this EQAS was expected to be approx. 10^3 CFU/g meat, for the samples EURL-M-5.1, M-5.2, M-5.3 and M-5.4. The sample M-5.5 was spiked as mentioned above, however with a susceptible *E. coli* strain (ATCC 25922) and therefore expected to be negative.

One slaughterhouse provided on Sept 2nd 15 batches of calf caecal samples from different herds. These samples were tested using the official selective isolation protocol for ESBL, AmpC and carbapenemase-producing *E. coli*.

One ESBL-negative caecal batch was chosen for preparation of the matrix caecal samples for the EQAS strains. Thereby 1 g aliquots of caecal content was spiked with 10 µl of a dilution containing 10^6 CFU/ml, causing an expected spiking level of 10^4 CFU/g for the samples M-5.6, M-5.7 and M-5.8. In contrast to previous years, all three caecal samples were spiked with resistant *E. coli*, as pre-testing of the survival of the test strains in matrix material was not included in the matrix EQAS preparation.

The minimal inhibitory concentrations (MIC) of selected antimicrobials were determined using broth microdilution method 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).

For follow-up on the stability of the inoculum in the matrix samples after shipping, repeated testing of isolation of test strains was performed on sets the eight samples in four time points after shipment (during two weeks). In this period, the meat and caecal samples were kept at 4°C, to mimic the conditions in the shipment parcel.

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. 4). For the confirmation of *E. coli* isolates, different methods were allowed as these are not specified in the legislation (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 proficiency test 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 (2018) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - 11th 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 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 the time for shipment. The caecal samples were sent shortly after preparation, and therefore kept at 4°C until the time for shipment. At the day of shipment, the samples were tightly packed in thermos boxes with cooling elements, frozen at -80°C. The parcels contained the eight samples in tubes, and an additional tube contained a temperature logger to register the temperature at 15 min intervals during transport. Furthermore, the parcel contained a welcome letter with the login and password to the online database for the data upload and a labelled envelope for returning the temperature logger to the EURL-AR.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2019 component

Escherichia coli EUVSEC	*Escherichia coli* EUVSEC2
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	

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

The thermos boxes 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 September 9th 2019.

2.6 Procedure

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 after sample shipment and until the reporting 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).

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 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.

3. Results

Upon arrival of the parcels, 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 registration of the temperature was extracted and read to provide the temperature ranges along the shipment and at sample reception/opening. All samples were registered to be between -1°C and 5°C at arrival inferred from the temperature at opening time from the temperature logger registration and thereby all samples were expected to be in good conditions for testing at the time for opening of the parcels.

3.1 Overall results of selective isolation

The number of possible test results for ESBL/AmpC qualitative isolation considered for this report was 248 tests; eight samples from each of 31 countries. As the meat and caecal matrices have a natural background of bacteria from the animal itself, there is a high possibility of presence of *E. coli* and other *Enterobacteriaceae*, which can even include ESBL producing bacteria, despite the pre-testing of both meat and caecal samples.

In the pork meat samples, some deviations were seen in the isolation of resistant *E. coli.* The sample M-5.5, which should be negative of ESBL/AmpC or carbapenemase producing *E. coli*, were in nine laboratories found positive, as growth occurred on the selective agar plates. In seven cases, the laboratories isolated carbapenemase producing *E. coli* from this sample, and further analysis revealed a resistance profile very comparable of the profile of the isolate from sample M-5.4. It seems unlikely that this mix-up or contamination of samples should occur simultaneously in seven of 31 laboratories, thus the most likely cause is mistakes in the sample preparation in the EURL-AR laboratory. These seven cases will not count as errors in the ESBL prediction. Further two laboratories isolated resistant bacteria from the sample spiked with susceptible *E. coli* ATCC 25922, which were categorized as ESBL and other phenotype, respectively. These two isolates did not have resistance profiles in accordance with any of the test strains, and could derive from the naturally occurring bacteria in the meat samples. Overall, these findings did not affect the AST results, as the M-5.5 samples were expected to contain susceptible *E. coli* only, which are not evaluated by AST.

It is well known that the inoculated bacteria might not survive well in the meat and especially the caecal matrix, and five laboratories failed to isolate the test strain from meat sample 5.4, whereas two laboratories failed to isolate the test strain from calf caecal sample 5.7.

As such, 241 tests were included in the evaluation, subtracted the seven erroneously spiked samples 5.5. These results are summarized in Table 2 and further discussed in section 3.3.

Table 2. The overall performance of ESBL/AmpC isolation and identification, 2019.

Isolation of ESBL /AMPC from samples		Correctly classified samples	
Number of performed tests		Number of correct tests	
N	%	N	%
241	100	230	95.4
Number of expected negative tests		Number of correctly identified negative tests	
N	%	N	%
24	10	22	91.7
Number of expected positive tests		Number of correctly identified positive tests	
N	%	N	%
217	90	208	95.9

3.2 Methods used by EQAS-participants

In this trial, 29 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). All 33 participating laboratories, which have submitted results, participated in the ESBL and AmpC isolation and performed the identification and susceptibility testing of the respective isolates. Three laboratories reported that they did not perform the optional carbapenemase selective isolation. The number of qualitative isolation tests results reported was variable, including results for three to eight samples, depending on how many samples were tested (four participants only tested meat or caecal samples), for the antimicrobial susceptibility test it depended on how many isolates were found and further tested in the MIC panels.

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

Most laboratories (n=33) reported that isolation had been performed following the exact procedures described in the protocol provided. One lab reported a small change in volume of enrichment broth, to allow for plate count. The species identification was performed using MALDI TOF (n=24), biochemical tests (n=9), or chromogenic agar plating (n=7), and PCR using specific targets to confirm the ID (n=3). Additionally, some laboratories reported using second and third identification methods as supplement.

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.

