DTU Food
National Food Institute

Survey on the practical application of the ESBL protocol in European laboratories

1 INTRODUCTION

To harmonize the monitoring of antimicrobial resistance in the European Union, the European Commission implemented in 2013 a regulation on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU). Hereby, ESBL- and AmpC-producing *E. coli* became mandatory, while carbapenemase-producing *E. coli* were voluntary, to monitor in meat and caecal samples in EU Member States. Isolation of these bacteria should be done according to the protocol suggested by the European Reference Laboratory for Antimicrobial Resistance (EURL-AR). At the current state, this protocol includes a non-selective pre-enrichment step, which is 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. However, in the recent years, alternative protocols have been launched suggesting the application of a selective pre-enrichment step.

2 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.

3 QUESTIONNAIRE SURVEY

In collaboration with the NRLs in Poland, Italy and the Netherlands, a questionnaire survey was designed by the EURL-AR regarding subjects on

1) whether or not the pre-enrichment broth for the isolation of ESBL, AmpC and carbapenemase producing *E. coli*, additionally is used in the laboratories for the isolation of other bacteria (e.g. *Salmonella*, commensal *E. coli* and enterococci)
2) methods applied in the laboratories for isolation of *Campylobacter, Salmonella*, enterococci, commensal *E. coli* and carbapenemase-producing *E. coli* from faecal and meat samples

A pilot testing of the questionnaire was conducted prior to the survey. The final questionnaire held twelve questions and is available in the Appendix. The questionnaire was send out per e-mail in March 2021 to the EURL-network, including NRLs and affiliated laboratories; holding a total of 45 contacts.

4 RESULTS

A total of 34 reponses from 32 countries were submitted, resulting in a survey response rate of 78%.

4.1 Reuse of the ESBL pre-enrichment broth for the isolation of other pathogens

Results on the re-use of the pre-enrichment broths produced for the isolation of ESBL, AmpC and Carbapenemase-producing *E. coli*, for either caecal content or meat samples, are presented in Table 1.

Table 1: Results from a questionnaire survey in 34 European NRLs and affiliated laboratories describing the re-use of the ESBL pre-enrichment broth (for caecal content or meat samples). For the isolation of *Salmonella*, commensal *E. coli* and enterococci, it is described whether the laboratories; re-use the ESBL-broth; produce another pre-enrichment broth; or perform direct plating. N indicates the total number of laboratories performing the given method. Results are stated in % (number of laboratories).

Bacteria	**Is the broth reused for the isolation of the following bacteria**		
ESBL pre-enrichment broth for caecal 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)
ESBL pre-enrichment broth for meat samples			
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)

[A] One laboratory reported two answers
[B] Two laboratories reported two answers

4.2 Laboratory procedures applied for the isolation of Campylobacter, Salmonella, enterococci, commensal E. coli and carbapenemase producing E.coli

For each pathogen, the laboratory procedures most commonly applied by the participating laboratories are presented in Table 2.

Table 2: Laboratory procedures applied by 34 European laboratories for the isolation of *Campylobacter*, *Salmonella*, commensal *E. coli* and enterococci from feacal and/or meat samples. N indicates the total number of laboratories performing the given method. Results are stated in % (number of laboratories).

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)

[A] One laboratory does not test for *Campylobacter*, while another laboratory responded with two answers (both "EN ISO 102-1-2017" and "EURL Campy method")

5 CONCLUSION

Results from the present questionnaire survey targeting the NRLs of the EURL-network indicates that the non-selective pre-enrichment broths produced for the isolation of ESBL, AmpC and carbapenemase-producing *E. coli* in caecal 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%). Hence, these results oppose a change in the current protocol by supplementing the pre-enrichment broth with a selective antimicrobial, which also would increase workload and expenses, as this would not be suitable for any reuse for other pathogens under survey.

