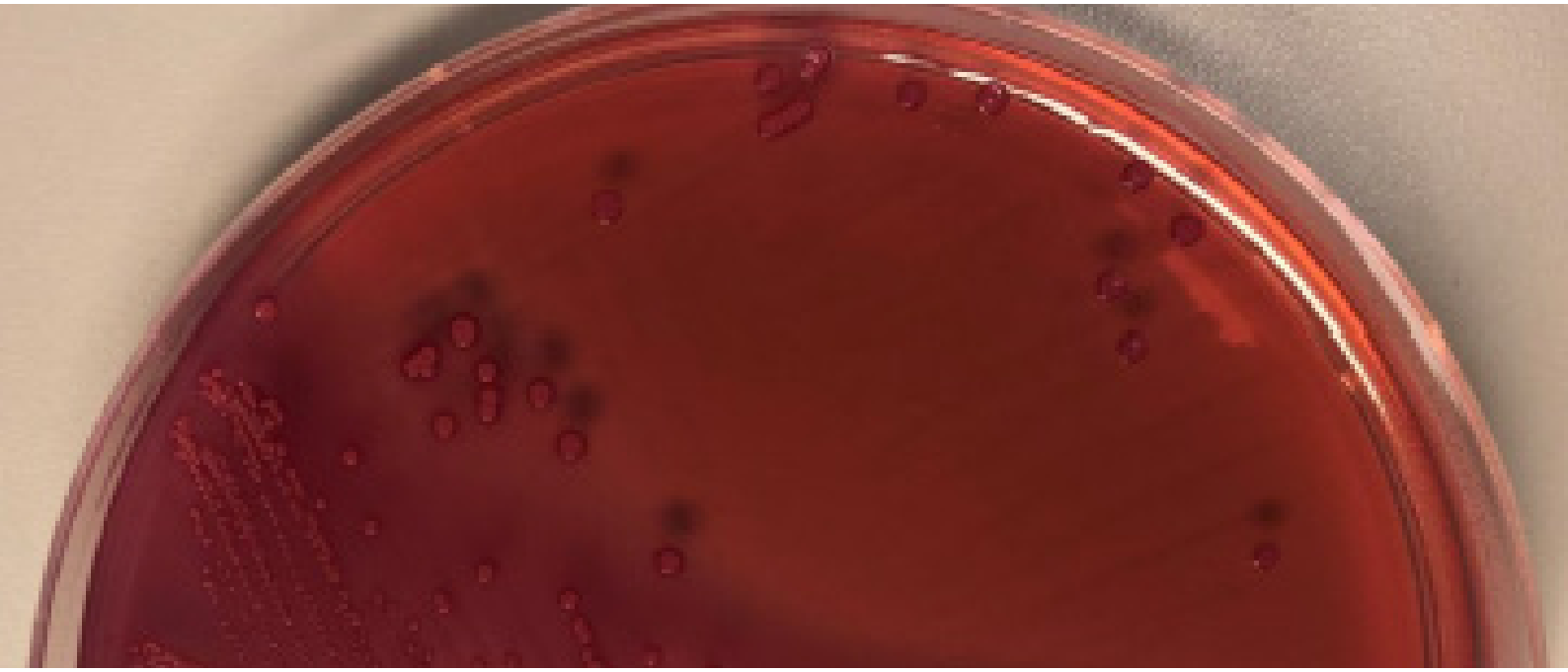
DTU National Food Institute

The 8th EURL-AR Proficiency Test on selective isolation of Escherichia coli with presumptive ESBL, AmpC or carbapenemase phenotypes from meat and caecal samples – 2022



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European Union Reference Laboratory –
Antimicrobial Resistance

The 8th EURL-AR Proficiency Test on selective isolation of Escherichia coli with presumptive ESBL, AmpC or carbapenemase phenotypes from meat and caecal samples – 2022

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1. Introduction

This report describes and summarises results from the eighth 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), AmpC and carbapenemase-producing *E. coli* from meat and caecal samples of animal origin and antimicrobial susceptibility testing (AST) of the isolated *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, 2019). In 2016, the EQAS was extended to include carbapenemase and *bla*$_{\text{OXA-48}}$-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, 2019). This was made mandatory from 2021 with the decision (2020/1729/EU).

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.

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.

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 level 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 eighth matrix EQAS was organized by the EURL-AR at the National Food Institute (DTU Food), Kgs. Lyngby, Denmark. Preliminary results were presented in slides available on the EURL-AR website prior to the annual workshop 2023 (held as a virtual meeting 23-24 May, 2023). The draft report was sent to the EURL-AR network for commenting in June 2023, and was hereafter approved with small editions.

2. Materials and Methods

2.1 Participants in EQAS 2022

A pre-notification (App. 1), announcing the matrix EQAS 2022, was distributed on 3 October 2022 by e-mail to the designated NRLs including all EU countries and Iceland, Norway, Switzerland and United Kingdom. In total, 37 laboratories participated in the matrix EQAS (App. 2) involving one NRL from the 27 EU countries and Iceland, Norway, Switzerland and United Kingdom. Four countries (Ireland, the Netherlands, Spain and Malta) had additional laboratories participating in the EQAS. 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. Two countries (Romania and Spain) had separate laboratories enrolled for handling meat and caecal samples and had two NRLs enrolled. Therefore, in total, 33 laboratory results from 31 countries are described. Participants from non-EU member states and additional laboratories were charged a fee for participation, whereas participation was free of charge for EU member states but each laboratory was expected to cover expenses associated with the analyses.

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 chicken meat and three chicken caeca samples which were either prepared by spiking with test strains or left unmodified.

The sample meat was minced chicken meat of Danish origin (raised, slaughtered and packed in Denmark) acquired in local supermarkets, and four different batches (based on production date and slaughterhouse) were bought in sufficient amount for covering both the pre-tests and preparation of the samples). The meat was pretested using the official method for selective isolation of *E. coli*-producing ESBL, AmpC or carbapenemases, to ensure that the batch used was negative for those,

and to test for the amount of conflicting background bacteria. A batch fulfilling these criteria was chosen for preparation of aliquots of 25 g of meat that were spiked as follows.

The test isolates used in the spiking of meat samples within the matrix EQA 2022 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. The final inoculum found in the samples in this EQA was expected to be approx. 10^3 CFU/g meat for samples EURL-M-8.1, M-8.2, M-8.3 and M-8.4. Sample EURL-M-8.5 was spiked as mentioned above but with a susceptible *E. coli* strain (ATCC 25922) and therefore expected to be negative for ESBL, AmpC or carbapenemase-producing *E. coli*.

One slaughterhouse provided on 26 October (2022) 40 chicken caecal samples from each of four different flocks. These samples were pooled per flock and tested using the official selective isolation protocol for ESBL, AmpC and carbapenemase-producing *E. coli*. One ESBL-negative caeca batch was chosen for preparation of the matrix caecal samples for the test strains. Thereby 1 g aliquots of pooled caecal content were used directly as blank sample or 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-8.7, and M-8.8. The sample M-8.6 was not inoculated and was expected to be negative. Selecting strains initially isolated from chicken caecal samples is expected to enhance the survival of the inoculum, as the sample matrix otherwise have a very poor support of the *E. coli* inoculum.

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 caeca 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 2020/1729/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 2020/1729/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 laboratory ID number and a labelled envelope for returning the temperature logger to the EURL-AR.

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 thermo 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 7 November 2022.

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 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). The database was delayed due to unexpected events hindering the final development and opening of the web tool. Eventually, the web tool was activated on 18 January 2023 and closed 8 February 2023.

For the first part of the results of the selective isolation procedure for ESBL/AmpC and carbapenemase producing *E. coli*, 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 (EUVSEC3 and EUVSEC2; Table 1) 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.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2022 component

Escherichia coli **EUVSEC3**	***Escherichia coli*** **EUVSEC2**
Ampicillin, AMP	Cefepime, FEP
Amikacin, AMK	Cefotaxime + clavulanic acid (F/C)
Azithromycin, AZI	Cefotaxime, FOT
Cefotaxime, FOT	Cefoxitin, FOX
Ceftazidime, TAZ	Ceftazidime, TAZ
Chloramphenicol, CHL	Ceftazidime+ clavulanic acid (T/C)
Ciprofloxacin, CIP	Ertapenem, ETP
Colistin, COL	Imipenem, IMI
Gentamicin, GEN	Meropenem, MERO
Meropenem, MERO	Temocillin, TRM
Nalidixic acid, NAL	
Sulfamethoxazole, SMX	
Tetracycline, TET	
Tigecycline, TGC	

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 from the returned temperature loggers to provide the temperature ranges along the shipment and at sample reception/opening. All samples were expected to be in good conditions for testing at the time of opening the parcels.