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

ESBL/AmpC

The total amount of test results was 241 tests for the ESBL/AmpC isolation qualitative results. All in all, 223 tests were assigned the correct ESBL/AmpC or carbapenemase phenotype, corresponding to 92.5 % correct interpretation results. For the sample M-5.1, which was expected to be ESBL+ AmpC due to presence of both CMY-2 and CTX-M-27 genes, nine laboratories reported a different ESBL phenotype. Four laboratories (#11, #22, #42 and #56) reported AmpC, despite the MIC phenotype showing ESBL+AmpC. Three additional laboratories (#6, #25 and #40) reported M-5.1 as AmpC, but in these cases the ESBL phenotypes were correct interpreted from the MICs. The reason for these isolates not expressing ESBL phenotype has not been identified. Further two laboratories found variating phenotypes in the isolate from M-5.1; lab #37 isolated a carbapenemase phenotype and lab #59 isolated an ESBL phenotype. Again, there was no obvious reason for these deviating phenotypes, and the resistance profiles do not match other of the test strains, which would have been the case if it was caused by mix-up of strains or contamination.

Additionally, there were seven other sporadic cases of unexpected phenotypes; one for M-5.2 (AmpC instead of ESBL), two for M-5.4 (loss of carbapenemase phenotype), two for M-5.6 (one loss of ESBL phenotype, one carbapenemase instead of ESBL), one for M-5.7 (ESBL+AmpC instead of AmpC) and finally one for M-5.8 (ESBL instead of carbapenemase). Two of these cases could be explained by an apparent mix of M-5.6 and M-5.8 by lab #39, which luckily can explain the unexpected presence of meropenem resistance in M-5.6. The remaining five cases appeared in different laboratories.

Other carbapenemases and OXA-48

The specific isolation of presumptive carbapenemase producing *E. coli* was performed by extending the protocol to include isolation on CARBA selective agar plates as described in the EURL-AR protocols. Three labs did not perform the optional carbapenemase selective isolation, but defined results based on the findings in the ESBL/AmpC selective method and AST results.

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 ChromID CARBA and ChromID OXA-48 or CARBA Smart combination plates (as reported by ten and eight participants, respectively), whereas two just indicated Biomerieux agar plates. The remaining participants did not report the brand of plates used for this purpose, but reported that the EURL-AR protocol was followed.

3.4 Antimicrobial susceptibility testing

Data omitted from evaluation

A total of 5208 MIC results were submitted to the EURL-AR EQAS database by the 33 labs. Hereof, there were initially 166 deviations (3.2%), but data analysis highlighted some problematic MICs. The EURL-AR lab originally tested a high MIC (>1024) of M-5.1 for sulfamethoxazole, but in the following MIC determinations after spiking into meat samples, MIC results of both 16 and >1024 were obtained. Only 3/31 laboratories obtained the high MIC value, which means that 90 % of participants obtained a low MIC (S) between 8-64 mg/L. As a concequence, the SMX MIC's for M-5.1 was omitted from further evaluation.

The strain M-5.6 did by repeated MIC testing in the EURL-AR lab, after spiking into caecal

samples, show cefoxitin MIC's of both 8 and 16, making it difficult to set both a reference value and an ESBL phenotype for this isolate. Although genotypically ESBL, also ESBL+AmpC is made a valid interpretation of this isolate.

Deviations in AST

Thus, 5177 AST results were included in this report and 5061 (97.8 %) of these were correct. The 33 labs uploaded a variable number of results, depending on the samples found positive and isolates tested in one or both panels, ranging from 48 to 147 test results per participant.

Of the 116 deviations detected, 26 were caused by 1-step MIC deviations, which is still regarded as acceptable deviations, although they resulted in a different susceptible/resistant interpretation. This was mainly seen for cefepime (M-5.7), ertapenem (M-5.1 and M-5.6) and temocillin (M-5.8) (Appendix 6). Thus, 90 deviations were regarded as true deviations, either due to incorrect MIC, compared to the expected results, or incorrect interpretation of susceptible/resistant phenotype (in 4 cases). A part of these deviations (27 deviations) was explained by a mix up of strains from one lab (M-5.6 and M-5.8 in Lab #39). Apart from this, there was a high number of non-systematic AST deviations, which was corresponding with the incoherent ESBL phenotypes reported by many participants.

The analysis per laboratory identified nine laboratories with no deviations, while the others had deviation percentages ranging from 0.5 % to 8.4 % (and 17.4 % in the case of strain mix-up). (Figure 2). 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. However, the AST results show more variation and more deviations compared to the ESBL/AmpC EQAS performed in previous years.

In the analysis of deviations per antimicrobial, it was observed that the highest deviation percentages was found for ertapenem, in part due to elevated ETP MIC in the AmpC isolate M-5.1 and the ESBL isolate M-5.6 (ETP: 5.5 %) followed by imipenem (IMI; 5.1 %). There was not a high variation between the antimicrobials, and the range of deviations was 0 – 5.5% (Figure 3).

Likewise, the deviations per matrix sample did not show high variation, and were in the range of 0.3 – 3.5 % and the highest level of deviations was observed for sample M-5.4 (Figure 4).

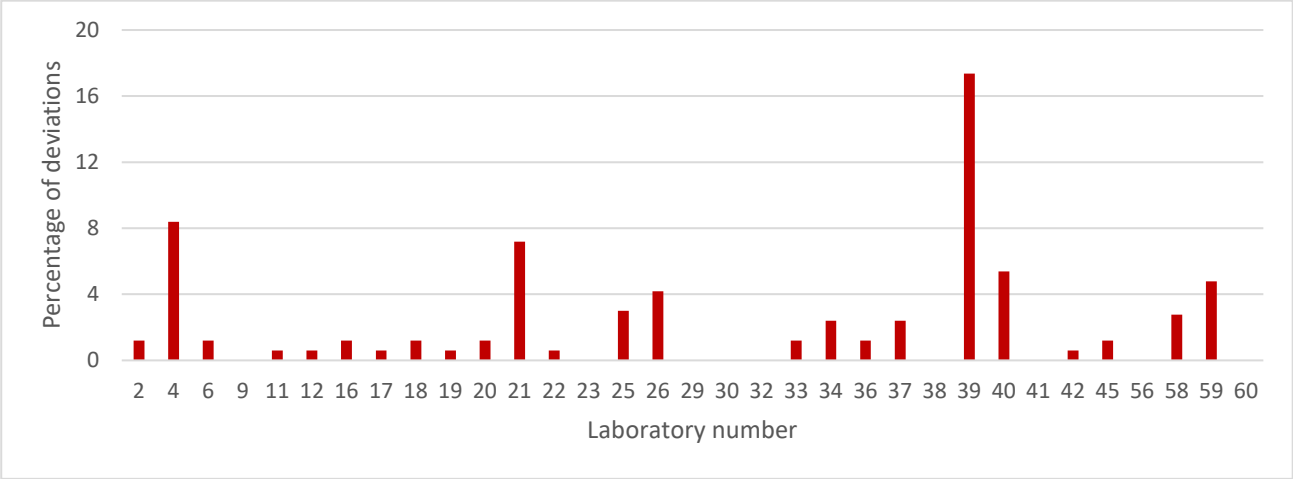


Figure 2. Percentages of deviations in antimicrobial susceptibility testing per participating laboratory

