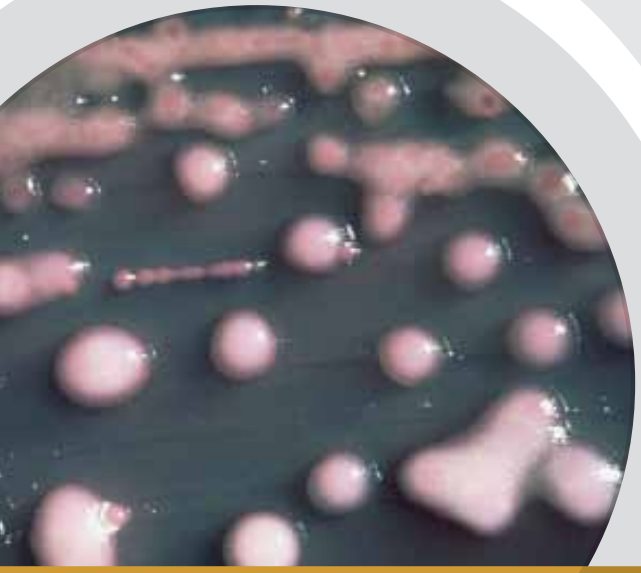

TECHNICAL REPORT

Expert consensus protocol on carbapenem resistance detection and characterisation for the survey of carbapenem- and/or colistin-resistant Enterobacteriaceae

Version 3.0

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ECDC TECHNICAL REPORT

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC) [FWC: OJ/2017/OCS/7530], coordinated by Anke Kohlenberg and Barbara Albiger and produced by Alma Brolund and Sara Byfors of the Public Health Agency of Sweden.
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Abbreviations

AST	Antimicrobial susceptibility testing
BMD	Broth microdilution
CCRE	Carbapenem- and/or colistin-resistant Enterobacteriaceae
E. coli	*Escherichia coli*
ESBL	Extended-spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURGenCCRE	Genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level
EURGen-Net	European Antimicrobial Resistance Genes Surveillance Network
EuSCAPE	European Survey of Carbapenemase-Producing Enterobacteriaceae
K. pneumoniae	*Klebsiella pneumoniae*
MIC	Minimum inhibitory concentration
NRL	National reference or expert laboratory
PCR	Polymerase chain reaction
WGS	Whole genome sequencing

Background

Carbapenem-resistant *E. coli* and *K. pneumoniae* are increasing globally including in Europe. Carbapenem use has increased since the mid-1990s because of the need to treat patients with documented or suspected infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and other third-generation cephalosporin-resistant Gram-negative bacteria [1]. The resulting increased selection pressure with carbapenems has driven the dissemination of carbapenem resistance and plasmid-mediated carbapenemases.

The increase in healthcare-associated infections caused by carbapenem-resistant bacteria, in particular *K. pneumoniae,* is of concern. Carbapenem resistance can be caused by the production of carbapenemases or chromosomal mechanisms such as altered permeability and porin loss. Carbapenemases hydrolyse the beta-lactam ring of the majority of beta-lactam antibiotics and thereby inhibit their action. Genes encoding for carbapenemase production are mostly located on a plasmid.

The European Centre for Disease Prevention and Control (ECDC) has developed a strategy for molecular surveillance of carbapenemase-producing Enterobacteriaceae [2]. This strategy, together with the experience from the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) project [3], informed the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) and the survey of carbapenem- and/or colistin-resistant Enterobacteriaceae (CCRE survey) in Europe.

CCRE survey

The primary objective of the CCRE survey is to determine the occurrence, geographic distribution and population dynamics within the healthcare setting of high-risk CCRE clones and/or transmissible resistance/genetic elements of critical public health importance in Europe in order to enable informed risk assessment and control policies.

This expert consensus protocol for carbapenem resistance detection and characterisation was jointly developed by the EURGenCCRE consortium, the scientific advisory board for EURGen-Net and ECDC to agree upon the protocol for phenotypic and genotypic carbapenem resistance detection and confirmation to be used for the CCRE survey. A separate expert consensus protocol was developed for colistin resistance detection and characterisation, as well as a laboratory manual with more detailed methodological information for characterisation of both carbapenem and colistin resistance in Enterobacteriaceae isolates.