3.1 Overall results of the selective isolation

The number of possible and evaluated test results for ESBL/AmpC qualitative isolation considered for this report was 248 tests, from eight samples for each of the 31 countries. These results are summarized in Figure 1 and further discussed in section 3.4. There was only one instance where an isolate was not recovered, as Lab #040 could not recover the isolate from sample M-8.2.

3.2 Methods used by EQAS-participants

In this trial, 29 participating NRL's reported results for all the eight samples. Two laboratories reported only results for the meat samples and two laboratories reported only results for the caecal samples. All 33 participating laboratories, which have submitted results, participated in the ESBL, AmpC and carbapenemase-producer isolation and performed the identification and susceptibility testing of the respective isolates. The number of qualitative isolation test results reported was variable, including results for three to eight samples, depending on how many samples were tested, and for the antimicrobial susceptibility tests it depended on how many isolates were isolated 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.

All laboratories reported that the selective isolation was performed according to the exact procedures described in the provided protocols, except one lab (#036) which reported that the pre-enrichment step was modified (1.7 g of caecal sample was used instead). Additionally, three labs (#012, #039 and #036) reported that they did not follow the EURL recommended protocol for isolation of carbapenem-producers, but only lab #012 stated that MacConkey was not used to avoid plasmid loss and instead all isolates were confirmed with MALDI-TOF directly from blood agar.

The species identification was performed using MALDI TOF (n=15), biochemical tests (n=8), chromogenic media (n=7) or PCR using published methods to confirm the ID (n=3). Additionally, five laboratories (#011, #018, #020, #037, #033) reported that second and third identification methods were used as supplements. From those labs that reported on the brand/plates used for selective isolation of carbapenem producers (n=15), CHROMID CARBA SMART Agar (n=4), CHROMID CARBA Agar (n=7), CHROMID OXA-48 (n=6) and CHROMagar mSuperCARBA (n=1) were used. Three laboratories did not specify the type of the plates, only the brand (BioMérieux).

The broth microdilution testing was performed using the antimicrobials and ranges defined under the EU Commission regulation 1729/2020 for testing the isolated and identified *E. coli* isolates using panel 1 (EUVSEC3). Additional AST of the presumptive ESBL/AmpC and/or carbapenemase isolates was performed using panel 2 (EUVSEC2), if relevant, and interpretation of the results was made according to the EFSA criteria for ESBL/AmpC and carbapenemase phenotypic classification.

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

The total amount of test results was 186 tests for the ESBL, AmpC and carbapenemase isolation qualitative results. Of them, 179 (96.2%) tests were obtained in concordance with the original strains correctly assigned ESBL, AmpC or carbapenemase phenotype (Table 2). All 62 samples expected to be negative (blank and susceptible) were correctly assigned. Regarding the 186 samples expected to be positive, all but seven (3.8%) were correctly assigned. Lab #040 could not recover the isolate from sample M-8.2.

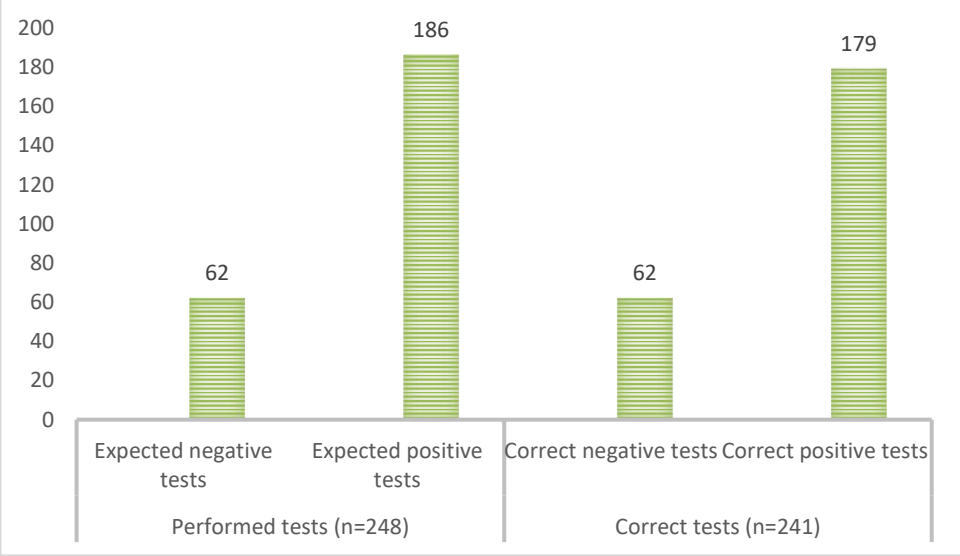

Figure 1. Overall performance of ESBL/AmpC/Carbapenemase isolation and identification, 2022

For the AmpC and ESBL+AmpC isolates (containing the *bla*$_{CMY-2}$ gene) spiked in samples M-8.1, M-8.4 and M-8.8, four labs (#017, #033, #004 and #042) observed in addition ertapenem resistance and hence reported it as "Other phenotype", which was according to the EFSA guidelines, and which is often observed in *bla*$_{CMY-2}$ -carrying isolates.

Table 2 Deviations in ESBL /AmpC and carbapenemase phenotype identification, 2022

Strain ID	Expected phenotype	*bla*-gene(s)	Deviations, %	Additional phenotype approved	Deviations (%) after changing phenotype
M-8.1	ESBL+AmpC	CMY-2, SHV-12	6.5	AmpC*	3.2
M-8.2	ESBL	CTM-X-14	6.5	None	6.5
M-8.3	Carbapenemase	OXA-48, CTX-M-27	0	None	0
M-8.4	AmpC	CMY-2	25.8	Other phenotype*	9.7
M-8.7	Carbapenemase	VIM-1	6.5	None	3.2
M-8.8	AmpC	CMY-2	3.2	Other phenotype*	0

*According to the EFSA guidelines for reporting antimicrobial resistance, AmpC + ETP resistance is reported as "Other phenotype" and approved in this report as an Other phenotype.

The analysis of deviations in ESBL/AmpC and carbapenemase phenotype categorisation per matrix sample indicates that the highest levels of deviations were observed for sample M-8.4 (9.7%; meat) due to the report of meropenem resistance in an originally expected AmpC isolate. Samples M-8.3 and M-8.8 had no deviations from the original phenotypes and for other samples the deviations ranged between 3.2 and 9.7% (Table 2).

3.4 Antimicrobial susceptibility testing

A total of 5,040 AST results were submitted. The 33 laboratories uploaded a variable number of results, depending on the samples found positive, isolates tested in one or both panels and isolates that could not be recovered, and ranged from 50 to 150 test results per participant. The deviation percentages per laboratory ranged from 0% to 10.4%. However, when the one-step dilution difference at the breakpoint was not considered, the deviations per laboratory decreased to 9.7% (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. Thus, in the analysis of MIC results, it was evident that both cefepime one two-fold dilution variations but also R/S interpreation gave the highest contribution to the deviations (15.3 % deviations for cefepime). Whereas the test strain cefepime MIC being close to the breakpoint is a common problem, this year it gave an additional disadvantage that the EUCAST ECOFF for cefepime was changed between the publication of the protocol, describing the interpretation criteria for this EQA (App. 4) and the EQA result submission date (cefepime ECOFF 0.25 changed 27-01-2023; eucast.org). As this seem to have caused a unusuallly high amount of deviations, the R/S interpretation for cefepime has been blanked for all samples. The main volume of issues occurred for samples M-8.4 and M-8.8. By blanking the cefepime results, a reduced number of 4,400 AST results were considered for this report.

A list of deviations in ESBL phenotype interpretations and AST results is available in App. 7.