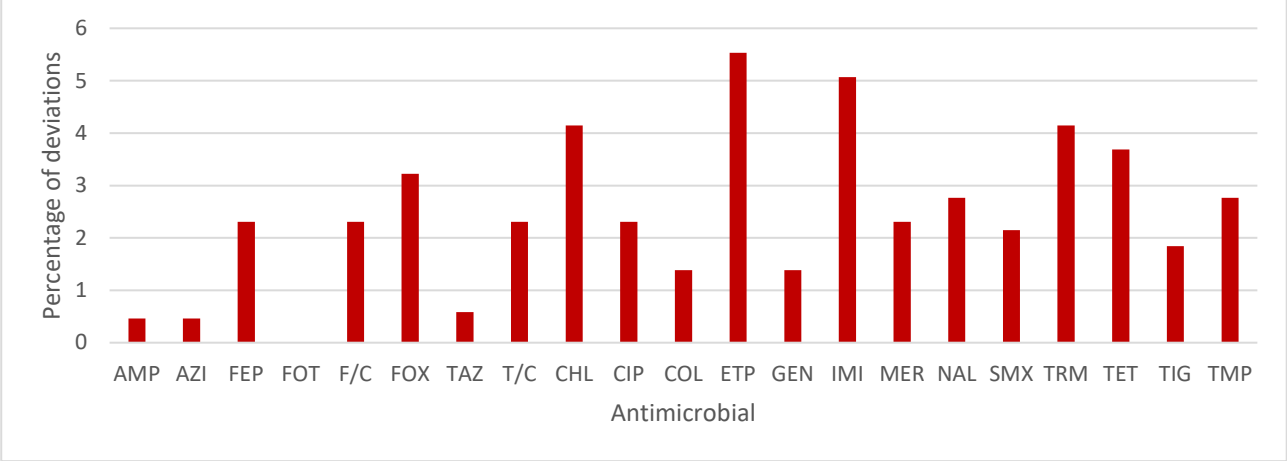


Figure 3. Percentages of deviations per antimicrobial in EQAS matrix 2019 (AST results).

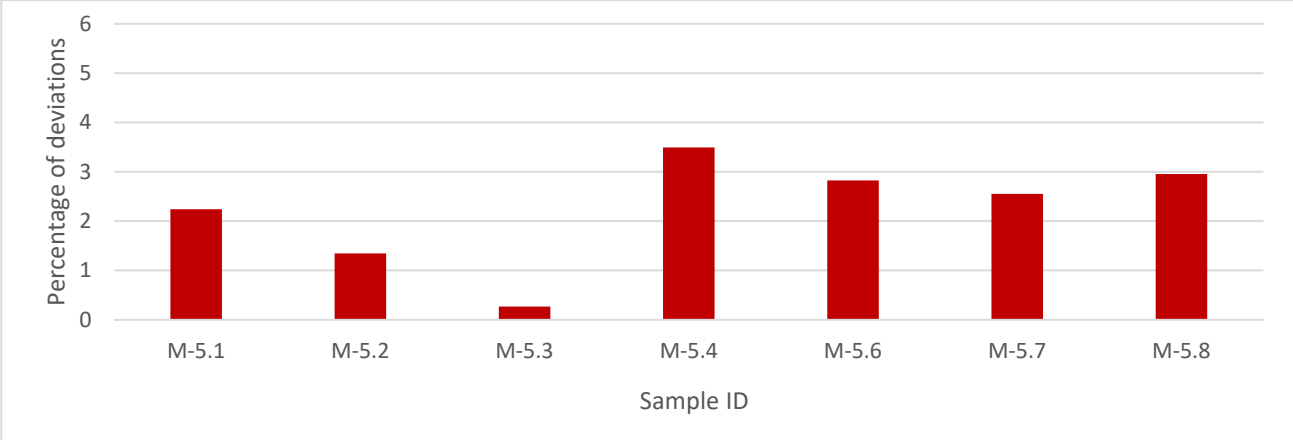


Figure 4. Percentages of deviations per sample in EQAS matrix 2019 (AST results).

3.5 ESBL/AmpC phenotypic testing conclusions

Five pork meat samples (M-5.1 – M-5.5) were included in this matrix EQAS. The sample M-5.1 contained an isolate expressing AmpC phenotype by CMY-2 gene and ESBL phenotype by CTX-M-27; sample M-5.2 and M-5.3 contained the ESBL genes CTX-M-32 and CTX-M-2, respectively, whereas M-5.4 expressed carbapenemase by OXA-162. M-5.5 contained a susceptible *E. coli*. In this EQAS round, all three calf caecal samples were spiked with resistant *E. coli*; M-5-6 carried the ESBL gene CARB-2, M-5.7 had an upregulated AmpC and M-5.8 had carbapenemase phenotype mediated by NDM-1.

Overall, an unusually high number of isolates did not express the expected phenotype. The AmpC isolate M-5.1 was misinterpreted in four labs, and exhibited additionally ESBL phenotype in three labs (See section 3.3). The ESBL strain M-5.2 exhibited in one case additionally AmpC phenotype (#40). The carbapenemase producing strain M-5.4 did in two cases not express carbapenemase resistance. And finally, nine laboratories isolated resistant *E. coli* from the meat sample inoculated with a susceptible strain; in seven cases likely due to mistakes made by the EURL-AR lab, and in two cases likely due to naturally occurring bacteria.

For the caecal samples, the ESBL isolate from M-5.6 did in some cases express an additional AmpC phenotype, but in one case, it did not express the expected ESBL synergy. Also for the strain M-5.7 the AmpC phenotype was unclear, and one lab seemed to have mixed up two of the caecal samples; M-5.6 and 5.8. This amounts to an extraordinary level of unclear or deviating ESBL/AmpC/carbapenemase phenotypes, compared to previous years.