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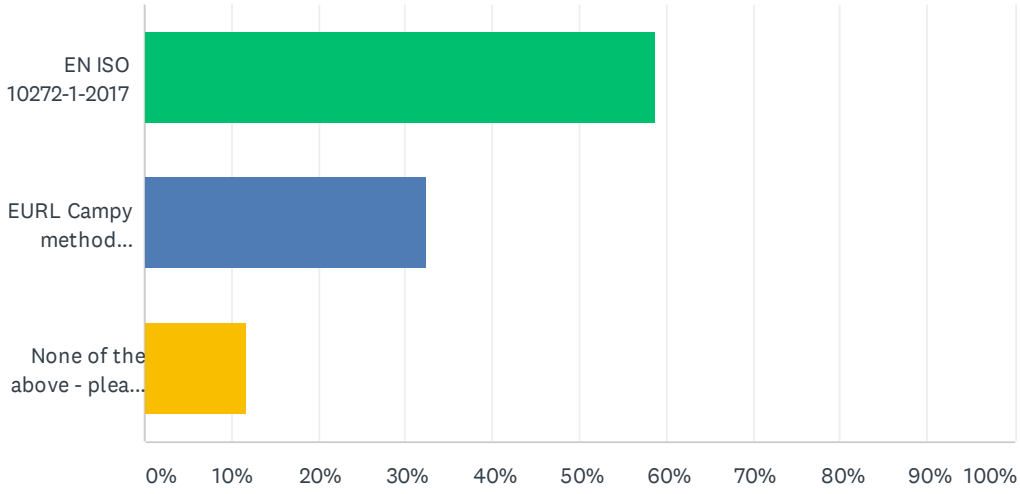
Q1 Contact information

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

Q2 Which method do you use for the isolation of Campylobacter

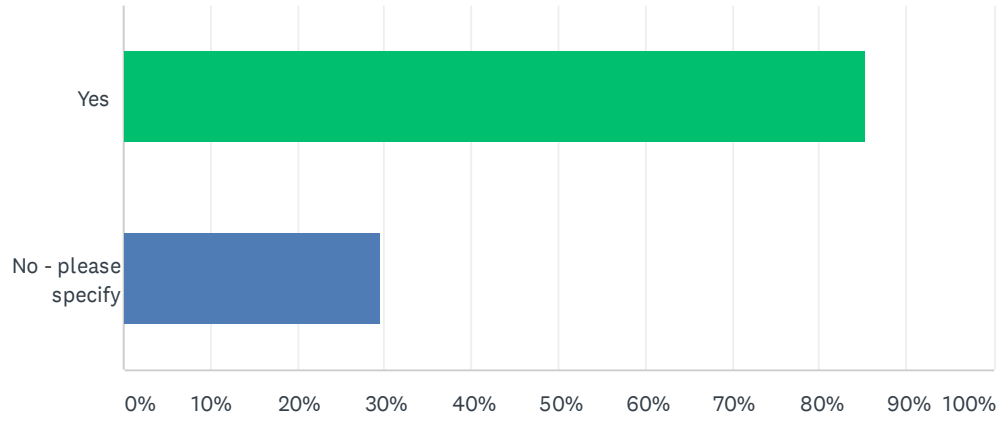
Answered: 34 Skipped: 0



ANSWER CHOICES	RESPONSES	
EN ISO 10272-1-2017	58.82%	20
EURL Campy method referring to EN ISO 10272	32.35%	11
None of the above - please specify what method being used	11.76%	4
Total Respondents: 34		

Q3 Do you follow ISO 6579-1:2017/Amd 1:2020 for the isolation of Salmonella from faecal and meat samples?

