Update on protocols

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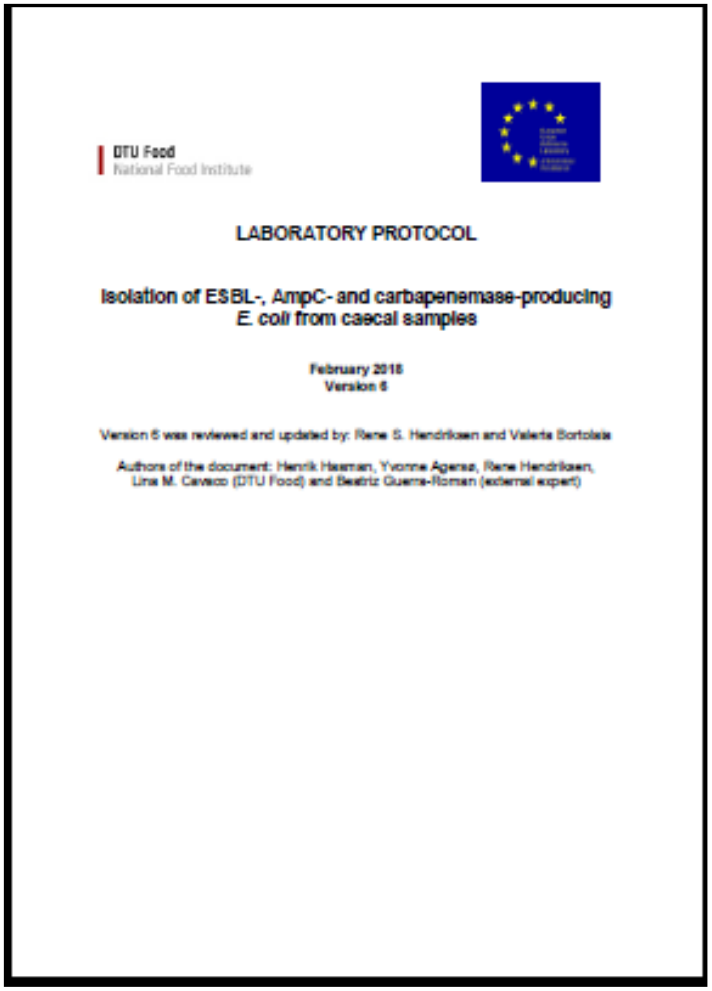
EURL-AR

REVISION:
Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal content and fresh meat samples



DTU Food
National Food Institute

LABORATORY PROTOCOL

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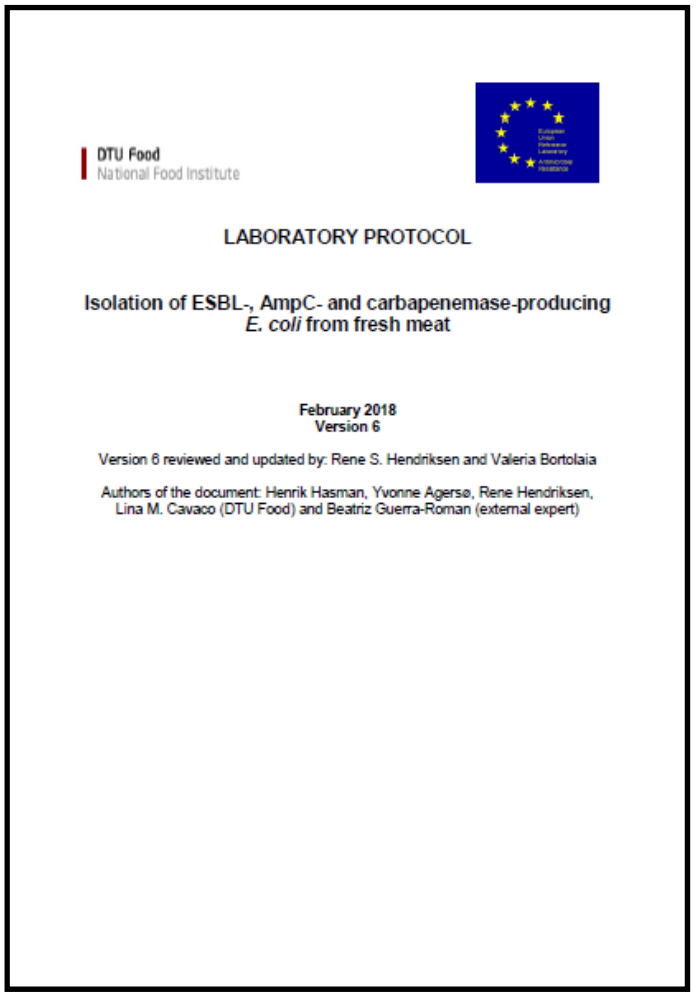
February 2018
Version 6

Version 6 was reviewed and updated by: Rene S. Hendriksen and Valeria Bortolaia

Authors of the document: Henrik Hasman, Yvonne Agersø, Rene Hendriksen, Lina M. Cavaco (DTU Food) and Beatriz Guerra-Roman (external expert)



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REVISION:
Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal content and fresh meat samples

Procedure (4, 5, 6, 7)		**Theory/comments**
1. Isolation and identification of ESBL-, AmpC-, and carbapenemase-producing *E. coli*		
1.1. Sample collection shall be randomized equally over all five business days of the week. It is recommended that samples should arrive at the laboratory within 36 hours after sampling. Samples which have not been stored appropriately (5°C ± 3°C) under transportation or storage shall be discarded.		It is necessary to keep the samples refrigerated to avoid unreliable results. During transport and storage prior to analysis, samples shall be handled according to the ISO/DIS 7218 standard: Microbiology of food and animal feeding stuffs – General rules for microbiological examinations. For further details, see chapter 5.7 of the Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs (8).