4. Discussion

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

The 2019 EURL-AR matrix EQAS trial was the fifth of its kind on samples of animal origin. Some challenges continue to be present; e.g. and adequate testing and selection of meat and caecal samples with a low level of background bacteria and absence of ESBL contamination. Despite the thorough pre-testing, some meat samples ended out with natural ESBL contamination. This year, four batches of minced pork meat were pre-tested, and only one batch seemed to be free of naturally occurring ESBL-producing bacteria, growing on MacConkey agar with CTX after enrichment. Although, after spiking and re-isolating test strains, the EURL-AR laboratory also noted a high level of competing bacteria. In the two previous rounds of pig/cattle samples (2015 and 2017), the matrix material had instead been beef meat and pig caecal. As the pre-screening of the matrix material only serves to reveal possible ESBL/AmpC contamination per batch and a rough estimation of the level of background bacteria, it is practically impossible to avoid having generic *Enterobacteriaceae* or *E. coli* and sometimes even ESBL bacteria in some parts of the meat matrix.

This is a great limitation for the matrix EQAS, compared to other EQAS' on pure isolates. In general, the ESBL/AmpC isolation was successful, as there were only seven instances where laboratories were not being able to isolate the resistant bacteria (five from meat sample M-5.4 and two from caecal sample M-5.7), but overall, there seemed to be unusually many sporadic cases of naturally occurring bacteria interfering with the isolation of test bacteria. It was also very unfortunate that there were seven cases of wrongly inoculated or marked samples being shipped, resulting in isolation of a carbapenem resistant *E. coli* from the sample that was supposed to be without resistant *E. coli* (M-5.5). It has not been possible to trace back the reason for the error in the EURL-AR laboratory.

4.2 Antimicrobial susceptibility testing

It is in general a problem for the AST interpretation, when the expected MIC values are close to the ECOFF's between susceptible and resistant. Although one MIC level deviation is accepted, it is problematic when it changes the susceptible/resistant interpretation. Thus, it can be difficult to select test strains with clear phenotypes, expected to survive in the matrix, without making compromises on this point. This issue gave rise to some of the AST deviations, but not to the same extent as in 2018, where the majority of the deviations were caused by this.

The remaining results, however, were generally hampered by sporadic incidences of unexpected phenotypes, which has not been easy to elucidate any further. It is difficult to interpret if the deviations in AST results are caused by misinterpretation of MIC or by variations in the phenotypes. As such, there were more widespread deviations in the AST results in 2019 than in previous years. This year, nine laboratories had no deviations at all, which is the same level as in 2018. Thus, the challenges met were not unexpected, as working with isolates in a matrix deriving from animals is likely to cause problems like retrieving the right isolates from the samples, or that 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 further caused by MIC results close to breakpoint, and this should carefully be considered when selecting the strains for spiking samples. Thus, some of the

deviations seem to derive from either mix of samples or by wrong interpretation of a correct MIC.

4.3 ESBL /AmpC phenotypic testing conclusions

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 strains that have different characteristics. Thus, the primary AST results, used for classification of ESBL, AmpC,

carbapenemases or other phenotypes were generally very good, with relatively few examples of wrong interpretation. It was seen that especially the AmpC prediction is at times difficult. Four laboratories reported that they did not perform the optional carbapenemase selective isolation, which is a decrease from eight labs in 2018.

5. Conclusion

In general, the results of this matrix EQAS demonstrate that most participating labs have well established methods to isolate ESBL and AmpC-carrying strains from meat or caecal samples, despite the difficult nature of the matrices.

The matrix samples continue to be difficult to work with, due to presence of background bacteria. Thus, there are still some preventable deviations, including mix of strains and wrong interpretation of susceptible or resistant phenotypes.

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.

7. Appendices

Appendix 1. Pre-notification EURL-AR EQAS matrix 2019
Appendix 2. List of participants
Appendix 3. Expected results
Appendix 4. Protocol EQAS matrix 2019
Appendix 5. Examples of Test forms EQAS matrix 2019
Appendix 6. List of deviations

EURL-AR EQAS pre-notification

G00-06-001/01.12.2014

EQAS 2019 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 third 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. The laboratories designated to be NRL-AR will 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 pork meat and/or calf caecal will be shipped. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures. 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 on **September 9th**. The protocol for this proficiency test is available for download from the website (https://www.eurl-ar.eu/protocols.aspx).

Submission of results: Results submission via a password-protected website will deadline to the National Food Institute will be in the beginning of **December 2019**, but as a new database is being generated, this deadline is not yet established. 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 *Salmonella*, *Campylobacter* and optional genotypic characterisation which will be carried out mid-October, 2019.

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

Sincerely,

Jette Kjeldgaard, EURL-AR EQAS-Coordinator

Appendix 2 List of participants

Meat	Caecal	Institute	Country
x	x	Austrian Agency for Health and Food Safety	Austria
x	x	Institute of Public Health	Belgium
x	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	Agence nationale de sécurité sanitaire alimentation, environnement, travail (ANSES) - Laboratoire de Fougères LERMVD	France
x	x	Federal Institute for Risk Assessment	Germany
x	x	Veterinary Laboratory of Chalkida	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	Veterinærinstituttet	Norway
x	x	National Veterinary Research Institute	Poland
x	x	Instituto Nacional de Investigação Agrária e Veterinária	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	Laboratorio Central de Veterinaria	Spain
x		Centro Nacional de Alimentación (AECOSAN)	Spain
x	x	Foodborne Zoonoses and Antimicrobial Resistance Unit (ZTA)*	Spain
x	x	National Veterinary Institute, SVA	Sweden
x	x	Institute of Veterinary Bacteriology, Vetsuisse Faculty Bern	Switzerland
x	x	Wageningen Bioveterinary Research (WBVR)	The Netherlands
x	x	The Netherlands Food and Consumer Product Safety Authority*	The Netherlands
x	x	Animal & Plant Health Agency (APHA)	United Kingdom
	x	Agri-Food and Biosciences Institute*	United Kingdom

Designated NRL-AR by the compentent authority of the member state
Non-NRL-AR enrolled by the EURL-AR
Not a Member State of the EU
* Submitted results were not included in the current report (one dataset per country, only)

Panel 1

Strain	AMP	MERO	COL	CHL	CIP	TAZ	FOT	GEN	NAL	SMX	TET	TMP	AZI	TGC	ESBL Conclusion
EURL-M-5.1	>64	≤0.03	≤1	>128	>8	>8	>4	1	>128	>1024	>64	>32	8	≤0.25	ESBL + AmpC
EURL-M-5.2	>64	≤0.03	≤1	≤8	≤0.015	2	>4	≤0.5	≤4	>1024	>64	0.5	4	≤0.25	ESBL
EURL-M-5.3	>64	≤0.03	4	128	≤0.015	2	>4	>32	≤4	>1024	>64	>32	4	≤0.25	ESBL
EURL-M-5.4	>64	0.5	8	>128	4	≤0.5	0.5	1	>128	>1024	>64	0.5	8	0.5	Carba
EURL-M-5.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
EURL-M-5.6	>64	≤0.03	≤1	>128	>8	4	>4	≤0.5	>128	>1024	>64	>32	16	0.5	ESBL (+ AmpC phenotype)
EURL-M-5.7	>64	≤0.03	≤1	≤8	0.03	4	2	≤0.5	≤4	≤8	≤2	0.5	8	≤0.25	ampC
EURL-M-5.8	>64	8	≤1	≤8	0.06	>8	>4	>32	≤4	>1024	≤2	≤0.25	8	≤0.25	Carba

Interpretation

Strain	AMP	MERO	COL	CHL	CIP	TAZ	FOT	GEN	NAL	SMX	TET	TMP	AZI	TGC	
EURL-M-5.1	R	S	S	R	R	R	R	S	R	R	R	R	S	S	CMY-2; CTX-M-27
EURL-M-5.2	R	S	S	S	S	R	R	S	S	R	R	S	S	S	CTX-M-32
EURL-M-5.3	R	S	R	R	S	R	R	R	S	R	R	R	S	S	CTX-M-2
EURL-M-5.4	R	R	R	R	R	S	R	S	R	R	R	S	S	S	OXA-162
EURL-M-5.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Susceptible
EURL-M-5.6	R	S	S	R	R	R	R	S	R	R	R	R	S	S	CARB-2
EURL-M-5.7	R	S	S	S	S	R	R	S	S	S	S	S	S	S	upregulated ampC
EURL-M-5.8	R	R	S	S	S	R	R	R	S	R	S	S	S	S	NDM-1

Code	FOX	TAZ	TAZ+CL	FOT	FOT+CL	FEP	MERO	IMI	ETP	TRM	ESBL conclusion	Gene
EURL-M-5.1	64	32	8/4	>64	4/4	32	≤0.03	0.25	0.06	16	ESBL + AmpC	CMY-2; CTX-M-27
EURL-M-5.2	4	2	≤0.12/4	32	≤0.06/4	4	≤0.03	0.25	≤0.015	8	ESBL	CTX-M-32
EURL-M-5.3	8	4	0.25/4	>64	≤0.06/4	32	≤0.03	0.25	0.03	4	ESBL	CTX-M-2
EURL-M-5.4	8	0.5	0.5/4	0.5	0.5/4	0.5	0.5	2	1	>128	Carba	OXA-162
EURL-M-5.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	Susceptible
EURL-M-5.6	8/16	16	0.25/4	>64	0.12/4	16	≤0.03	≤0.12	0.06	8	ESBL (+ AmpC)	CARB-2
EURL-M-5.7	32	8	4/4	2	1/4	0.12	≤0.03	0.25	≤0.015	8	AmpC	upregulated ampC
EURL-M-5.8	>64	>128	>128/4	>64	>64	>32	8	4	>2	32	Carba	NDM-1

Code	FOX	TAZ	T/C	FOT	FOT+CL	FEP	MERO	IMI	ETP	TRM	ESBL conclusion	Gene
EURL-M-5.1	R	R	NO SYNERGY	R	SYNERGY	R	S	S	S	NA	ESBL + AmpC	CMY-2; CTX-M-27
EURL-M-5.2	S	R	SYNERGY	R	SYNERGY	R	S	S	S	NA	ESBL	CTX-M-32
EURL-M-5.3	S	R	SYNERGY	R	SYNERGY	R	S	S	S	NA	ESBL	CTX-M-2
EURL-M-5.4	S	S	NO SYNERGY	R	NO SYNERGY	R	R	R	R	NA	Carba	OXA-162
EURL-M-5.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	Susceptible
EURL-M-5.6	S/R	R	SYNERGY	R	SYNERGY	R	S	S	S	NA	ESBL (+ AmpC)	CARB-2
EURL-M-5.7	R	R	NO SYNERGY	R	NO SYNERGY	S	S	S	S	NA	AmpC	upregulated ampC
EURL-M-5.8	R	R	NO SYNERGY	R	NO SYNERGY	R	R	R	R	NA	Carba	NDM-1

DTU Food
National Food Institute

PROTOCOL

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

Table of contents

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 antimicrobial susceptibility testing (AST) of obtained isolates of eight samples of either meat or caecal content. In 2019, these eight samples will include five 25-g samples of pork meat and three 1-g samples of cattle caecal content. These samples may contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes.

It is expected that the participating laboratories apply the same analysis procedures used in the monitoring, described by the regulation EC/652/2013, and perform the selective isolation following the by EU recommended methods, published on the EURL-AR website 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 September 2019, 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 matrices.

The samples will be spiked matrices of either beef meat or pig caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 5.1 to 5.8. Hereof 5.1 to 5.5 being samples of meat (each 25 g) and 5.6 to 5.8 being samples of caecal content (each 1 g).

The matrix samples will be shipped on September 9th in chilled state in separate tubes and contained in a cooling box with a temperature logging devices and cooling elements.