Answered: 34 Skipped: 0



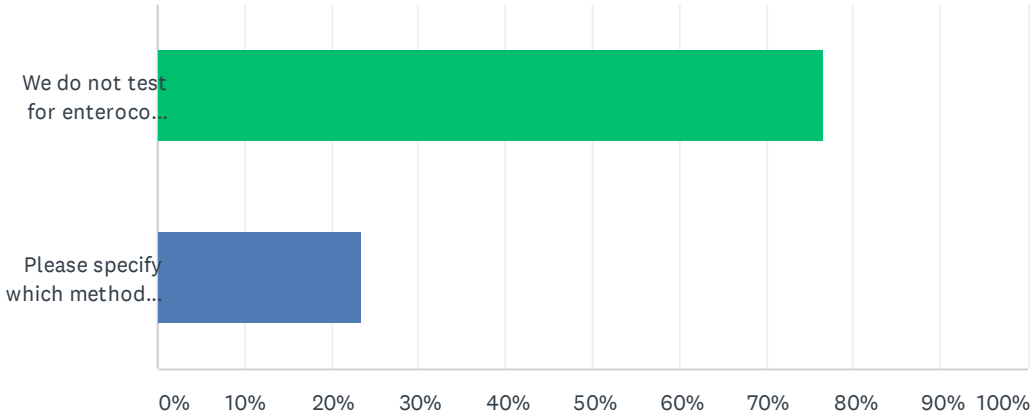
ANSWER CHOICES	RESPONSES	
Yes	85.29%	29
No - please specify	29.41%	10
Total Respondents: 34		

Q4 Which method do you use for the isolation of commensal E. coli from faecal and meat samples? Please specify.

Answered: 34 Skipped: 0

Q5 Which method do you use for the isolation of enterococci?

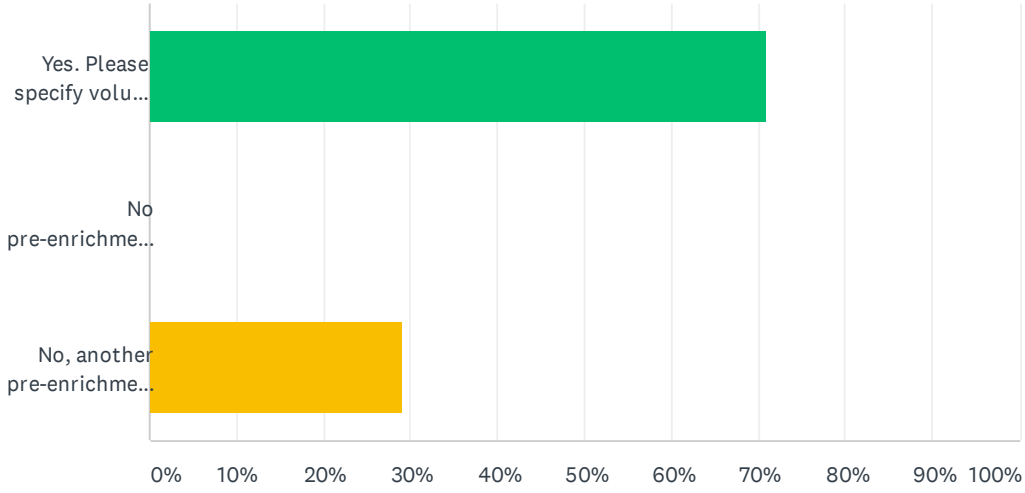
Answered: 34 Skipped: 0



ANSWER CHOICES	RESPONSES	
We do not test for enterococci in the frame of the EU harmonised AMR monitoring	76.47%	26
Please specify which method being used	23.53%	8
Total Respondents: 34		

Q6 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in meat samples - is the same pre-enrichment also used for the isolation of Salmonella?