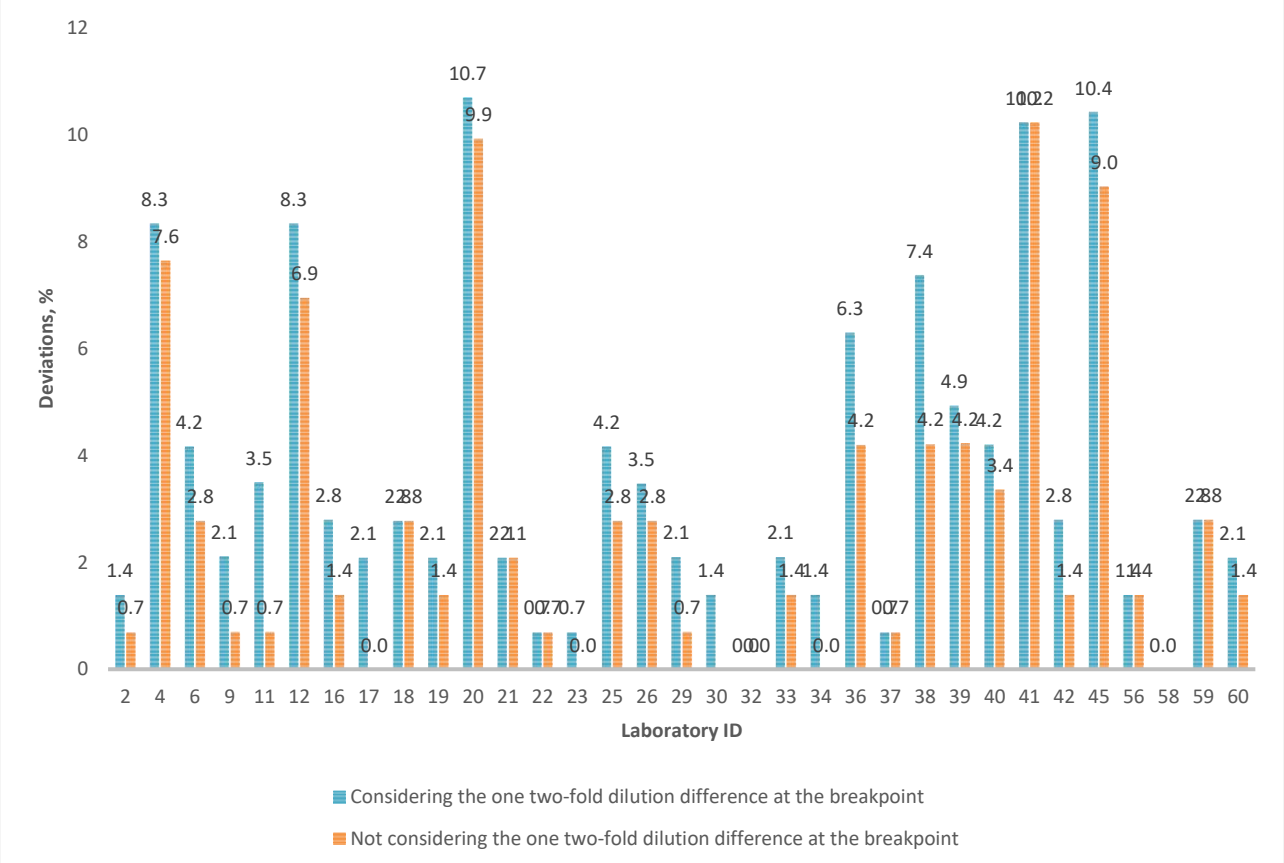

Figure 2. Percentages of deviations in AST results per participating laboratory in the EQAS Matrix 2022

In the analysis of deviations per antimicrobial, the highest deviation percentage was for nalidixic acid (12.7%), imipenem (12%), chloramphenicol (10.3%) and ceftazidime (2.7%) (Figure 3). However, if we do not consider the one two-fold dilution differences, which are accepted in the MIC method, the percentage deviations decrease to 8.8%, 6.3%, 2.7% and 1.6%, respectively, all the remaining deviations caused by more than one two-fold dilution MIC difference. For some antimicrobials (e.g., tetracycline, sulfamethoxazole, nalidixic acid, ciprofloxacin), the deviations from the expected MIC values were caused by more than one two-fold dilution difference which completely changed the phenotype and could be explained by either acquisition of plasmids carrying genes for resistance to these antimicrobials or through isolation of background bacteria or contaminated isolates.

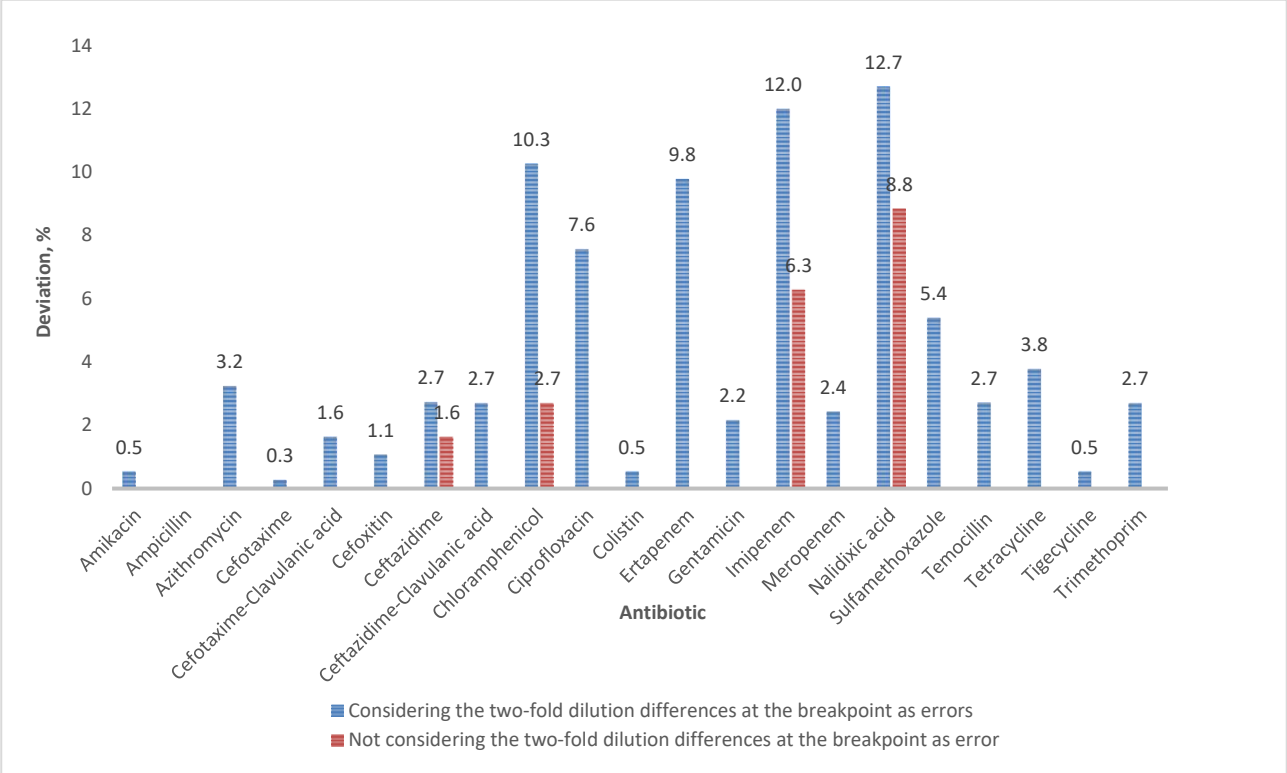

Figure 3. Percentages of deviations in AST results per antimicrobial in the Matrix EQAS 2022

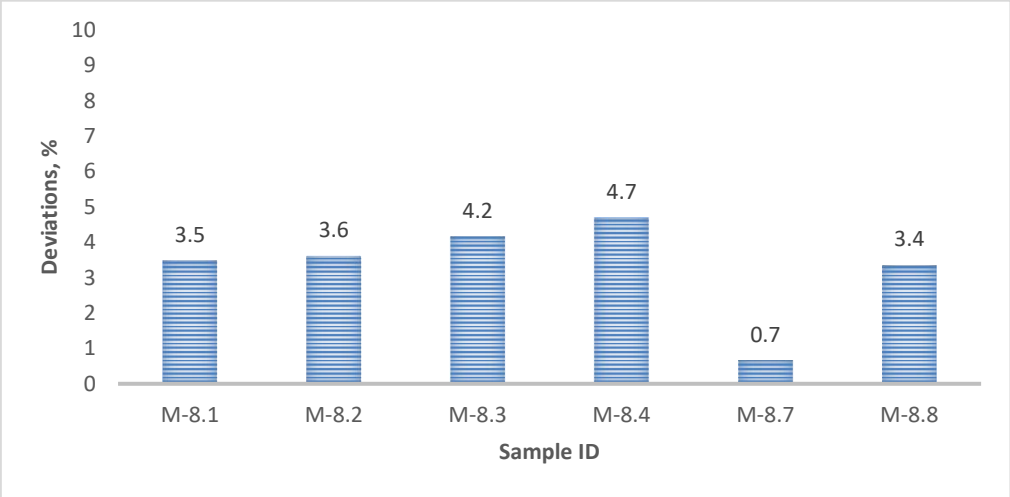

Figure 4. Percentage of deviations in MIC results per sample in the Matrix EQAS 2022

When deviations in the MIC interpretations per sample were considered (Figure 4), the deviations were widely distributed and all samples have deviations of less than 5%, with least deviations found for the caecal samples. These results are excluding the cefepime deviations, and thereby relating to 24 antimicrobials and 31 datasets (except that 30 datasets were used for sample 8.2 as lab #040 could not recover it).

