Survey on the application of the ESBL pre-enrichment in European laboratories

ESBL protocol update

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Background

- The protocol for isolation and identification of ESBL, AmpC and carbapenemase-producing *E. coli* follows the principal of the Scientific Opinion's from EFSA
  - *Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals*
  - *Scientific Opinion on carbapenem resistance in food animal ecosystems*

- Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* is conducted according to the protocol suggested by the EURL-AR

- At the current state, this protocol includes a non-selective pre-enrichment step
  - based on the assumption that the pre-enrichment broth produced for the isolation of ESBL, AmpC and carbapenemase producing *E. coli,* would be re-used for the isolation of other bacteria, e.g. *Salmonella,* commensal *E. coli* and enterococci

- In the recent years, alternative protocols have been published with the application of selective pre-enrichment steps to increase the sensitivity and specificity

Objective

- The primary aim of the questionnaire survey was to identify to what extent the pre-enrichment broth, produced for the isolation of ESBL, AmpC and carbapenemase producing *E. coli*, is re-used for the isolation of other bacteria in the laboratories of the EURL-network.
- Secondary, to identify routine procedures applied in the laboratories for the isolation of other bacteria

Questionnaire survey

- In collaboration with the NRLs in Poland, Italy and the Netherlands, a questionnaire survey was developed and piloted

- The final questionnaire contained twelve questions
  – Dispatched by e-mail in March 2021 to a total of 45 contacts

- 34 replied from 32 countries, resulting in a survey response rate of 78%

ESBL protocol_survey

Q1 Contact information

Answered: 34 Skipped: 0

ANSWER CHOICES	RESPONSES	
Institute name	100.00%	34
Country	100.00%	34
Contact person	100.00%	34
e-mail	100.00%	34

Re-use of the ESBL pre-enrichment broth for the isolation of other pathogens

Bacteria	Is the broth reused for the isolation of the following bacteria		
ESBL pre-enrichment broth for ceacal content	Yes	No (other pre-enrichment)	No (direct plating)
Salmonella (n=29)	66% (19)	34% (10)	0% (0)
Commensal *E. coli* (n=28)[A]	32% (9)	4% (1)	68% (19)
Enterococci (n=10)[A]	50% (5)	20% (2)	40% (4)

Bacteria	Is the broth reused for the isolation of the following bacteria		
ESBL pre-enrichment broth for meat samples	Yes	No (other pre-enrichment)	No (direct plating)
Salmonella (n=24)	71% (17)	29% (7)	0% (0)
Commensal *E. coli* (n=24)[B]	58% (14)	13% (3)	38% (9)
Enterococci (n=6)[A]	50% (3)	33% (2)	33% (2)

Lab procedures applied for the isolation

Bacteria	Laboratory procedure	Number of responses
Campylobacter (n=33)[A]	EN ISO 10272-1-2017	62% (21)
	EURL Campy method (EN ISO 10272)	32% (11)
	Other	9% (2)
Salmonella (n=33) faecal and/or meat samples	ISO 6579-1:2017/Amd 1:2020	91% (30)
	Other	9% (3)
Enterococci (n=8)	Slanetz-Barley medium (+/- pre-enrichment)	75% (6)
	Other	25% (2)
Commensal *E. coli* (n=34) faecal samples	Direct plating	53% (18)
	EURL-AR protocol	18% (6)
	Other	29% (10)
Carbapenemase-producing *E. coli* (n=33)	Chromogenic agar plates, as suggested in the EURL AMR-protocol	97% (33)
	Screening by PCR/other molecular method prior to isolation	5% (2)

In summary

- The NRLs indicated that the non-selective pre-enrichment broths produced for the isolation of ESBL, AmpC and carbapenemase-producing *E. coli* in ceacal and meat samples, to a large extent are re-used for the isolation of other pathogens; especially Salmonella (66-71%), but also commensal E. coli (32-58%) and enterococci (50%)

- The EURL are hesitating to change the current protocol by supplementing the pre-enrichment broth with a selective antimicrobial of various reasons
  - A shift in the selective procedure will discontinue the trend data
  - Increase workload at NRLs
  - Increase expenses at NRLs – EC
    - There should be a very good reasons to make changes - pros and cons should be taken into considerations – potential a discuss for EFSA

- Seems as a lack of harmonization of the isolation procedures for enterococci and commensal *E. coli*

Thank you for your attention

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