Upon arrival, 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 start of analysis for each sample**
- **collect the temperature logging 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 return it 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 volume 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 5.1 to 5.5
- Follow the protocol for caecal content when testing samples 5.6 to 5.8

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 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 comparing to 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 *E. coli*	MIC (mg/L) **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

Plasmid-mediated quinolone resistance

When performing AST 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 *E. coli*	MIC (mg/L) **R is >**
Cefepime, FEP	0.125
Cefotaxime, FOT	0.25
Cefotaxime + clavulanic acid (F/C)	**0.25**
Cefoxitin, FOX	8
Ceftazidime, TAZ	0.5
Ceftazidime+ clavulanic acid (T/C)	**0.5**
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 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. the MIC of the agent when tested alone (MIC FOT : FOT/CL or TAZ : TAZ/CL ratio ≥ 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; https://www.eurl-

ar.eu/CustomerData/Files/Folders/3-workshop-kgs-lyngby-april2016/25_efsa-eusr-amr-workflow-and-criteria-for-esbl-ampc-carbapenemase-phenotypes.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 6th, December, 2019.** 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:

Jette Sejer Kjeldgaard
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204,

DK-2800 Lyngby

Denmark

Tel: +45 3588 6269

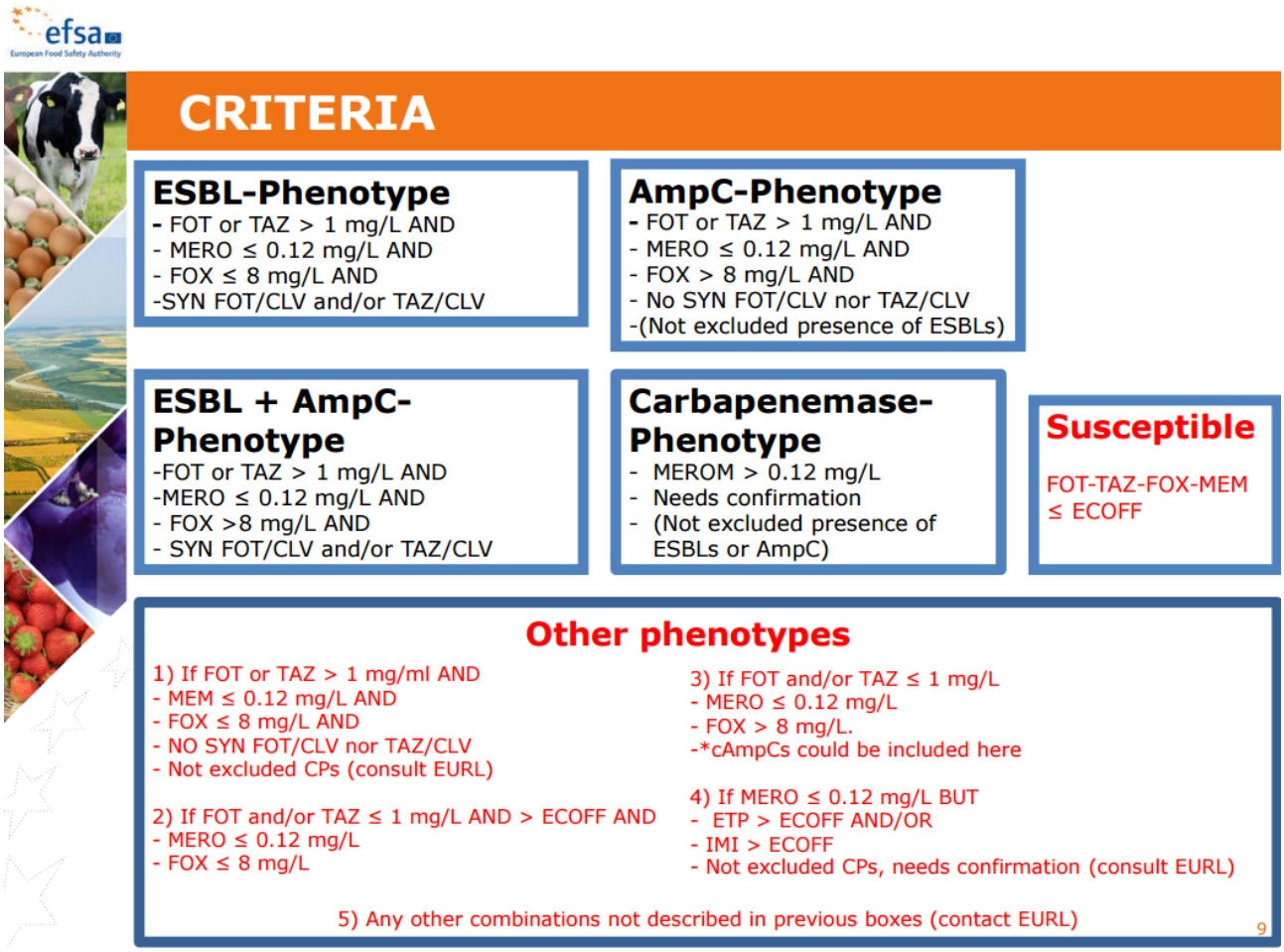
E-mail: jetk@food.dtu.dk

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

As the EURL-AR EQAS database is undergoing major changes, instructions on how to submit results for evaluation will be provided as soon as the database has been validated. Information will be sent via email to the EURL-AR EQAS participants.

APPENDIX

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



Please refer to the presentation at https://www.eurl-ar.eu/CustomerData/Files/Folders/3-workshop-kgs-lyngby-april2016/25_efsa-eusr-amr-workflow-and-criteria-for-esbl-ampc-carbapenemase-phenotypes.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 2019 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 2019 for monitoring of ESBL/AmpC-detection in relation to 2013/652/EU? (you may choose more than one option)

- ☐ caecal, calfes
- ☐ meat, pork
- ☐ other matrices, please specify:

Did you process samples for carbapenemase-selective isolation?