3.5 ESBL/AmpC and carbapenemase phenotypic testing conclusions

Five chicken meat samples (M-8.1 – M-8.5) were included in this matrix EQAS. Sample M-8.1 contained an isolate expressing ESBL+ AmpC phenotype due to the presence of both *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{SHV-12}}$ genes; sample M-8.2 was inoculated with a strain carrying the ESBL gene *bla*$_{\text{CTX-M-14}}$, whereas M-8.3 had carbapenemase phenotype due to the presence of *bla*$_{\text{OXA-48}}$ and additionally *bla*$_{\text{CTX-M-27}}$. Sample M-8.4 was spiked with a *bla*$_{\text{CMY-2}}$ -carrying *E. coli* (AmpC phenotype) and 8.5 was inoculated with the susceptible *E. coli* ATCC 29522 strain (Table 2).

Three chicken caeca samples were included in this matrix EQAS (M-8.6 – M-8.8). One of the three caecal samples (M-8.6) was not spiked (blank), while samples M-8.7 and M-8.8 contained isolates that express a carbapenemase phenotype mediated by VIM-1 and an AmpC phenotype due to the presence of *bla*$_{\text{CMY-2}}$, respectively.

Overall, there were almost no discrepancies in differentiating between ESBL and AmpC phenotypes. Lab #019 reported isolation of an *E. coli* isolate from sample M-8.2 with an ESBL + AmpC phenotype instead of an ESBL phenotype. More discrepancies were observed when reporting carbapenem resistant isolates of expected AmpC, ESBL and ESBL+AmpC isolates. Lab #004 isolated a carbapenem resistant strain from sample M-8.1 with MERO MIC = 0.12 mg/L and 0.25 mg/L for Panel 1 and 2, respectively, and ETP MIC = 1 mg/L. The strain also showed resistance to azithromycin, chloramphenicol, nalidixic acid, sulfamethoxazole, tetracycline, trimethoprim, temocillin, for which reason the phenotype could be due to an acquisition of a plasmid(s) carrying genes for resistance to all of the above antibiotics. Also, the possibility of isolation of, or contamination by, background bacteria cannot be ruled out. Similarly, three laboratories (#012, #020

and #041) recovered isolates from sample M-8.4 with resistance to meropenem, which also showed altered phenotype with resistance to other antibiotics with very similar resistance profiles. The same issue was the case for lab #045, which recovered a carbapenem resistant isolate from sample M-8.2, with a completely different resistance phenotype. These strains are causing a high proportion of the total deviations, but still the incidences seem sporadic. The EURL-AR will initiated a follow-up on these isolates during the period of commenting in June 2023, and invited laboratories to retest their MIC and *E. coli* species confirmation for specific isolates, to elucidate this problem.

4. Discussion

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

The 2022 EURL-AR Matrix EQAS trial was the eighth of its kind on samples of animal origin, since the first round of this EQAS in 2015. All laboratories recovered presumptive isolates from the caecal samples and only one laboratory (#040) could not recover one isolate from a meat sample (M-8.2). Additionally, in this round, five laboratories recovered carbapenem resistant isolates instead of ESBL+AmpC (M-8.1, lab #004) and AmpC (M-8.4, labs #020, #012, #041 and #039). The EURL-AR laboratory recorded findings of high level of background bacteria in meat sample M-8.2 (identified as *Aeromonas veronii* (Figure 5) by MALDI-TOF) and in caecal sample M-8.8 (identified as *Escherichia fergusonii* by MALDI-TOF), after refrigerated storage of the samples at the EURL-AR in the first days and weeks after shipment of samples. As all the meat and caecal samples are prepared from the same batch of meat and from caeca samples pooled from the same farm, this effect could apply to a proportion of samples with any sample number. The unusually high level of competing bacteria, growing on the selective agar plates, might be one of the reasons for observing other phenotypes than the expected. Additionally, it is expected to see deviations in the resistance phenotypes, and possibly even in the ESBL categorisations, due to sporadic interactions with bacteria and/or mobile genetic elements in the microbiota of the matrix samples, and these cannot be accounted for, as they are not necessarily detected in the tests performed by the EURL-AR after the preparation and shipment of samples. As the screening of matrix material only serves to reveal possible ESBL/AmpC/carbapenemase 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 portions of the meat matrix.

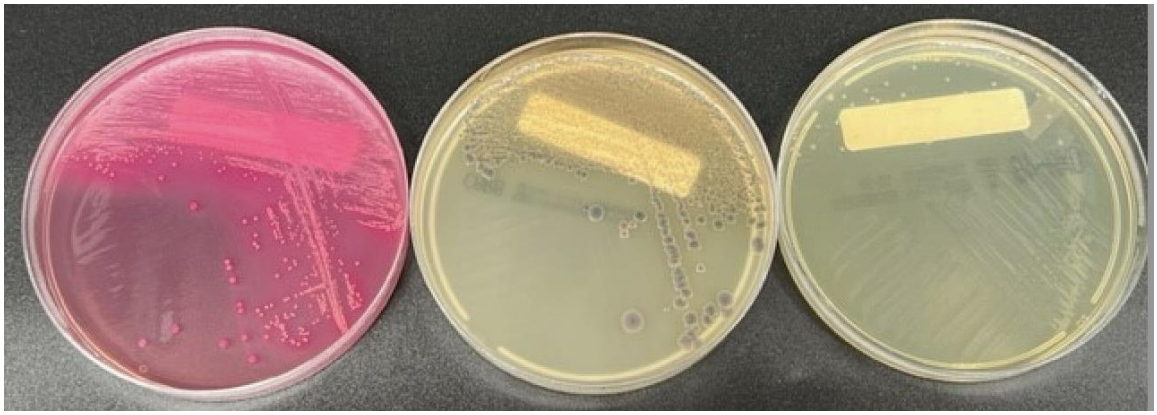

Figure 5. Selective isolation from sample M-8.2 plated on MacConkey+CTX, and CHROMID CARBA (with non-*E. coli* background bacteria – purple instead of mauve) and OXA-48 plates at the EURL-AR

4.2 Antimicrobial susceptibility testing

In the 2022 iteration of the matrix EQAS, only 2 laboratories had no deviations in their AST results compared to EQAS 2021 (n=11) and EQAS 2020 (n=15). The AST deviations were caused by i) one two-fold dilution difference, which is allowed for the broth microdilution method, around the breakpoint, which changes the phenotype interpretation, and is not necessarily due to acquisition of genes, and ii) more than one two-fold dilution difference which is either caused by true errors or by variation in MIC values caused by either gene acquisition (especially to certain antimicrobials) or recovery/contamination of contaminating isolates present in the matrix. As it can be difficult to select test strains with clear phenotypes for all antibiotics and expected to survive in the matrix, this issue gave rise to the majority of AST deviations, but especially for cefepime, imipenem, chloramphenicol and nalidixic acid in this year's set of samples. Some deviations came also from a wrong interpretation of the phenotype based on the obtained MIC values, which could be avoided in relation to data handling. Finally, the use of different ECOFFs for cefepime caused additional deviations, which caused that cefepime were blanked from scoring.

Thus, the challenges seen in this and previous matrix EQAS are not unexpected, as working with isolates in a matrix is likely to cause problems, such as retrieving the inoculated isolates from the samples, changes in the sample composition during enrichment which might favour isolation of one over other strain, or changes in the isolate characteristics (plasmid exchanges).

4.3 ESBL /AmpC and carbapenemase phenotypic testing conclusions

Due to the reasons mentioned above, the phenotypic interpretation of the isolated strains is not straightforward. In relation to the ESBL classification, this EQAS is dependent on the EFSA Criteria for interpretation of *Escherichia coli* (panel 2 results; see protocol), and an issue in this year's Matrix EQAS was the ertapenem resistance in *bla*$_{\text{CMY-2}}$ carrying AmpC-type *E. coli* (M-8.1, M-8.4 and M-8.8) which had an elevated MIC to ertapenem, but not to meropenem. Following the EFSA classification, the strain should be considered 'Other phenotype' if meropenem ≤ 0.12 mg/L but ertapenem (or imipenem) is above the ECOFF. It is a well-known issue for *bla*$_{\text{CMY-2}}$ isolates and therefore it is generally accepted to classify this phenotype as 'AmpC-type', although 'Other phenotype' is expected and also accepted.