The authors of the expert consensus protocol support and recommend the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 2.0 published in July 2017 [4]. A description of the workflow for collecting isolates for inclusion into the CCRE survey is described in the following paragraphs.

Definitions

Carbapenem non-susceptibility and detection, confirmation and differentiation of carbapenemase-producing *E. coli* and *K. pneumoniae* for inclusion in CCRE survey

The meropenem breakpoints for *E. coli* and *K. pneumoniae* are S ≤2 mg/L and R>8 mg/L. The corresponding breakpoints for ertapenem are S ≤0.5 mg/L and R>0.5 mg/L. Isolates with meropenem minimum inhibitory concentration (MIC)>2 mg/L and/or ertapenem MIC>0.5 mg/L are considered resistant or susceptible, increased exposure[1] and should be investigated for carbapenem resistance mechanisms. This approach will not identify all carbapenemase-producing *E. coli* and *K. pneumoniae* isolates, but will detect most isolates with clinically significant carbapenem non-susceptibility.

Local clinical microbiology laboratories

As specified in the ECDC study protocol for genomic-based surveillance of CCRE at the EU level [5], the participating local clinical microbiology laboratories are asked during the study period to select the first 10 non-duplicate isolates of either *E. coli* or *K. pneumoniae* that are resistant or susceptible increased exposure (MIC above the susceptible break point) to at least one of the tested carbapenems. In addition, the next carbapenem-susceptible isolate (MIC below the susceptible breakpoint) of the same species should be collected. Antimicrobial susceptibility testing (AST) should be done according to EUCAST guidelines [4] using clinical breakpoints. The

[1] New EUCAST definition of previous intermediate category applicable from January 2019: http://www.eucast.org/newsiandr.

results from the performed AST, as well as the hospital and patient data as specified in the ECDC study protocol [5], should be entered by local clinical microbiology laboratories into the database for the CCRE survey.

National reference or expert laboratories (NRLs)

By confirming the results obtained by local clinical microbiology laboratories, NRLs provide important quality assurance at the national level. In addition to information reported by local clinical microbiology laboratories, the results from all tests performed at the NRL (mandatory and voluntary, Figure 1) should be entered into the database for the CCRE survey. Detection of carbapenemases as well as other predicted resistance mechanisms will be confirmed by central whole genome sequencing (WGS). WGS results will be reported back to the NRL of each country.

Mandatory confirmation of carbapenem susceptibility testing results for CCRE survey

According to the ECDC study protocol for genomic-based surveillance of CCRE at the EU level [5], the NRL should confirm the carbapenem susceptibility testing results of all isolates. This confirmation is crucial for quality assurance and obtaining the best possible susceptibility categorisation of all isolates in the collection and subsequent analysis. Broth microdilution (BMD) and disk diffusion for meropenem and disk diffusion for ertapenem and imipenem must be performed for all isolates. BMD to test for susceptibility to imipenem and ertapenem is encouraged, but not mandatory. EUCAST clinical breakpoints should be used for the interpretation of AST results. Methods should be quality controlled using strains with known MIC values and resistance mechanisms.

If a NRL is unable to comply with mandatory testing for the CCRE survey described above, this needs to be communicated to the EURGenCCRE consortium in advance. In this communication, two questions should be addressed:

- description of the reason(s) why this protocol cannot be adhered to; and
- description of the methodological routine of the NRL upon local requests (e.g. for confirmation of clinical samples).