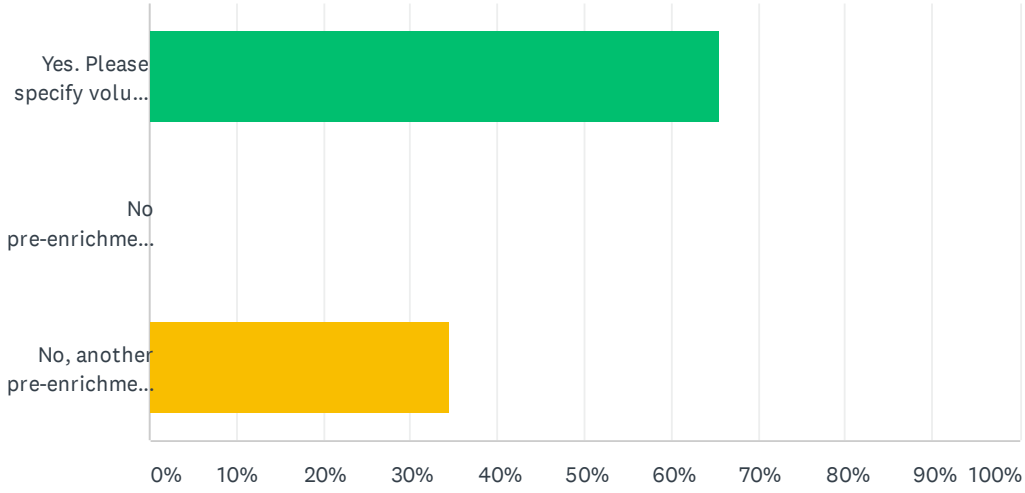
Answered: 24 Skipped: 10



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	70.83%	17
No pre-enrichment is used - direct plating is performed	0.00%	0
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify name of the pre-enrichment and ratio between sample and buffer (sample weight/ml buffer) in the text box below	29.17%	7
Total Respondents: 24		

Q7 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in caecal content - is the same pre-enrichment also used for the isolation of Salmonella?

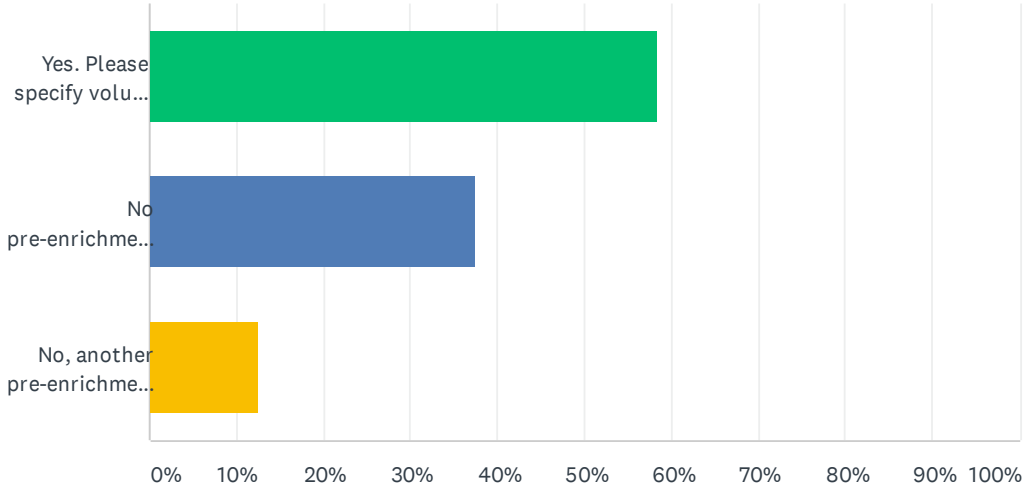
Answered: 29 Skipped: 5



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	65.52%	19
No pre-enrichment is used - direct plating is performed	0.00%	0
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify name of the pre-enrichment and ratio between sample and buffer (sample weight/ml buffer) in the text box below	34.48%	10
Total Respondents: 29		

Q8 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in meat samples - is the same pre-enrichment also used for the isolation of commensal E. coli?