4.4 Performance in AST of the quality control strains

Antimicrobial susceptibility test results for the *Escherichia coli* ATCC 25922 quality control strain were evaluated based on the CLSI quality control ranges (App. 6). For a second year, the *E. coli* ATCC 25922 QC strain was included in the reporting of the EQAS results. All 33 participating labs tested *E. coli* ATCC 25922 by MIC determination and reported a total of 865 out of 900 test results. Of the reported results, 99.4% were within the acceptable range (App. 6). The five deviations were reported for trimethoprim, tigecycline, cefoxitin and sulfamethoxazole by labs #017, #029, #039, #042 and #061. Additionally, Labs #026 and #037 did not provide data for Panel 2 and Lab #029 for antimicrobials from Panel 1 and 2.

5. Conclusion

The Matrix EQAS 2022 report demonstrates that most participating labs have well established methods to isolate ESBL/ AmpC and carbapenemase-carrying strains from meat or caecal samples, and in only one instance, one meat isolate was not recovered. Due to the difficult nature of the matrices and the background microflora, apparently not all laboratories were recovering the original spiked isolates. High levels of background microflora (*Aeromonas veronii* and *Escherichia fergusonii*) was observed in some samples after storage at the EURL-AR, of which the first type grew well on selective carbapenem agar. This might have caused problems with isolating the inoculated *E. coli* for some laboratories, especially if they are not using the specific chromogenic agar type as routine. Otherwise, exchange of genetic material, including resistance determinants, between the bacteria in the matrix could have impacted the phenotypic outcome. Additionally, ertapenem non-susceptibility was observed in several cases, which changed the interpretation of 'AmpC phenotype' to "Other phenotypes".

The EURL-AR will follow up on the unexpected carbapenem resistant strains isolated from meat samples, to elucidate this problem further. The susceptibility testing results were in general very satisfactory, with exception of one two-fold dilution differences at the breakpoint which changed the interpretation and more than one two-fold dilution differences that could be derived from genes acquisition. Also, an issue occurred by participants using the updated EUCAST ECOFFS, instead of those listed in the EQAS protocol, which are incorporated in the web-tool, and this caused additional deviations, leading to a subset of scores being blanked.

As part of the follow-up, some participants reported the high level of background bacteria, and that these were identified (by MALDI-TOF) as *Aeromonas veronii* and *Escherichia fergusonii* and additionally *A. bestiarum.* It was also reported that they obtained several strains on the ESBL or carbapenemase selective agar from the same matrix sample, which differed in the overall resistance profile. The participants are expected to report the phenotype of the carbapenemase producing strains in these cases, which, together with the interaction with carbapenem resistant background bacteria can explain the high number of unexpected carbapenemase producers.

6. References

Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU (relevant as of 01.01.2021).

7. Appendices

Appendix 1. Pre-notification EURL-AR EQAS matrix 2022
Appendix 2. List of participants
Appendix 3. Test strains and reference values (MIC in mg/L)
Appendix 4. Protocol EQAS matrix 2022
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EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2022

DTU Food
National Food Institute

EURL-AR EQAS pre-notification

G00-06-001/26.10.2020

EQAS 2022 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 another 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*. Additionally, quality control (QC) strains *E. coli* ATCC 25922 and *A. baumannii* 2012-70-100-69 will be included.

This EQAS is targeted NRL's on antimicrobial resistance involved in the monitoring according to the EU Commission decision 2020/1729 and specifically processing meat and/or caecal samples in the specific monitoring for ESBL. You may contact the EQAS-Coordinator if you wish to inform of changes in relation to your level of participation in compared to previous years.

Participation is free of charge for all above-mentioned designated laboratories. The invitation to participate in the proficiency test is extended to additional participants besides official NRLs and to participants from laboratories which are involved in the network but are not designated NRLs (cost for participation will be 150 EUR).

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 chicken meat and/or chicken 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 the second week of November 2022. The protocol for this proficiency test will be available for download from the website (https://www.eurl-ar.eu/protocols.aspx).

Submission of results: Results must be submitted to the National Food Institute **no later than 16 January 2023** via the password-protected webtool. 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.

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

Sincerely,

Jette Kjeldgaard, EURL-AR EQAS-Coordinator

Appendix 2 - 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, NRL	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)	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	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	Public Health Laboratory/National Veterinary Laboratory *	Malta
x	x	Wageningen Bioveterinary Research (WBVR)	Netherlands
x	x	The Netherlands Food and Consumer Product Safety Authority *	Netherlands
x	x	Veterinærinstituttet	Norway
x	x	National Veterinary Research Institute	Poland
x	x	Instituto Nacional de Investigação Agrária e Veterinária	Portugal
x		Institute for Hygiene and Veterinary Public Health	Romania
	x	Institute for Diagnosis and Animal Health	Romania
x	x	State Veterinary and Food Institute (SVFI)	Slovakia
x	x	National Veterinary Institute	Slovenia
x		Centro Nacional de Alimentación (AECOSAN)	Spain
x	x	Foodborne Zoonoses and Antimicrobial Resistance Unit *	Spain
	x	Laboratorio Central de Veterinaria	Spain
x	x	National Veterinary Institute, SVA	Sweden
x	x	Institute of Veterinary Bacteriology, Vetsuisse Faculty Bern	Switzerland
x	x	Animal & Plant Health Agency (APHA)	United Kingdom

Designated NRL-AR by the compentent authority of the member state

Non-NRL-AR enrolled by the EURL-AR

Non-MS NRL-AR

* Submitted results were not included in the current report (one dataset per country, only)

Appendix 3 Test strain reference values 1/2

Panel 1 =EUVSEC3

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Gene	Prediction
EURL-M 8.1	>32	4	≤4	1	≤ 0.25	>8	>4	≤1	8	≤2	0.5	≤8	≤8	≤0.03	0.5	CMY-2, SHV-12	ESBL + AmpC OR AmpC
EURL-M 8.2	>32	4	≤4	≤0.5	≤ 0.25	1	>4	≤1	>64	≤2	>16	>512	>64	≤0.03	8	CTM-X-14	ESBL
EURL-M 8.3	>32	>64	≤4	≤ 0.5	≤ 0.25	>8	>4	≤1	≤4	>32	>16	>512	16	0.5	0.5	OXA-48, CTX-M-27,	Carbapenemase
EURL-M 8.4	>32	4	≤4	≤ 0.5	≤ 0.25	>8	>4	≤1	>64	≤2	≤0.25	≤8	≤8	≤0.03	4	CMY-2	AmpC*
EURL-M 8.5																	Susceptible
EURL-M 8.6																	
EURL-M 8.7	>32	>64	8	2	≤ 0.25	>8	>4	≤1	>64	>32	>16	>512	32	2	>8	VIM-1	Carbapenemase
EURL-M 8.8	>32	8	≤ 4	≤0.5	≤ 0.25	> 8	> 4	≤ 1	≤4	> 32	> 16	> 512	> 64	≤ 0.03	0.03	CMY-2	AmpC*

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP
EURL-M 8.1	R	S	S	S	S	R	R	S	S	S	S	S	S	S	R
EURL-M 8.2	R	S	S	S	S	R	R	S	R	S	R	R	R	S	R
EURL-M 8.3	R	R	S	S	S	R	R	S	S	R	R	R	S	R	R
EURL-M 8.4	R	S	S	S	S	R	R	S	R	S	S	S	S	S	R
EURL-M 8.5															
EURL-M 8.6															
EURL-M 8.7	R	R	S	S	S	R	R	S	R	R	R	R	R	R	R
EURL-M 8.8	R	S	S	S	S	R	R	S	S	R	R	R	R	S	S

* For sample 8.4 and 8.8 'Other phenotype' is additionally accepted

Appendix 3 Test strain reference values 2/2

Panel 2=EUVSEC2

Strain	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	ESBL conc phenptypic	Gene
EURL-M 8.1	64	16	0.03	0.25	≤0.03	32	0.5	8	8	8	ESBL+AmpC or AmpC	CMY-2, SHV-12
EURL-M 8.2	8	16	≤0.015	0.25	≤0.03	1	4	≤0.06	0.25	8	ESBL	CTX-M-14
EURL-M 8.3	>64	>64	2	1	0.5	16	16	4	8	>128	Carbapenemase	OXA-48, CTX-M-27
EURL-M 8.4	>64	16	0.12	≤0.12	≤0.03	16	0.5	8	8	8	AmpC*	CMY-2
EURL-M 8.5											Susceptible	
EURL-M 8.6											Blank	
EURL-M 8.7	> 64	> 64	0.5	4	2	>128	32	64	>128	128	Carbapenemase	VIM-1
EURL-M 8.8	> 64	8	0.03	0.25	≤ 0.03	16	0.25	8	16	8	AmpC*	CMY-2

Strain	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	ESBL conclusion	Gene
EURL-M 8.1	R	R	S	S	S	R	R	R	R	S	ESBL+AmpC or AmpC	CMY-2, SHV-12
EURL-M 8.2	S	R	S	S	S	R	R	S	S	S	ESBL	CTX-M-14
EURL-M 8.3	R	R	R	R	R	R	R	R	R	R	Carbapenemase	OXA-48, CTX-M-27
EURL-M 8.4	R	R	R	S	S	R	R	R	R	S	AmpC*	CMY-2
EURL-M 8.5											Susceptible	
EURL-M 8.6											Blank	
EURL-M 8.7	R	R	R	R	R	R	R	R	R	R	Carbapenemase	VIM-1
EURL-M 8.8	R	R	S	S	S	R	S	R	R	S	AmpC*	CMY-2

* For sample 8.4 and 8.8 'Other phenotype' is additionally accepted

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2022

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 2022, these eight samples will include five 25-g samples of chicken meat and three 1-g samples of chicken 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 2020/1729/EU, 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 MATRIX EQAS 2022

3.1 Shipping, receipt and storage of samples

In November 2022, 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 chicken meat or pooled chicken caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 8.1 to 8.8. Hereof 8.1 to 8.5 being samples of meat (each 25 g) and 8.6 to 8.8 being samples of caecal content (each 1 g).