1.2 The samples shall be stored at the laboratory at a temperature between 5°C ± 3°C until microbiological analysis. This should be initiated as soon as possible after receipt in the laboratory, preferably within 24 hours. It is recommended that analysis is started, as a rule, within 48 hours after collecting the sample. Exceptions to this rule are samples collected on Thursdays and Fridays, these samples may be analyzed on the following Monday at the latest, i.e. within 96 hours after collecting the sample. It must be ensured that the cold chain is kept at all times between sample collection and analysis.

For further details, see chapter 5.7 of the Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs (8).

This protocol has been validated for storage of samples in the laboratory for up to 24 hours. **Validation of the 96-hour time interval between collection and analysis of samples is planned to be performed by the EURL-AR in 2018.**

1.2 The samples shall be stored at the laboratory at a temperature of 5°C ± 3°C until microbiological analysis. This should be initiated as soon as possible after receipt in the laboratory, preferably within 24 hours. It is recommended that analysis is started, as a rule, within 48 hours after collecting the sample. Exceptions to this rule are samples collected on Thursdays and Fridays: these samples may be analyzed on the following Monday at the latest, i.e. within 96 hours after collecting the sample. It must be ensured that the cold chain is kept at all times between sample collection and analysis. Microbiological analysis shall always be **initiated prior to the expiration date** as indicated on the label for meat samples. Meat samples without an expiration date shall always be analysed on the same day as collected.		For further details, see chapter 5.7 of the Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs (8). This protocol has been validated for storage of samples in the laboratory for up to 24 hours. Validation of the 96-hour time interval between collection and analysis of samples is planned to be performed by the EURL-AR in 2018.

REVISION:

FLOW DIAGRAM
for detection ESBL/AmpC/carbapenemases
(including OXA-48 and OXA-48-like enzymes) in caecal samples

Non-selective pre-enrichment [item 1.4-1.5]

1 g of caecal sample in 9 mL of buffered peptone water (37°C ± 1°C, 18-22 h)

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FLOW DIAGRAM
for detection of *E. coli* producing ESBL/AmpC/carbapenemases
(including OXA-48 and OXA-48-like enzymes) in meat samples

Non-selective pre-enrichment [item 1.4-1.5]

25 g of meat sample in 225 mL of buffered peptone water
(incubate at 37°C± 1°C for 18-22 h)

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Selective isolation

➔ <u>of presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* [item 1.6]</u>
10 µL overnight pre-enrichment culture streaked on **MacConkey agar plate supplemented with 1 mg/L of cefotaxime** (incubate at 44°C ± 0.5°C for 18-22 h)

➔ <u>of presumptive carbapenemase (including OXA-48- and OXA-48-like)-producing *E. coli* [item 3.1 + 3.2]</u>
10 µL overnight pre-enrichment culture streaked **on suitable selective agar plate(s).** (Commercially available chromogenic agar for isolation of carbapenemase-producing *E. coli* (including isolates producing only OXA-48 and/or OXA-48-like enzymes). (incubation according to manufacturer's instructions)

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3. Specific isolation of carbapenemase-producing *E. coli*		
3.1. To specifically isolate carbapenemase-producing *E. coli* (including strains producing OXA-48 and OXA-48-like enzymes) from the caecal samples, one loopful (10 µL loop) of pre-enrichment culture in BPW should be inoculated onto suitable selective agar(s) as shown in Figure 2 (see also flow diagram in Appendix 2). In details, the 10 µL of the pre-enrichment culture are plated for confluent growth on ¼ of a plate and further streaking is performed using either the same loop or a 1-µL loop within an additional ¼ of the plate to obtain single colonies. In this way, each plate can be used for cultures from two samples (Figure 2).		It is important to choose selective agar plates that have been validated with regard to specificity and sensitivity of detection of carbapenemase-producing *E. coli* using the control strains described below. Preferably, a commercially available chromogenic agar for isolation of carbapenemase-producing *E. coli* (including isolates producing only OXA-48 and/or OXA-48-like enzymes) shall be used. If two different plates are required to accomplish this, 10 µL are spread on each type of plate.

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Sub-cultivation

Presumptive *E. coli* colonies from [item 1.6] onto **MacConkey agar plate supplemented with 1 mg/L cefotaxime to maintain the selective pressure** (incubate at 37°C ± 1°C for 18-22 h) [item 1.7]

Presumptive *E. coli* colonies from [item 3.2] onto commercially available chromogenic agar for isolation of carbapenemase-producing *E. coli* (including isolates producing only OXA-48 and/or OXA-48-like enzymes)" or MacConkey agar without antibiotic supplements (**as cefotaxime would not be the optimal substrate for all carbapenemases potentially present)** (incubate at 37°C ± 1°C for 18-22 h) [item 3.3]

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Identification and storage of isolates [item 1.8; 2.1 + 3.4]

Species ID by use of appropriate method
Subculture to ensure purity
Storage: Suitable method for keeping isolates viable for at least five years [see item 1.8 for details]

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Antimicrobial susceptibility testing [item 1.9]

Testing on the first panel (Table 1 of Commission Implementing Decision 2013/652/EU) and, if resistant to cefotaxime, ceftazidime and/or meropenem, further testing on the second panel (Table 4 of Commission Implementing Decision 2013/652/EU).

THANK YOU FOR YOUR ATTENTION!