DTU Food
National Food Institute

European Union Reference Laboratory Antimicrobial Resistance

Laboratory Protocol

Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*

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Version 2

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HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Approval
1	New document	-	14 Jan 2015	Authors
2	Page 3, item 6	In this step, use selective and indicative agar plates	21 Jan 2015	Henrik Hasman

Background

It is important for the harmonized monitoring of carbapenemase producing *E. coli* to ensure the validity of the selective and indicative agar plates used to detect presumptive carbapenemase-producers. If these plates do not contain the correct concentration of the selective agent, false positive or false negative results can occur.

Based on Expert advice, the EURL AR has identified and validated a set of test strains with MIC values close to the EUCAST breakpoints of the carbapenems ertapenem (ERT), meropenem (MERO) and imipenem (IMI) included in the second panel (Table 4) of the 2013/652/EU decision. The control set consists of two positive control strains: a strain producing the carbapenemase GES-5 (*E. coli* TZ 3638) and a strain producing the carbapenemase OXA-48 (*E. coli* 16874). These two positive strains are included as not all carbapenemases have the same spectrum in relation to the carbapenems in some of the commercial selective and indicative agar systems. In addition, a third strain is supplied (*E. coli* TZ 116; VIM-1, CMY-13). This strain serves as a backup strain of the TZ 3638 strain and shall not be used unless the EURL-AR request so. As a negative control, we recommend to use the *E. coli* control strain ATCC 25922, which is traditionally used for QC when testing antimicrobial susceptibility.

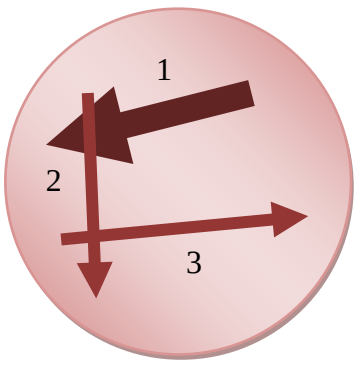
It is recommended that this protocol is carried out and validation of the plates is completed prior to performing the selective enrichment for presumptive carbapenemase-producing *E. coli* from meat and caecal samples. This is to avoid a situation where samples have been inoculated on plates that do not pass the QC validation.

Please note that bacterial growth on the indicative and selective agar plates for detection of carbapenemase-producing Enterobacteriaceae does not necessarily mean that the growing bacteria actually produce a carbapenemase enzyme. This is because other molecular mechanisms such as over-production of AmpC enzymes in combination with porin deficiencies might lead to reduced susceptibility towards one or more of the carbapenems. Please read the scientific opinion on carbapenem resistance in food animal ecosystems in the references section below for more details.

References:

Scientific Opinion on Carbapenem resistance in food animal ecosystems. EFSA Journal 2013; 11(12):3501 [70 pp.]. doi:10.2903/j.efsa.2013.3501.

Contents

Procedure	Theory/comments
1. The two positive test strains supplied by the EURL-AR should be stored at -80ºC in glycerol upon arrival from the EURL-AR. The negative control strain ATCC 25922 should already be stored at the NRL as part of the phenotypic susceptibility testing programs.	If the negative control strain is not present in the testing laboratory, it can be acquired from the ATCC strain collection directly.
2. It is the intention that this protocol shall be used each time the protocols for selective enrichment of carbapenemase-producing *E. coli* in either fresh meat or caecal material from production animals are carried out.	The activity of the carbapenem in the plates might decrease upon prolonged storage. Therefore, it is recommended that this protocol is used each time any of the two protocols for selective enrichment of carbapenemase producing *E. coli* is performed.
3. Inoculate the two positive control strains as well as the ATCC 25922 strain to single colonies from the -80ºC. freezing stock onto fresh blood agar plates and incubate at 37ºC for 18-24 hours.	Other nutrient agar plates can be used. However, blood agar is often helpful to identify bacterial contaminants based on colony morphology.
4. After incubation, resuspend the three test strains in 0.9% saline separately and adjust the cell density to McFarland 0.5.	
5. Dilute each test strain a 1000-fold in fresh Buffered Peptone Water (BPW) and incubate 18-22 hours at 37 ºC.	E.g. 9 µL McFarland-adjusted sample in 9 mL BPW or 10 µL in 10 mL BPW.
6. After this incubation, use a 10 µl loop to streak each of the test strains on (a) selective and indicative agar plate(s) for detection of carbapenemase-producing *E. coli* in a single streak (1; Figure 1). From this streak, perform two additional streaks (2 and 3; Figure 1) with 1 µl loops to obtain single colonies.	 Figure 1

7. Incubate the selective and indicative agar plate(s) according to the manufacturer's instructions.

This will most often **NOT** be the 44ºC used in the ESBL/AmpC validation protocol.

8. After incubation of the plates, assess growth.

Full inhibition of growth should be obtained for the negative control and single colonies should be obtained in at least streak 1 but preferably also in streak 2 according to Figure 1 for the two positive controls. If different agar plates are used for detection of the GES-5 producing strain and for the OXA-48 producing strain, then please note that the GES-5 strain will not grow on agar plates developed to detect OXA-48 while the OXA-48 might (or might not, depending on the agar system used) grow on agar plates designed for detection of presumptive carbapenemase-producers.

APPENDIX 1

Composition and preparation of culture media and reagents

Buffered peptone Water is available from several companies. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturer's description if it differs from the description given here.

Buffered peptone water

Formula	g/litre
Enzymatic digest of casein	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9.0
Potassium dihydrogen phosphate (KH_2PO_4)	1.5
pH 7.0 +/- 0.2 @ 25°C	

Dissolve the components in water by heating if necessary. Adjust the pH, so that after sterilization it is 7.0+/- 0.2 at 25°C. Dispense the medium into flasks of suitable capacity to obtain the portions necessary for the test. Sterilize for 15 min in the autoclave set at 121°C.