The matrix samples will be shipped on November 7th in frozen/chilled state in separate tubes and contained in a cooling box with a temperature logging device and freezing 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 from the parcel (small discoid device located in a bag inserted in a labelled tube);** 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 QC reference strains

Include the *E. coli* ATCC25922 and *Acinetobacter baumannii* (2012-70-100-69) reference strains in the MIC testing, and report results of these together with the isolates obtained from the EQAS samples. Note that, for the testing of the *E. coli* ATCC25922 reference strain, the two compounds, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole.

3.3 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. **Results should be produced according to the laboratory's routine procedures for antimicrobial susceptibility testing by MIC determination.** All the following procedures should follow the methods used in the monitoring for ESBL and AmpC *E. coli* according to the 2020/1729/EU Decision. 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 https://www.eurl-ar.eu/protocols.aspx).

According to the 2020/1729/EU Decision, **the monitoring of carbapenemase-producing *E. coli* from the samples is now mandatory**, and should be performed 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 8.1 to 8.5
- Follow the protocol for caecal content when testing samples 8.6 to 8.8

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-, AmpC-, and carbapenemase 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).

3.4 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 a presumptive carbapenemase positive *E. coli* isolate was detected.
- An ESBL- or AmpC-presumptive isolate (if you do not have a carbapenemase positive isolate) 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 Decision 2020/1729/EU (using the two-step approach, i.e. both testing panels) and applying the interpretative criteria listed below.

Table 1: Panel 1 antimicrobials recommended for AST of *E. coli* spp. and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2022) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	8
Ampicillin (AMP)	8
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.25
Ceftazidime (TAZ or CAZ)	0.5
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.064
Colistin (COL)	2
Gentamicin (GEN)	2
Meropenem (MERO or MEM)	0.06
Nalidixic acid (NAL)	8
Sulfonamides (SMX)	64*
Tetracycline (TET)	8
Tigecycline (TGC)	0.5
Trimethoprim (TMP)	2

* EFSA Technical Report (doi: 10.2903/sp.efsa.2021.EN-6652)

Beta-lactam resistance

Confirmatory testing for ESBL and carbapenemase 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: Panel 2 antimicrobials recommended for AST of *E. coli* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2022)

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.25
Cefotaxime (FOT or CTX)	0.25
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.25
Cefoxitin (FOX)	8
Ceftazidime (TAZ or CAZ)	0.5
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	0.5
Ertapenem (ETP)	0.03
Imipenem (IMI)	0.5
Meropenem (MERO or MEM)	0.06
Temocillin (TRM)	16

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 a 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 (See the appendix to this protocol). Importantly: Note that for *E. coli*, two cut-off values apply for cefotaxime and ceftazidime: the EUCAST cut-off values, those that define R/S (see Tables 1 and 2), and the screening cut-off values (cefotaxime >1 and ceftazidime >1) which are those applied to categorise bacterial phenotypes as ESBL, AmpC, carbapenemase, etc., based on panel 2 results (see Appendix). Likewise this is the situation for the *E.coli* meropenem cut-off values/screening cut-off value.

4 REPORTING OF RESULTS AND EVALUATION

Test forms are available for recording your results before you enter them into the web tool.

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 16th January, 2023.** 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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5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

The 'guideline for submission of results via webtool' is available for download directly from the EURL-AR website (https://www.eurl-ar.eu/eqas.aspx).

Access the webtool using this address: https://amr-eqas.dtu.dk. Please follow the guideline carefully and **remember to access the webtool via an 'incognito' website**.

When you submit your results, remember to have by your side the completed test forms.

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting your input please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once!** 'Final submit' blocks data entry.

⇨ About login to the webtool:

When first given access to login to the webtool, your **personal** loginID and password were sent to you by email. This is relevant for two email addresses connected to each NRL-AR (the EURL-AR defined a primary and a secondary contact).
Note that:

a) If the EURL-AR has only one contact person for an NRL, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact jetk@food.dtu.dk.
b) If your laboratory has two or more contact points on the EURL-AR contact list, two have been defined as the primary and secondary contact. Should you like to make changes to the primary and secondary contact or should you like more than the two persons to be able to access the webtool, please contact jetk@food.dtu.dk.

All participants registered with an account in the submission webtool will receive a separate email presenting the relevant personal username and password. The email will be sent by the time when the webtool has gone through internal quality control and has been approved for user access. The EQAS Coordinator will let all participants know when to look out for it.

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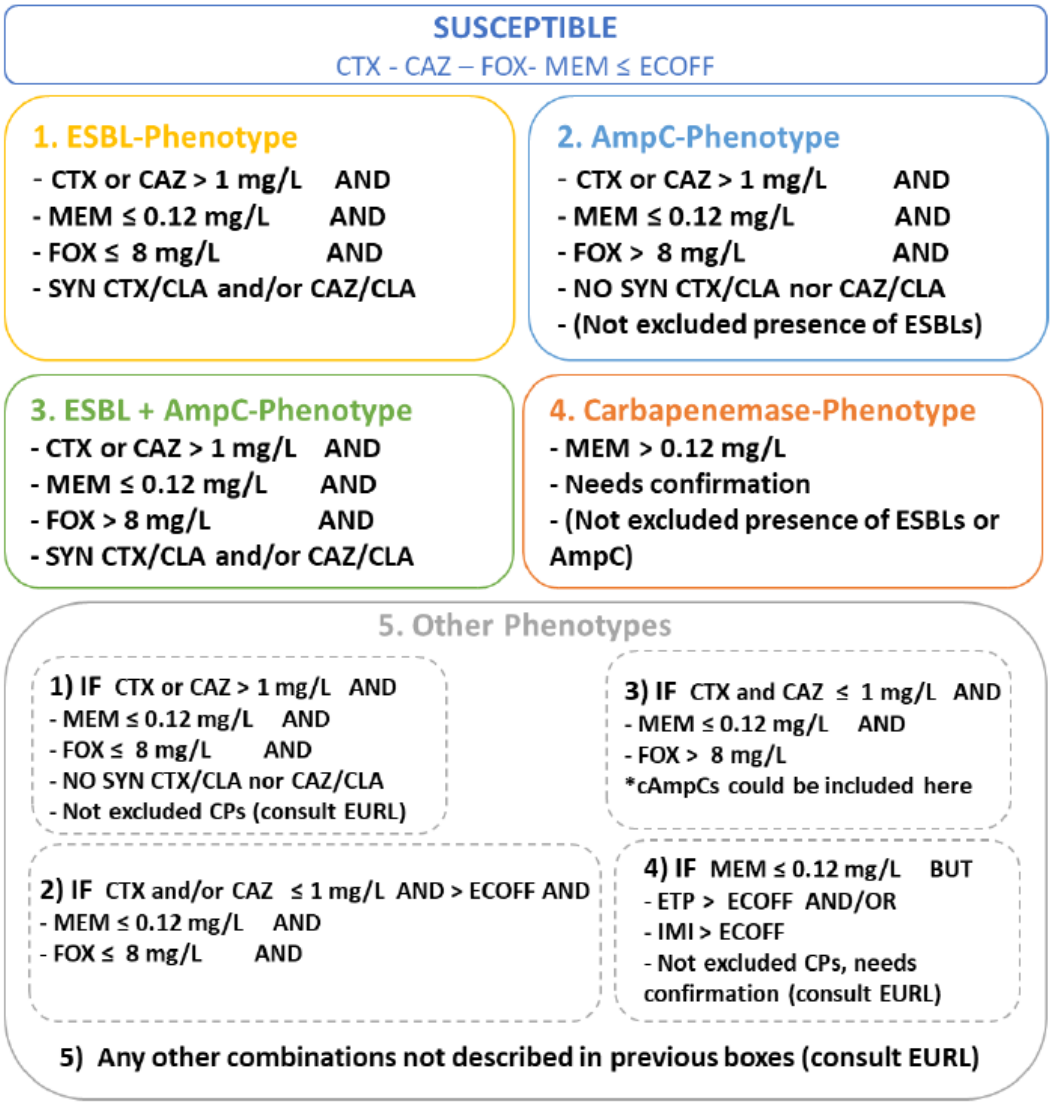
APPENDIX

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



Presumptive ESBL-producers include isolates exhibiting Phenotype 1 or 3.
Presumptive AmpC producers include isolates exhibiting Phenotype 2 or 3.