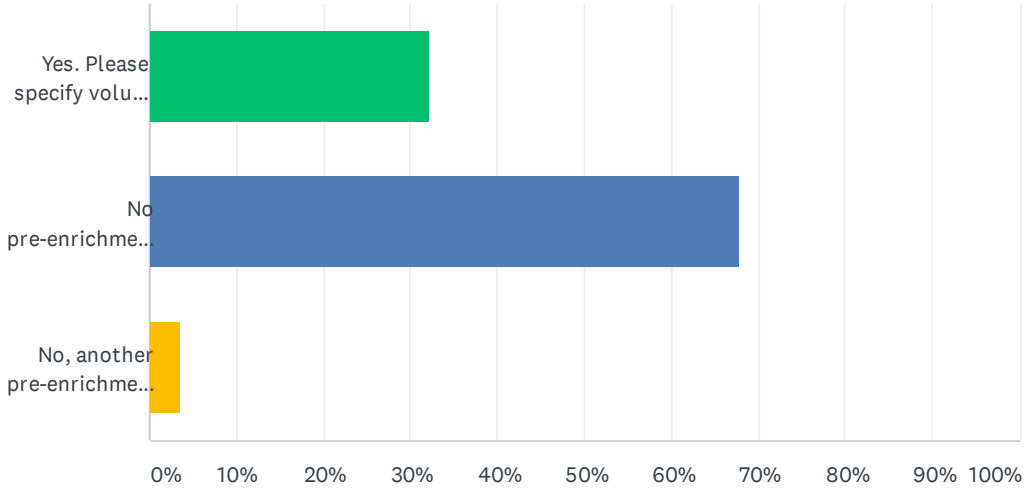
Answered: 24 Skipped: 10



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	58.33%	14
No pre-enrichment is used - direct plating is performed	37.50%	9
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify name of the pre-enrichment and ratio between sample and buffer (sample weight/ml buffer) in the text box below	12.50%	3
Total Respondents: 24		

Q9 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in caecal content - is the same pre-enrichment also used for the isolation of commensal E. coli?

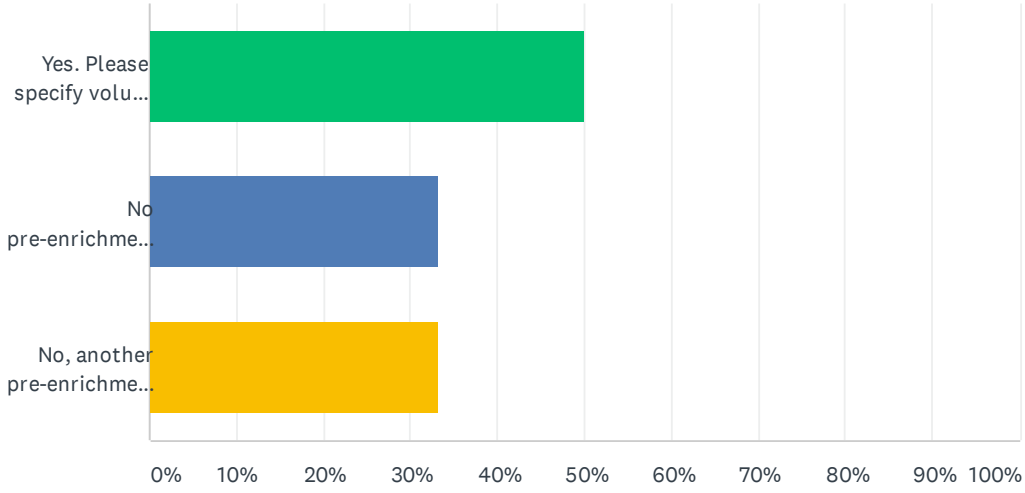
Answered: 28 Skipped: 6



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	32.14%	9
No pre-enrichment is used - direct plating is performed	67.86%	19
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify the name of the pre-enrichment and ratio between sample and buffer (sample weight/ml buffer) in the text box below	3.57%	1
Total Respondents: 28		

Q10 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in meat samples - is the same pre-enrichment also used for the isolation of enterococci?