- ☐ Yes
- ☐ No

How many samples did your laboratory process in 2019 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 2019 for monitoring of carbapenemase-production in relation to 2013/652/EU? (you may choose more than one option)

- ☐ caecal, calfes
- ☐ meat, pork
- ☐ 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-5.1'

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 MacConkey 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 (mg/L)	S / R
E. coli EURL M-5.1	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 (mg/L)	S / R
E. coli EURL M-5.1	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:

☐ Presumptive ESBL
☐ Presumptive ESBL+ AmpC
☐ Presumptive AmpC
☐ Presumptive carbapenemase
☐ Other phenotype
☐ Susceptible

Comments (include optional genotype or other results):

Appendix 6 List of deviations - ESBL predictions

Lab #	Strain	Obtained value	Expected value	Score
4	EURL-M-5.7	ESBL+AmpC-phenotype	AmpC-phenotype	0
6	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
11	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
21	EURL-M-5.6	AmpC-phenotype	ESBL-phenotype; ESBL+AmpC-phenotype	0
22	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
25	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
26	EURL-M-5.4	Other phenotypes	Carbapenemase-phenotype	0
26	EURL-M-5.5	Other phenotypes	Susceptible (to panel 2 antimicrobials)	0
33	EURL-M-5.4	Other phenotypes	Carbapenemase-phenotype	0
37	EURL-M-5.1	Carbapenemase-phenotype	ESBL+AmpC-phenotype	0
39	EURL-M-5.6	Carbapenemase-phenotype	ESBL-phenotype; ESBL+AmpC-phenotype	0
39	EURL-M-5.8	ESBL-phenotype	Carbapenemase-phenotype	0
40	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
40	EURL-M-5.2	AmpC-phenotype	ESBL-phenotype	0
42	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
56	EURL-M-5.1	AmpC-phenotype	ESBL+AmpC-phenotype	0
59	EURL-M-5.1	ESBL-phenotype	ESBL+AmpC-phenotype	0
59	EURL-M-5.5	ESBL-phenotype	Susceptible (to panel 2 antimicrobials)	0
2	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
4	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
12	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
19	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
20	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
30	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
33	EURL-M-5.5	Carbapenemase-phenotype	Susceptible (to panel 2 antimicrobials)	0
59	EURL-M-5.5	ESBL-phenotype	Susceptible (to panel 2 antimicrobials)	0
26	EURL-M-5.5	Other phenotypes	Susceptible (to panel 2 antimicrobials)	0

Appendix 6 List of deviations -AST results

Lab #	Strain ID	Panel	Antimicrobial	Operator to obtained value	Obtained value	Operator to expected value	Expected value	Obtained Interpretation	Expected Interpretation	Score
2	EURL-M-5.6	2	Ertapenem	=	0.12	=	0.06	R	S	0
2	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
4	EURL-M-5.7	1	Azithromycin	=	64	=	8	R	S	0
4	EURL-M-5.4	1	Ceftazidime	>	8	<=	0.5	R	S	0
4	EURL-M-5.4	1	Gentamicin	>	32	<=	0.5	R	S	0
4	EURL-M-5.7	1	Sulfamethoxazole	>	1024	<=	8	R	S	0
4	EURL-M-5.3	1	Tetracycline	>	64	>	64	S	R	0
4	EURL-M-5.7	1	Tetracycline	=	64	<=	2	R	S	0
4	EURL-M-5.4	1	Trimethoprim	>	32	=	0.5	R	S	0
4	EURL-M-5.7	1	Trimethoprim	>	32	<=	0.25	R	S	0
4	EURL-M-5.7	2	Cefepime	=	4	=	0.12	R	S	0
4	EURL-M-5.4	2	Cefoxitin	=	64	=	8	R	S	0
4	EURL-M-5.4	2	Ceftazidime	>	128	=	0.5	R	S	0
4	EURL-M-5.4	2	Ceftazidime/clavulanic acid	=	128	=	0.25	R	S	0
4	EURL-M-5.1	2	Ertapenem	=	0.12	=	0.06	R	S	0
4	EURL-M-5.4	2	Imipenem	=	0.5	=	2	S	R	0
6	EURL-M-5.7	2	Cefepime	=	0.25	=	0.12	R	S	0
6	EURL-M-5.6	2	Ertapenem	=	0.12	=	0.06	R	S	0
11	EURL-M-5.6	1	Chloramphenicol	<=	8	>	128	S	R	0
12	EURL-M-5.4	2	Cefoxitin	=	16	=	8	R	S	0
16	EURL-M-5.1	2	Ertapenem	=	0.12	=	0.06	R	S	0
16	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
17	EURL-M-5.6	2	Ertapenem	=	0.12	=	0.06	R	S	0
18	EURL-M-5.4	1	Ampicillin	>	64	>	64	S	R	0
18	EURL-M-5.4	2	Cefoxitin	=	16	=	8	R	S	0
19	EURL-M-5.4	2	Imipenem	=	0.25	=	2	S	R	0

20	EURL-M-5.4	2	Imipenem	=	0.5	=	2	S	R	0
20	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
21	EURL-M-5.2	1	Chloramphenicol	>	128	<=	8	R	S	0
21	EURL-M-5.7	1	Chloramphenicol	=	64	<=	8	R	S	0
21	EURL-M-5.2	1	Ciprofloxacin	=	4	<=	0.015	R	S	0
21	EURL-M-5.7	1	Ciprofloxacin	=	8	=	0.03	R	S	0
21	EURL-M-5.1	1	Colistin	=	8	<=	1	R	S	0
21	EURL-M-5.2	1	Colistin	=	8	<=	1	R	S	0
21	EURL-M-5.7	1	Colistin	=	4	<=	1	R	S	0
21	EURL-M-5.2	1	Nalidixic acid	>	128	<=	4	R	S	0
21	EURL-M-5.7	1	Nalidixic acid	>	128	<=	4	R	S	0
21	EURL-M-5.7	1	Sulfamethoxazole	>	1024	<=	8	R	S	0
21	EURL-M-5.7	1	Tetracycline	=	32	<=	2	R	S	0
21	EURL-M-5.6	1	Trimethoprim	>	32	>	32	S	R	0
22	EURL-M-5.7	2	Cefepime	=	0.25	=	0.12	R	S	0
25	EURL-M-5.4	1	Tigecycline	=	1	=	0.5	R	S	0
25	EURL-M-5.6	1	Tigecycline	=	1	=	0.5	R	S	0
25	EURL-M-5.1	1	Tigecycline	=	1	<=	0.25	R	S	0
25	EURL-M-5.3	1	Tigecycline	=	1	<=	0.25	R	S	0
25	EURL-M-5.6	2	Ertapenem	=	0.12	=	0.06	R	S	0
26	EURL-M-5.4	1	Meropenem	=	0.12	=	1	S	R	0
26	EURL-M-5.4	2	Cefotaxime/clavulanic acid	=	0.25	=	0.5	S	R	0
26	EURL-M-5.4	2	Cefoxitin	=	16	=	8	R	S	0
26	EURL-M-5.4	2	Imipenem	=	0.25	=	2	S	R	0
26	EURL-M-5.8	2	Imipenem	=	0.5	=	4	S	R	0
26	EURL-M-5.4	2	Meropenem	=	0.12	=	1	S	R	0
26	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
33	EURL-M-5.4	1	Meropenem	=	0.12	=	1	S	R	0
33	EURL-M-5.4	2	Meropenem	=	0.12	=	1	S	R	0
34	EURL-M-5.4	2	Ceftazidime/clavulanic acid	=	0.25	=	0.25	R	S	0
34	EURL-M-5.1	2	Ertapenem	=	0.12	=	0.06	R	S	0
34	EURL-M-5.4	2	Imipenem	=	0.5	=	2	S	R	0
34	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0