Please refer to: EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal 2022; 20 (3):7209, 197 pp. https://doi.org/10.2903/j.efsa.2022.7209, Figure F.1.

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 2022 for monitoring of ESBL/AmpC detection in relation to 2020/1729/EU? (Choose only one option)

- ☐ none
- ☐ less than 100
- ☐ 101-200
- ☐ 201-300
- ☐ 301-400
- ☐ 401- 1000
- ☐ more than 1000

Which kind of samples did your laboratory process in 2022 for monitoring of ESBL/AmpC detection in relation to 2020/1729/EU? (You may choose more than one option)

- ☐ caecal, poultry
- ☐ meat, poultry
- ☐ none
- ☐ other matrices, please specify:

How many samples did your laboratory process in 2022 for monitoring of carbapenemases in relation to 2020/1729/EU? (Choose only one option)

- ☐ none
- ☐ less than 100
- ☐ 101-200
- ☐ 201-300
- ☐ 301-400
- ☐ 401- 1000
- ☐ more than 1000

Which kind of samples did your laboratory process in 2022 for monitoring of carbapenemase-production in relation to 2020/1729/EU? (you may choose more than one option)

- ☐ caecal, poultry
- ☐ meat, poultry
- ☐ none
- ☐ other matrices, please specify:

Any other comments:

METHODS

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

Regarding the methods used for selective isolation of ESBL/AmpC in this EQAS, please indicate if you had any modifications to the selective isolation procedure using the EURL recommended protocols that refer to the EU decision 2020/1729/EU:

☐ 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)

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- Did you make changes in the selective isolation procedure:

Different sample amount (weight) used for the enrichment procedure

for meat samples

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 this EQAS:

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

☐ The protocol was used without modifications
☐ The protocol was modified

Plates used (brand/type)

Volume of sample plated

Please justify any changes:

Comments:

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 EUVSEC3 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/Carbapenemase presumptive strains (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 2020/1729/EU:

TEST FORM – SAMPLE 'EURL M-8.1'

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:

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-8.1	Amikacin AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT or CTX			
	Ceftazidime, TAZ or CAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO or MEM			
	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-8.1	Cefepime, FEP			
	Cefotaxime + clavulanic acid F/C or CTX/CLA			
	Cefotaxime, FOT or CTX			
	Cefoxitin, FOX			
	Ceftazidime, TAZ or CAZ			
	Ceftazidime+ clavulanic acid T/C or CAZ/CLA			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO or MEM			
	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 AmpC	☐ Other phenotype
☐ Presumptive ESBL+ AmpC	☐ Presumptive carbapenemase	☐ Susceptible

Comments (include optional genotype or other results):

Appendix 6 - QC ranges

***Escherichia coli* ATCC 25922**

Panel	Antimicrobial	Abbreviation	Acceptable range	
			Min	Max
Panel 1	Ampicillin	AMP	2	8
Panel 1	Amikacin	AMI	NA	NA
Panel 1	Azithromycin	AZI	NA	NA
Panel 1	Cefotaxime	FOT	0.03	0.12
Panel 1	Ceftazidime	TAZ	0.06	0.5
Panel 1	Chloramphenicol	CHL	2	8
Panel 1	Ciprofloxacin	CIP	0.004	0.016
Panel 1	Colistin	COL	0.25	2
Panel 1	Gentamicin	GEN	0.25	1
Panel 1	Meropenem	MER	0.008	0.06
Panel 1	Nalidixic acid	NAL	1	4
Panel 1	Sulfamethoxazole	SMX	8	32
Panel 1	Tetracycline	TET	0.5	2
Panel 1	Tigecycline	TGC	0.03	0.25
Panel 1	Trimethoprim	TMP	0.5	2
Panel 2	Cefepime	FEP	0.016	0.12
Panel 2	Cefotaxime/clavulanic	F/C	NA	NA
Panel 2	Cefotaxime	FOT	0.03	0.12
Panel 2	Cefoxitin	FOX	2	8
Panel 2	Ceftazidime	TAZ	0.06	0.5
Panel 2	Ceftazidime/clavulanic	T/C	NA	NA
Panel 2	Ertapenem	ETP	0.004	0.016
Panel 2	Imipenem	IMI	0.06	0.25
Panel 2	Meropenem	MER	0.008	0.06
Panel 2	Temocillin	TRM	NA	NA

NA: Not available

Appendix 7 - List of deviations

Deviations in ESBL categorisation - full list

Lab number	Strain	Obtained ESBL-phenotype	Expected ESBL-phenotype	Score ESBL-phenotype
NRL-AR-004	EURL M-8.1	Carbapenemase-phenotype	ESBL+AmpC-phenotype, AmpC-phenotype	0
NRL-AR-012	EURL M-8.4	Carbapenemase-phenotype	AmpC-phenotype, Other phenotypes	0
NRL-AR-019	EURL M-8.2	ESBL+AmpC-phenotype	ESBL-phenotype	0
NRL-AR-020	EURL M-8.4	Carbapenemase-phenotype	AmpC-phenotype, Other phenotypes	0
NRL-AR-040	EURL M-8.7	Other phenotypes	Carbapenemase-phenotype	0
NRL-AR-041	EURL M-8.4	Carbapenemase-phenotype	AmpC-phenotype, Other phenotypes	0
NRL-AR-045	EURL M-8.2	Carbapenemase-phenotype	ESBL-phenotype	0

Deviations in AST results - full list

Grey markings refer to 1 two-fold dilution differences

Lab number	Strain	Panel	Antimicrobial	Obtained operator	Expected operator	Obtained MIC value	Expected MIC value	Obtained interpretation	Expected interpretation	Score interpretation
NRL-AR-002	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-002	EURL M-8.3	Panel1	Nalidixic acid	=	<=	16	4	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Azithromycin	>	=	64	8	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Chloramphenicol	=	<=	64	8	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Meropenem	=	<=	0.12	0.03	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Nalidixic acid	>	=	64	8	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Tetracycline	>	<=	32	2	R	S	0
NRL-AR-004	EURL M-8.1	Panel1	Trimethoprim	>	=	16	0.5	R	S	0
NRL-AR-004	EURL M-8.1	Panel2	Ertapenem	=	=	1	0.03	R	S	0
NRL-AR-004	EURL M-8.1	Panel2	Meropenem	=	<=	0.25	0.03	R	S	0
NRL-AR-004	EURL M-8.1	Panel2	Temocillin	=	=	128	8	R	S	0
NRL-AR-004	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-004	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-006	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-006	EURL M-8.4	Panel1	Ciprofloxacin	=	=	0.03	2	S	R	0

