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)

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1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on selective isolation of presumptive extended spectrum beta-lactamase (ESBL)-, AmpC- or carbapenemase-producing *E. coli* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), and will include the selective isolation procedures and

antimicrobials susceptibility testing (AST) of obtained isolates of eight samples of either meat or caecal content. In 2016, these eight samples will include five samples of 25g meat and three samples of 1g of caecal content, both of poultry origin. These samples may or may not contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes.

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

2 OBJECTIVES

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

3 OUTLINE OF THE EQAS

3.1 Shipping, receipt and storage of samples

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

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

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

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

It is required that participants

- **when opening the parcel, note the date and exact time at opening (this data is very important to follow the temperature data checks)**
- **proceed to sample analysis immediately after opening the parcel**
- **register the date for analysis start for each sample**
- **collect the temperature control device (small discoid device located in a bag inserted in a labelled tube, located inside the parcel);** open the tube and take out the bag with the

device inside. Place this bag with the device in the labelled bubble envelope provided and send it back to the EURL-AR as soon as possible. Please note that you will have to arrange for stamps/postage (the post systems differ from country to country, why this cannot be arranged and paid from the EURL-AR in advance).

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

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

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

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

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

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

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

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

3.3 Antimicrobial susceptibility testing

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

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

AST results to be reported should be from:

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

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

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

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

* Tentative ECOFF
** EUCAST.org

Plasmid-mediated quinolone resistance

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

Beta-lactam resistance

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

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

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Cefepime, FEP	0.125
Cefotaxime, FOT	0.25
Cefotaxime + clavulanic acid (F/C)	Not applicable
Cefoxitin, FOX	8
Ceftazidime, TAZ	0.5
Ceftazidime+ clavulanic acid (T/C)	Not applicable
Ertapenem, ETP	0.064
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, http://www.crl-ar.eu/data/images/ws_april-2016/f11_efsa_criteria.pdf and in the appendix to this protocol).

4 REPORTING OF RESULTS AND EVALUATION

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

4.1 General recommendations for data upload

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

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

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

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

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5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read carefully this paragraph before entering the web page.
Remember that you need by your side the completed test forms.

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

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

5.1 Sample reception/testing

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

5.2 Selective enrichment methods

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

5.3 Test results

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

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

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

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

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

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

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

5.3.3 AST of *E. coli*

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

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

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

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

Remember to report also the conclusion of the phenotypic testing on the second panel (will be evaluated separately).
If you did not test for susceptibility to a given antimicrobial, please leave the field empty.

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

Click on "save".

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

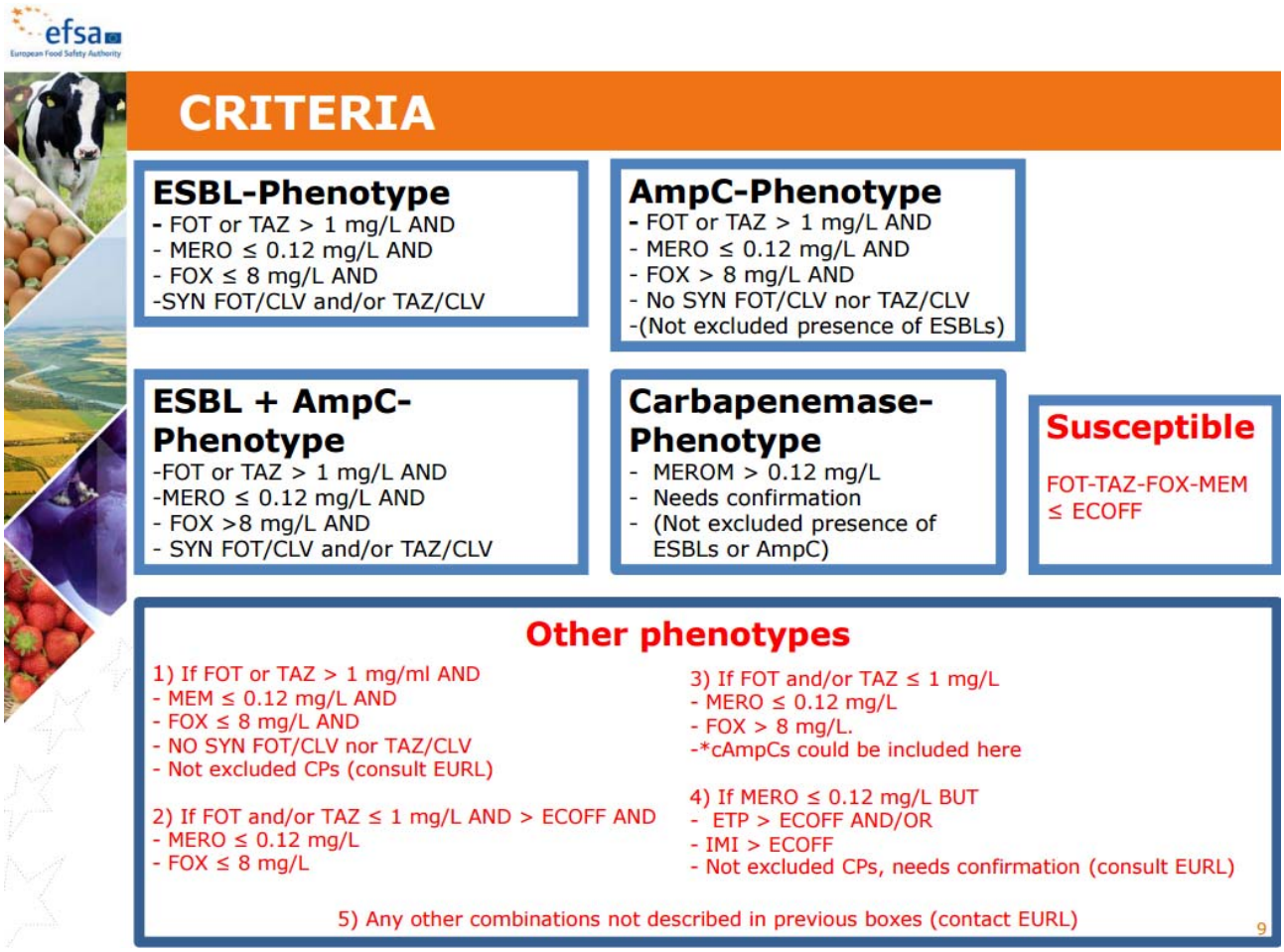
Review the input pages by browsing through the pages and make corrections if necessary. Remember to save a page if you make corrections. If you press home a page without saving changes, you will see an error screen. In this case, click on "save" to save your results, browse back to the page and then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

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

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

APPENDIX

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



Please refer to the full presentation at http://www.crl-ar.eu/data/images/ws_april-2016/f11_efsa_criteria.pdf