36	EURL-M-5.4	2	Imipenem	=	0.5	=	2	S	R	0
36	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
37	EURL-M-5.1	1	Meropenem	=	0.25	<=	0.03	R	S	0
37	EURL-M-5.6	1	Tetracycline	=	8	>	64	S	R	0
37	EURL-M-5.1	2	Ertapenem	=	2	=	0.06	R	S	0
37	EURL-M-5.1	2	Meropenem	=	0.25	<=	0.03	R	S	0
39	EURL-M-5.6	1	Chloramphenicol	<=	8	>	128	S	R	0
39	EURL-M-5.8	1	Chloramphenicol	>	128	<=	8	R	S	0
39	EURL-M-5.6	1	Ciprofloxacin	=	0.06	>	8	S	R	0
39	EURL-M-5.8	1	Ciprofloxacin	>	8	=	0.06	R	S	0
39	EURL-M-5.6	1	Gentamicin	>	32	=	1	R	S	0
39	EURL-M-5.8	1	Gentamicin	=	1	>	32	S	R	0
39	EURL-M-5.6	1	Meropenem	>	16	<=	0.03	R	S	0
39	EURL-M-5.8	1	Meropenem	<=	0.03	=	16	S	R	0
39	EURL-M-5.6	1	Nalidixic acid	<=	4	>	128	S	R	0
39	EURL-M-5.8	1	Nalidixic acid	>	128	<=	4	R	S	0
39	EURL-M-5.7	1	Sulfamethoxazole	>	1024	<=	8	R	S	0
39	EURL-M-5.6	1	Tetracycline	=	4	>	64	S	R	0
39	EURL-M-5.8	1	Tetracycline	=	16	<=	2	R	S	0
39	EURL-M-5.6	1	Trimethoprim	=	0.5	>	32	S	R	0
39	EURL-M-5.8	1	Trimethoprim	>	32	<=	0.25	R	S	0
39	EURL-M-5.7	2	Cefepime	=	0.25	=	0.12	R	S	0
39	EURL-M-5.2	2	Cefotaxime/clavulanic acid	=	0.25	<=	0.06	R	S	0
39	EURL-M-5.6	2	Cefotaxime/clavulanic acid	>	64	=	0.12	R	S	0
39	EURL-M-5.8	2	Cefotaxime/clavulanic acid	=	0.12	>	64	S	R	0
39	EURL-M-5.8	2	Cefoxitin	=	8	>	64	S	R	0
39	EURL-M-5.6	2	Ceftazidime/clavulanic acid	>	128	=	0.25	R	S	0
39	EURL-M-5.8	2	Ceftazidime/clavulanic acid	=	0.5	>	128	S	R	0
39	EURL-M-5.1	2	Ertapenem	=	0.12	=	0.06	R	S	0
39	EURL-M-5.6	2	Ertapenem	>	2	=	0.06	R	S	0
39	EURL-M-5.6	2	Imipenem	=	4	<=	0.12	R	S	0
39	EURL-M-5.8	2	Imipenem	=	0.25	=	4	S	R	0
39	EURL-M-5.6	2	Meropenem	=	16	<=	0.03	R	S	0

39	EURL-M-5.8	2	Meropenem	<=	0.03	=	16	S	R	0
39	EURL-M-5.8	2	Temocillin	=	8	=	64	S	R	0
40	EURL-M-5.2	1	Chloramphenicol	=	128	<=	8	R	S	0
40	EURL-M-5.4	1	Chloramphenicol	<=	8	>	128	S	R	0
40	EURL-M-5.7	1	Chloramphenicol	=	32	<=	8	R	S	0
40	EURL-M-5.2	1	Ciprofloxacin	>	8	<=	0.015	R	S	0
40	EURL-M-5.2	1	Nalidixic acid	=	128	<=	4	R	S	0
40	EURL-M-5.2	1	Trimethoprim	>	32	=	0.5	R	S	0
40	EURL-M-5.2	2	Cefoxitin	=	32	=	4	R	S	0
40	EURL-M-5.4	2	Imipenem	=	0.25	=	2	S	R	0
40	EURL-M-5.8	2	Temocillin	=	16	=	64	S	R	0
42	EURL-M-5.4	2	Imipenem	=	0.5	=	2	S	R	0
45	EURL-M-5.1	2	Ertapenem	=	0.12	=	0.06	R	S	0
45	EURL-M-5.6	2	Ertapenem	=	0.25	=	0.06	R	S	0
58	EURL-M-5.7	2	Cefepime	=	0.25	=	0.12	R	S	0
58	EURL-M-5.8	2	Temocillin	=	32	=	64	S	R	0
59	EURL-M-5.1	1	Chloramphenicol	<=	8	>	128	S	R	0
59	EURL-M-5.1	1	Nalidixic acid	<=	4	>	128	S	R	0
59	EURL-M-5.7	1	Sulfamethoxazole	>	1024	<=	8	R	S	0
59	EURL-M-5.1	1	Tetracycline	<=	2	>	64	S	R	0
59	EURL-M-5.7	1	Tetracycline	>	64	<=	2	R	S	0
59	EURL-M-5.1	2	Cefotaxime/clavulanic acid	<=	0.06	=	4	S	R	0
59	EURL-M-5.1	2	Cefoxitin	=	4	=	64	S	R	0
59	EURL-M-5.1	2	Ceftazidime/clavulanic acid	<=	0.12	=	8	S	R	0

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