NRL-AR-006	EURL M-8.4	Panel1	Nalidixic acid	<=	>	4	64	S	R	0
NRL-AR-006	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-006	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-006	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-009	EURL M-8.2	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-009	EURL M-8.2	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-009	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-011	EURL M-8.1	Panel1	Nalidixic acid	>	=	16	8	R	S	0
NRL-AR-011	EURL M-8.2	Panel2	Cefoxitin	=	=	8	8	R	S	0
NRL-AR-011	EURL M-8.3	Panel1	Meropenem	=	=	0.5	0.25	S	R	0
NRL-AR-011	EURL M-8.7	Panel1	Tigecycline	=	<=	0.5	0.25	R	S	0
NRL-AR-011	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-012	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Azithromycin	>	=	64	4	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Chloramphenicol	=	<=	32	8	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Meropenem	=	<=	0.25	0.03	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Nalidixic acid	=	>	8	64	S	R	0
NRL-AR-012	EURL M-8.4	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Tetracycline	>	<=	32	2	R	S	0
NRL-AR-012	EURL M-8.4	Panel1	Trimethoprim	>	<=	16	0.25	R	S	0
NRL-AR-012	EURL M-8.4	Panel2	Imipenem	=	<=	2	0.12	R	S	0
NRL-AR-012	EURL M-8.4	Panel2	Meropenem	=	<=	1	0.03	R	S	0
NRL-AR-012	EURL M-8.4	Panel2	Temocillin	>	=	128	8	R	S	0
NRL-AR-012	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-016	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-016	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-016	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.5	0.03	R	S	0
NRL-AR-016	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-017	EURL M-8.1	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-017	EURL M-8.7	Panel1	Gentamicin	=	=	4	2	R	S	0
NRL-AR-017	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-018	EURL M-8.2	Panel2	Cefotaxime	<=	=	0.06	16	S	R	0
NRL-AR-018	EURL M-8.2	Panel2	Cefotaxime-clavulanic acid	=	<=	64	0.06	R	S	0
NRL-AR-018	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-018	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-019	EURL M-8.2	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-019	EURL M-8.2	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-019	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-020	EURL M-8.3	Panel1	Ceftazidime	=	>	0.5	8	S	R	0
NRL-AR-020	EURL M-8.3	Panel1	Ciprofloxacin	=	=	0.03	0.5	S	R	0
NRL-AR-020	EURL M-8.3	Panel1	Tetracycline	<=	>	2	32	S	R	0

NRL-AR-020	EURL M-8.3	Panel2	Ceftazidime	=	=	0.5	16	S	R	0
NRL-AR-020	EURL M-8.3	Panel2	Ceftazidime-clavulanic acid	=	=	0.5	8	S	R	0
NRL-AR-020	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-020	EURL M-8.4	Panel1	Azithromycin	>	=	64	4	R	S	0
NRL-AR-020	EURL M-8.4	Panel1	Meropenem	=	<=	0.25	0.03	R	S	0
NRL-AR-020	EURL M-8.4	Panel1	Nalidixic acid	<=	>	4	64	S	R	0
NRL-AR-020	EURL M-8.4	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-020	EURL M-8.4	Panel1	Tetracycline	>	<=	32	2	R	S	0
NRL-AR-020	EURL M-8.4	Panel1	Trimethoprim	>	<=	16	0.25	R	S	0
NRL-AR-020	EURL M-8.4	Panel2	Meropenem	=	<=	0.25	0.03	R	S	0
NRL-AR-020	EURL M-8.4	Panel2	Temocillin	>	=	128	8	R	S	0
NRL-AR-021	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-021	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-021	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-022	EURL M-8.4	Panel2	Ertapenem	=	=	0.03	0.12	S	R	0
NRL-AR-023	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-025	EURL M-8.1	Panel1	Ciprofloxacin	=	=	1	0.5	S	R	0
NRL-AR-025	EURL M-8.2	Panel1	Sulfamethoxazole	=	>	16	512	S	R	0
NRL-AR-025	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-025	EURL M-8.4	Panel1	Ciprofloxacin	<=	=	0.015	2	S	R	0
NRL-AR-025	EURL M-8.4	Panel1	Nalidixic acid	<=	>	4	64	S	R	0
NRL-AR-025	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-026	EURL M-8.2	Panel2	Cefotaxime-Clavulanic acid	=	<=	0.12	0.06	R	S	0
NRL-AR-026	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-026	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-026	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-026	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-029	EURL M-8.2	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-029	EURL M-8.2	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-029	EURL M-8.3	Panel2	Imipenem	=	=	0.25	1	S	R	0
NRL-AR-030	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-030	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-033	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-033	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-033	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-034	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-034	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-036	EURL M-8.1	Panel1	Amikacin	=	<=	32	4	R	S	0
NRL-AR-036	EURL M-8.1	Panel1	Colistin	=	<=	4	1	R	S	0
NRL-AR-036	EURL M-8.1	Panel1	Gentamicin	=	=	4	1	R	S	0
NRL-AR-036	EURL M-8.1	Panel1	Nalidixic acid	=	=	16	8	R	S	0

NRL-AR-036	EURL M-8.1	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-036	EURL M-8.1	Panel1	Tetracycline	=	<=	32	2	R	S	0
NRL-AR-036	EURL M-8.1	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-036	EURL M-8.1	Panel2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-036	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-037	EURL M-8.2	Panel1	Sulfamethoxazole	=	>	16	512	S	R	0
NRL-AR-038	EURL M-8.1	Panel1	Gentamicin	=	=	4	1	R	S	0
NRL-AR-038	EURL M-8.1	Panel1	Nalidixic acid	=	=	16	8	R	S	0
NRL-AR-038	EURL M-8.1	Panel1	Sulfamethoxazole	=	<=	128	8	R	S	0
NRL-AR-038	EURL M-8.1	Panel1	Trimethoprim	=	=	4	0.5	R	S	0
NRL-AR-038	EURL M-8.1	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-038	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-038	EURL M-8.4	Panel1	Gentamicin	=	<=	8	0.50	R	S	0
NRL-AR-039	EURL M-8.2	Panel1	Chloramphenicol	<=	>	8	64	S	R	0
NRL-AR-039	EURL M-8.3	Panel1	Ciprofloxacin	=	=	0.03	0.5	S	R	0
NRL-AR-039	EURL M-8.3	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	8	S	R	0
NRL-AR-039	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-039	EURL M-8.4	Panel2	Ertapenem	<=	=	0.015	0.12	S	R	0
NRL-AR-039	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-039	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-040	EURL M-8.1	Panel1	Sulfamethoxazole	=	<=	128	8	R	S	0
NRL-AR-040	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-040	EURL M-8.4	Panel1	Sulfamethoxazole	=	<=	512	8	R	S	0
NRL-AR-040	EURL M-8.4	Panel2	Ertapenem	=	=	0.03	0.12	S	R	0
NRL-AR-040	EURL M-8.7	Panel2	Imipenem	=	=	0.5	2	S	R	0
NRL-AR-041	EURL M-8.4	Panel1	Azithromycin	>	=	64	4	R	S	0
NRL-AR-041	EURL M-8.4	Panel1	Meropenem	=	<=	0.5	0.03	R	S	0
NRL-AR-041	EURL M-8.4	Panel1	Nalidixic acid	=	>	8	64	S	R	0
NRL-AR-041	EURL M-8.4	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-041	EURL M-8.4	Panel1	Tetracycline	>	<=	32	2	R	S	0
NRL-AR-041	EURL M-8.4	Panel1	Trimethoprim	>	<=	16	0.25	R	S	0
NRL-AR-041	EURL M-8.4	Panel2	Imipenem	=	<=	1	0.12	R	S	0
NRL-AR-041	EURL M-8.4	Panel2	Meropenem	=	<=	0.5	0.03	R	S	0
NRL-AR-041	EURL M-8.4	Panel2	Temocillin	>	=	128	8	R	S	0
NRL-AR-042	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-042	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-042	EURL M-8.8	Panel1	Ciprofloxacin	=	=	0.25	0.03	R	S	0
NRL-AR-042	EURL M-8.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-045	EURL M-8.1	Panel1	Ciprofloxacin	<=	=	0.015	0.5	S	R	0
NRL-AR-045	EURL M-8.2	Panel1	Azithromycin	>	=	64	8	R	S	0
NRL-AR-045	EURL M-8.2	Panel1	Chloramphenicol	=	>	16	64	S	R	0

NRL-AR-045	EURL M-8.2	Panel1	Meropenem	=	<=	1	0.03	R	S	0
NRL-AR-045	EURL M-8.2	Panel1	Tetracycline	>	<=	32	2	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Cefotaxime-Clavulanic acid	=	<=	8	0.06	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Cefoxitin	>	=	64	8	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Ceftazidime-Clavulanic acid	=	=	16	0.25	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Ertapenem	>	<=	2	0.015	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Imipenem	=	<=	1	0.12	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Meropenem	=	<=	1	0.03	R	S	0
NRL-AR-045	EURL M-8.2	Panel2	Temocillin	>	=	128	8	R	S	0
NRL-AR-045	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-045	EURL M-8.3	Panel1	Nalidixic acid	=	<=	16	4	R	S	0
NRL-AR-045	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-056	EURL M-8.3	Panel1	Chloramphenicol	>	=	64	16	R	S	0
NRL-AR-056	EURL M-8.4	Panel2	Ertapenem	=	=	0.03	0.12	S	R	0
NRL-AR-059	EURL M-8.1	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-059	EURL M-8.2	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-059	EURL M-8.2	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-059	EURL M-8.3	Panel2	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-060	EURL M-8.3	Panel1	Chloramphenicol	=	=	32	16	R	S	0
NRL-AR-060	EURL M-8.7	Panel1	Azithromycin	=	>	8	64	S	R	0
NRL-AR-060	EURL M-8.8	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0

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