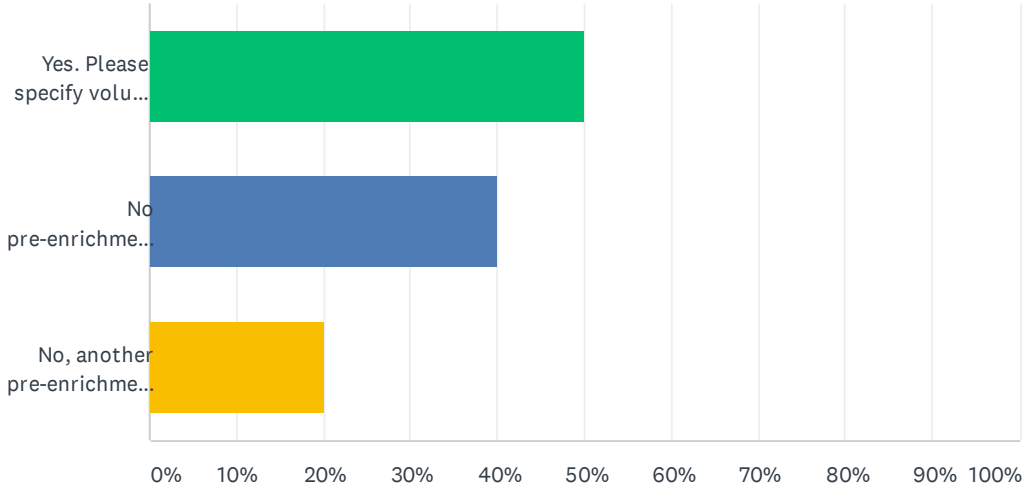
Answered: 6 Skipped: 28



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	50.00%	3
No pre-enrichment is used - direct plating is performed	33.33%	2
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify the name of the pre-enrichment and ratio between sample and buffer (sample weight/ml buffer) in the text box below	33.33%	2
Total Respondents: 6		

Q11 The pre-enrichment used for the isolation of ESBL, AmpC and carbapenemase producing E. coli in caecal content - is the same pre-enrichment also used for the isolation of enterococci?

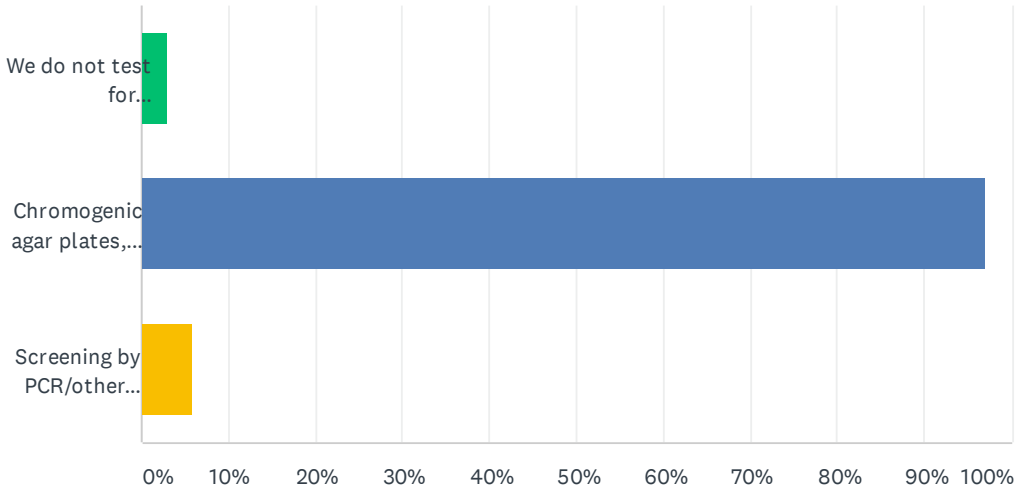
Answered: 10 Skipped: 24



ANSWER CHOICES	RESPONSES	
Yes. Please specify volume and ratio (sample:broth) in the text box below	50.00%	5
No pre-enrichment is used - direct plating is performed	40.00%	4
No, another pre-enrichment is used due to the ratio between sample and buffer. Please specify the name of the pre-enrichmenr and ratio between sample and buffer (sample weight/ml buffer) in the text box below	20.00%	2
Total Respondents: 10		

Q12 Which selective agar plates do you use for detecting carbapenemase producing E. coli?

Answered: 34 Skipped: 0



ANSWER CHOICES	RESPONSES	
We do not test for carbapenemase producing E. coli in the frame of the EU harmonized AMR monitoring	2.94%	1
Chromogenic agar plates, as suggested in the EURL AMR protocol	97.06%	33
Screening by PCR/other molecular methods before attempting isolation	5.88%	2
Total Respondents: 34		

Q13 We sincerely thank you for taking the time to participate in this survey.To finally submit your answers, press 'Submit'Any comments?

Answered: 13 Skipped: 21