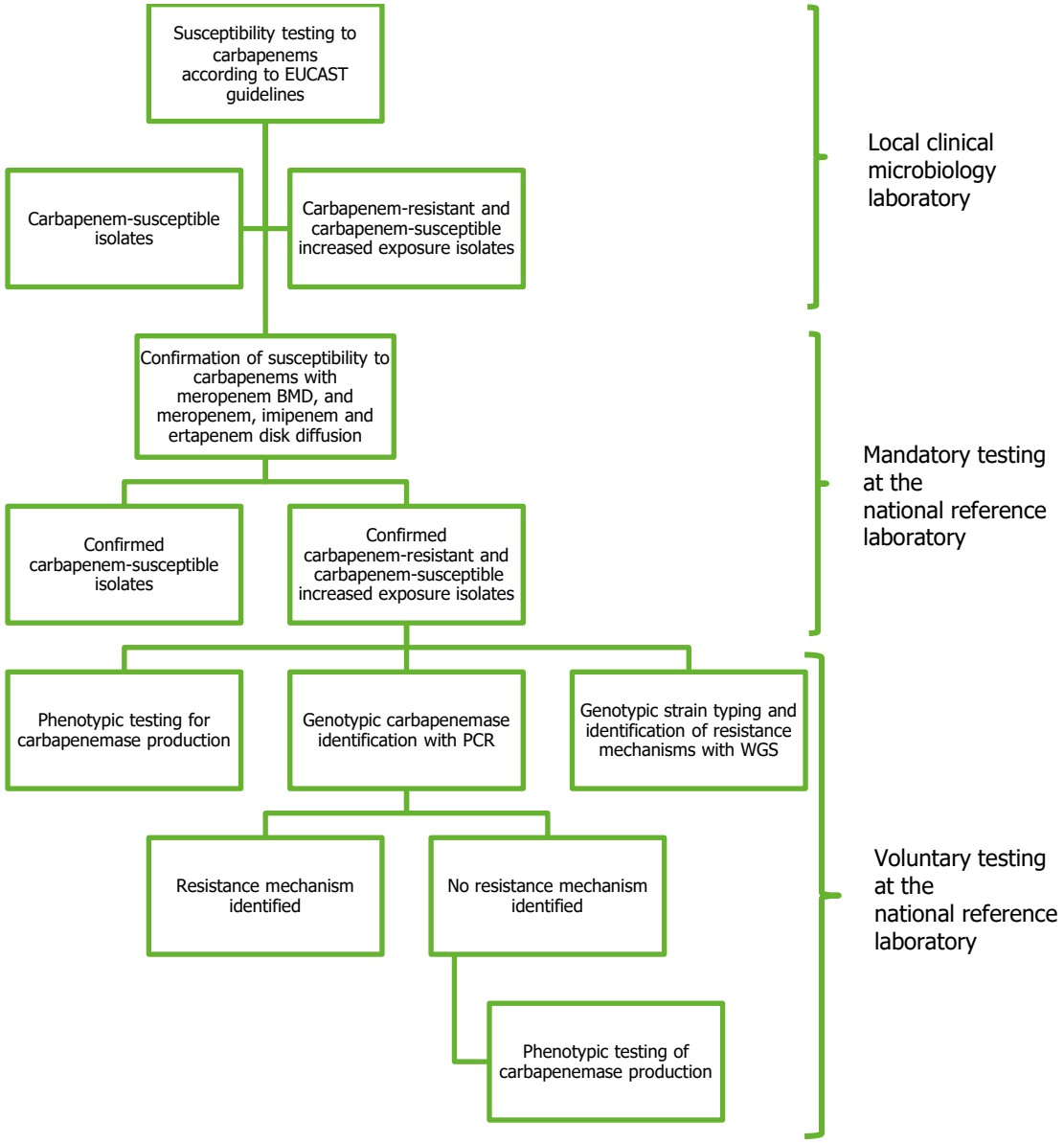
Voluntary phenotypic and genotypic confirmation and differentiation

NRLs are encouraged to proceed with the detection of carbapenemase production in carbapenem non-susceptible isolates with MIC values above the susceptible break point. This can be done by either phenotypic carbapenemase confirmation tests and/or molecular tests. If polymerase chain reaction (PCR) is used as a first step, the carbapenemase-negative isolates should be retested with a phenotypic method for carbapenemase production confirmation (Figure 1).

Laboratory procedures

Details of the laboratory methods and procedures suitable for the CCRE survey are outlined in the separate laboratory manual.

Figure 1: Overview of the isolate detection and characterisation workflow for CCRE survey



EUCAST: European Committee on Antimicrobial Susceptibility Testing
BMD: broth microdilution
PCR: polymerase chain reaction
WGS: whole genome sequencing.

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