PROTOCOL FOR ISOLATION, IDENTIFICATION AND STORAGE OF *CAMPYLOBACTER JEJUNI* AND/OR *C. COLI* FOR THE EU MONITORING OF ANTIMICROBIAL RESISTANCE

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Version 1

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1. CONTEXT

In June 2019, the EFSA proposed technical specifications on harmonised monitoring of antimicrobial resistance (AMR) in zoonotic and indicator bacteria from food-producing animals and food [1] in order to support the review of the corresponding EU legislation. It was considered highly desirable that the harmonised monitoring be based on harmonised methods for both isolation and antimicrobial susceptibility testing in order to improve the comparability of *Campylobacter* prevalence and AMR data between the MSs. It was proposed that a harmonised protocol based on the European standard EN ISO 10272-1 [2], detection procedure C, should be provided for the purpose and consequently the EURL-*Campylobacter* performed in 2020 the requested pilots to finalise the setting up of the harmonised protocol. A better knowledge of the prevalence of *Campylobacter* spp., *C. jejuni* and *C. coli* in animal production and food of animal origin in the different MSs and of the prevalence of resistance in these two species will help understanding the epidemiology and especially the potential sources of human *C. jejuni* and *C. coli* infections, and the differences in proportions of *C. jejuni* and *C. coli* in human cases between European countries implementing Commission Decision 2020/1729. For the sake of ensuring harmonisation of the AMR monitoring, the use of this harmonised protocol is recommended by EFSA, the EURL for antimicrobial resistance (EURL-AR) and the European Commission within the framework of the harmonised monitoring of AMR in *C. jejuni* and *C. coli* from food-producing animals in the EU.

2. SCOPE OF THE METHOD

The protocol describes the detection and identification of *C. jejuni* and *C. coli* by direct plating from caecal content of broilers, fattening turkeys, and cattle of under one year of age and *C. coli* by direct plating from caecal content of fattening pigs to be analysed within the EU monitoring of antimicrobial resistance.

The aim of this protocol is to support national reference laboratories for AMR obtaining the number of *Campylobacter* isolates referred to in point 4.1 of Part A of Annex to Commission Decision 2020/1729.

3. REFERENCES

This protocol is based on the EN ISO 10272-1: 'Microbiology of the food chain – horizontal method for detection and enumeration of *Campylobacter* spp.' [2], the EFSA Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food (2019) [1] and studies carried out within the framework of EURL-*Campylobacter* work programme of 2019-2020.

4. DETECTION AND IDENTIFICATION OF THERMOTOLERANT *CAMPYLOBACTER*

4.1. Transport of samples and storage before analysis

Samples should be maintained at a temperature of 5 ±3°C and stored as set out in the latest version of standard ISO 17604 [3]. Analysis should begin as soon as possible, preferably within 72 hours but up to 96 hours after collecting the samples. Samples should not be allowed to dry.

4.2. Inoculation

Using a sterile loop of 10 microliters, the well mixed samples are plated directly onto the first half of modified Charcoal Cefoperazone Deoycholate agar (mCCDA) [2] and Butzler [4] selective agars. A second sterile loop is used to streak out on the second half of the plates.

A quality control procedure to validate the productivity and selectivity of the agar media should be carried out as described in Annex B of the EN ISO 10272-1 [2] using reference strains of *C. jejuni, C. coli, E. coli* and *S. aureus*.

4.3. Incubation

The plates are incubated at 41.5°C +/-1°C in a microaerobic atmosphere and examined after 44h +/-4h to detect the presence of suspect *Campylobacter* colonies. Plates can be stored refrigerated in microaerobic atmosphere for 72 h (over the weekend) before proceeding with 4.4.

4.4. Purification before identification and storage

Based on colony morphology and typical microscopic appearance, four typical or suspect colonies are selected for identification. For pig samples, at least two typical or suspect colonies are selected. If possible, the selection of colonies should be equally distributed between the selective agars. The agar from which the colony originated should be noted.

Tests should be carried out without delay to avoid loss of cultivability of *Campylobacter* isolates in air.

Re-streak each selected colony to purify on a non-selective blood agar plate (e.g. Columbia blood agar), in order to obtain well isolated colonies.

Incubate at 41.5°C +/-1°C for 24–48 hours in microaerobic atmosphere. Plates can be stored refrigerated in microaerobic atmosphere over the weekend (i.e. for up to 72 hours) before re-streak on a new non-selective media.

From each plate of non-selective blood agar, select one well-isolated colony to streak again onto a plate of non-selective blood agar in order to obtain a heavy growth of a pure culture for identification and storage. Incubate at 41.5°C +/-1 for 24–48 hours under microaerobic atmosphere. Plates can be stored refrigerated in microaerobic atmosphere over the weekend (i.e. for up to 72 hours) before proceeding with identification tests if appropriate for the identification method.

4.5. Identification

Analyse selected subcultures for the identification of isolates of *C. jejuni* and *C. coli*. However, for samples from pigs, only identification of *C. coli* is necessary. Identification can be performed using either matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) according to the manufacturer's instructions, PCR according to the protocol recommended by the EURL-AR (https://www.eurl-ar.eu/protocols.aspx) or by the EURL-*Campylobacter (http://www.sva.se/en/about-us/eurl-campylobacter/laboratory-procedures/)*. Other methods for identification may be used provided the suitability of the method is verified [5].

4.6. Expression of results

For each sample of caeca, the results of the identification of the typical or suspect colonies are indicated as follows:

- Presence of *C. jejuni* detected or not detected (for samples from chicken, turkey and cattle)
- Presence of *C. coli* detected or not detected (for samples from chicken, turkey, pigs and cattle)

The media from which each isolate was obtained should be recorded for reporting with data submission to EFSA.

4.7. Selection of isolates for MIC testing

Determination of MICs should be performed on a maximum of one *C. jejuni* and one *C. coli* per epidemiological unit (flock for chicken and turkey, and slaughter batch for cattle and pigs) in accordance with Commission Implementing Decision 2020/1729. In case more than one isolate of the same species is found in the same sample, one is randomly chosen for MIC testing.

The AST can be performed either directly after identification or after appropriate storage.

Storage

If storage is needed for isolates selected for MIC testing, identified or presumptive, it should be at –70°C or colder in glycerol peptone water or beads until testing. Measures need to be taken to ensure viability of the cultures during storage and possible transport to another laboratory.

Commission Implementing Decision 2020/1729 requires that resistant isolates shall be stored by the Member State laboratories at a temperature of – 80 °C for a minimum period of five years. Other temperatures of storage may be used provided that they ensure viability and absence of changes in strain properties.

[1] EFSA (European Food Safety Authority), Aerts M, Battisti A, Hendriksen R, Kempf I, Teale C, Tenhagen B-A, Veldman K, Wasyl D, Guerra B, Liebana E, Thomas-Lopez D and Belœil P-A, 2019. Scientific report on the technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA Journal 2019;17(6):5709, 122 pp. https://doi.org/10.2903/j.efsa.2019.5709

[2] ISO 10272-1:2017: Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method. International Organization for Standardization.

[3] ISO 17604: Microbiology of the food chain – Carcass sampling for microbiological analysis. International Organization for Standardization.

[4] Lauwers S, De Boeck M, Butzler JP. *Campylobacter enteritis* in Brussels. Lancet. 1978 Mar 18;1(8064):604-5. doi: 10.1016/s0140-6736(78)91045-0.

[5] EN ISO 7218: Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. International Organization for Standardization.