DETECTION OF ESBL, AMPC- AND CARBAPENEMASE PRODUCING *E.COLI* IN CAECA

Ring trial 28th of March 2018

Sciensano

Infectious diseases in humans - Foodborne pathogens

April 2018 • Elsene • Belgium

RESPONSIBLE SCIENTIST: FLORENCE CROMBÉ

•

RESPONSIBLE TECHNICIAN: CÉLINE DESTERCK

•

SCIENTIFIC APPROVER: NADINE BOTTELDOORN

The present trial was organised upon request of FAVV-AFSCA. The study focused on the detection of presumptive ESBL-, AmpC- and/or carbapenemase (CPE) producing *E. coli* in caeca according to decision 2013/652/EU.

1. PARTICIPATING LABORATORIES

Five laboratories participated to the study.

Nr. of participating laboratory
11
17
21
32
33

2. PLANNING OF THE STUDY

27/03/2018: sample preparation and transport to the participating laboratories
28/03/2018: start of the study for all laboratories
20/04/2018: deadline submission of results

3. MATERIALS AND METHODS

Strains used:

- ESBL producing *E. coli* TIAC 809
- OXA-48 producing *E. coli* TIAC 2892
- CPE producing *E. coli* TIAC 2893

The strains used in the study are well-characterized and confirmed reference strains received from the EURL-AR.

Tested matrix: chicken faeces (S17FP09303)

As caeca is difficult to obtain, surrogate caeca was made by mixing chicken faeces with physiological water. The faecal samples provided to the laboratories initially consisted of a pool of faeces that was thoroughly mixed, divided in smaller aliquots and stored at -20°C in our laboratory until usage.

The chicken faeces were not naturally contaminated with EBSL/AmpC producing *E. coli* neither with CPE/OXA-48 producing *E. coli*.

Spiking of the samples:

- Inoculum preparation: the strains were grown in BHI at 37°C for 24h. The concentration of the strains was adjusted to OD_{600nm} 1 before diluting.
- Sample preparation: five samples (10g) were prepared and aliquoted for each participating laboratory, e.g. 5 samples of 1g chicken faeces in a stomacher bag with filter.
- Contamination of the samples: four samples (10g) were spiked with 1000µl of the 10^{-6}-fold dilution, which corresponds to a theoretical bacterial concentration of 100cfu/100µl. One sample (10g) was considered as blank for ESBL/CPE/OXA-48 as it was not spiked (Table 1).
- The five samples were sent to the participating laboratory.
- Determination of the inoculum concentration: for all strains the inoculum concentration was determined by plating in triplicate on nutrient agar plates.

Table 1: Sample composition per participating laboratory

Sample nr.	Matrix	Strain	Inoculum
E1	1 g of surrogate caeca	TIAC 809, TIAC 2892, TIAC 2893	100 cfu/g
E2	1 g of surrogate caeca	TIAC 809, TIAC 2893	100 cfu/g
E3	1 g of surrogate caeca	/	/
E4	1 g of surrogate caeca	TIAC 809	100 cfu/g
E5	1 g of surrogate caeca	TIAC 809, TIAC 2892	100 cfu/g

Method: laboratory protocol: Isolation of ESBL-, AmpC- and carbapenemase-producing E. coli from caecal samples, January 2017, version 4. DTU Food - EURL-AR.

4. RESULTS

Inoculum concentration

The concentration of the inoculum of all strains, TIAC 809, TIAC 2892 and 2893, is presented in table 2. The obtained results correspond to the theoretical bacterial concentrations of, respectively, 100cfu/100µl, 10cfu/100µl and 1cfu/100µl for dilutions 10^{-6}, 10^{-7} and 10^{-8}. Consequently, the samples were spiked with 59 ± 3 cfu/g of TIAC 809, 70 ± 3 cfu/g of TIAC 2892 and 57 ± 6 cfu/g of TIAC 2893.

Table 2: Inoculum concentration

E. coli ESBL TIAC 809	Results			Mean	SD	Conc.
	plate 1	plate 2	plate 3			
Dil -6	58	56	62	59	3	59 cfu/100µl
Dil -7	8	2	6			
Dil -8	0	0	0			

E. coli Oxa-48 TIAC 2892	Results			Mean	SD	Conc.
	plate 1	plate 2	plate 3			
Dil -6	67	73	70	70	3	70 cfu/100µl
Dil -7	4	5	5			
Dil -8	1	1	0			

E. coli CPE TIAC 2893	Results			Mean	SD	Conc.
	plate 1	plate 2	plate 3			
Dil -6	55	53	64	57	6	57 cfu/100µl
Dil -7	8	17	6			
Dil -8	0	0	0			

Study results

The expected results of the present study are presented in table 3.

Table 3: Expected results

(D: detected/g or ND: not detected/g)

Sample nr.	*E. coli* ESBL/AmpC	*E. coli* Oxa-48	*E. coli* CPE
E1	D	D	D
E2	D	ND	D
E3	ND	ND	ND
E4	D	ND	ND
E5	D	D	ND

All laboratories, except one, detected ESBL/AmpC producing *E. coli*, CPE producing *E. coli* and/or OXA-48 producing *E. coli*, in the four spiked samples (Table 4). Laboratory nr. 17 did not detect ESBL/AmpC producing *E. coli* in sample E4. No bacteria were detected on the fifth blank sample.

Table 4: Observed results
(D: detected/g or ND: not detected/g)

Nr. of laboratory	Sample nr. (ESBLAmpC/Oxa-48/CPE)				
	E1	E2	E3	E4	E5
11	D/D/D	D/ND/D	ND/ND/ND	D/ND/ND	D/D/ND
17	D/D/D	D/ND/D	ND/ND/ND	**ND**/ND/ND	D/D/ND
21	D/D/D	D/ND/D	ND/ND/ND	D/ND/ND	D/D/ND
32	D/D/D	D/ND/D	ND/ND/ND	D/ND/ND	D/D/ND
33	D/D/D	D/ND/D	ND/ND/ND	D/ND/ND	D/D/ND

5. CONCLUSION

All laboratories detected ESBL/AmpC producing *E. coli* and/or CPE (including OXA-48) producing *E. coli* in surrogate caeca. Yet, one laboratory did not detect ESBL/AmpC producing *E. coli* in one out of the four samples spikes with ESBL/AmpC producing *E. coli*. No false-positive resultats were obtained for the blank samples.

6. REFERENCES

Décision d'exécution de la Commission du 12 novembre 2013 concernant la surveillance et la présentation de rapports relatifs à la résistance aux antimicrobiens chez les bactéries zoonotiques et commensales (2013/652/UE).

Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from caecal samples. January 2017. Version 4. DTU Food EURL-AR

CONTACT
Florence Crombé • Florence.Crombe@sciensano.be • T +32 2 642 53 35

Sciensano • Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium • T + 32 2 642 51 11 • T press + 32 2 642 54 20 • info@sciensano.be • **www.sciensano.be**

Responsible publisher(s): Pierre Kerkhofs, Managing director • Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium •