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

Version 3.0

www.ecdc.europa.eu
Expert consensus protocol on carbapenem resistance detection and characterisation for the survey of carbapenem- and/or colistin-resistant Enterobacteriaceae

Version 3.0
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.

*Contributing authors (alphabetical order):*

This protocol was sent for consultation to the Members of the EURGen-Net Scientific Advisory Board: Sylvain Brisse (Institut Pasteur, France), Alessandra Carattoli (Istituto Superiore de Sanità, Italy), Corinna Glasner (University Medical Center Groningen, The Netherlands), Hajo Grundmann (University Medical Center Freiburg, Germany), Alexander Kallen (Centers for Disease Control and Prevention, US), Gunnar Skov Simonsen (University Hospital of North Norway, Norway), Nicole Stoesser (Nuffield Department of Medicine, University of Oxford/Oxford University Hospitals NHS Trust, UK) and Neil Woodford (Public Health England, UK).


Stockholm, January 2019

DOI: 10.2900/52645
Catalogue number: TQ-02-19-014-EN-N

© European Centre for Disease Prevention and Control, 2019

Cover picture: © Centers for Disease Prevention and Control

Reproduction is authorised, provided the source is acknowledged.

For any use or reproduction of photos or other material that is not under the EU copyright, permission must be sought directly from the copyright holders.
Contents

Abbreviations ............................................................................................................................................ iv
Background ................................................................................................................................................ 1
CCRE survey ............................................................................................................................................... 1
   Definitions ....................................................................................................................................... 1
   Local clinical microbiology laboratories........................................................................................... 1
   National reference or expert laboratories (NRLs) ............................................................................... 2
   Mandatory confirmation of carbapenem susceptibility testing results for CCRE survey .................... 2
   Voluntary phenotypic and genotypic confirmation and differentiation ................................................ 2
Laboratory procedures ............................................................................................................................. 2
References ................................................................................................................................................. 4

Figures

Figure 1: Overview of the isolate detection and characterisation workflow for CCRE survey .................... 3
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>Antimicrobial susceptibility testing</td>
</tr>
<tr>
<td>BMD</td>
<td>Broth microdilution</td>
</tr>
<tr>
<td>CCRE</td>
<td>Carbapenem- and/or colistin-resistant Enterobacteriaceae</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>ESBL</td>
<td>Extended-spectrum beta-lactamase</td>
</tr>
<tr>
<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
</tr>
<tr>
<td>EURGenCCRE</td>
<td>Genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level</td>
</tr>
<tr>
<td>EURGen-Net</td>
<td>European Antimicrobial Resistance Genes Surveillance Network</td>
</tr>
<tr>
<td>EuSCAPE</td>
<td>European Survey of Carbapenemase-Producing Enterobacteriaceae</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>NRL</td>
<td>National reference or expert laboratory</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
</tr>
</tbody>
</table>
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 exposure1 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 break point) 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/newsandpress](http://www.eucast.org/newsandpress).
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

**Susceptibility testing to carbapenems according to EUCAST guidelines**

- Carbapenem-susceptible isolates
- Carbapenem-resistant and carbapenem-susceptible increased exposure isolates

**Confirmation of susceptibility to carbapenems with meropenem BMD, and meropenem, imipenem and ertapenem disk diffusion**

- Confirmed carbapenem-susceptible isolates
- Confirmed carbapenem-resistant and carbapenem-susceptible increased exposure isolates

**Phenotypic testing for carbapenemase production**

- Resistance mechanism identified
- No resistance mechanism identified

**Genotypic carbapenemase identification with PCR**

- Genotypic strain typing and identification of resistance mechanisms with WGS

**Local clinical microbiology laboratory**

- Mandatory testing at the national reference laboratory

- Voluntary testing at the national reference laboratory

---

*EUCAST: European Committee on Antimicrobial Susceptibility Testing*

*BMD: broth microdilution*

*PCR: polymerase chain reaction*

*WGS: whole genome sequencing.*
References


European Centre for Disease
Prevention and Control (ECDC)

Address:
Gustav III:s boulevard 40, SE-169 74 Solna,
Sweden

Tel. +46 8 58601000
Fax +46 8 58601001
www.ecdc.europa.eu

An agency of the European Union
www.europa.eu

ECDC is committed to ensuring the transparency and independence of its work

In accordance with the Staff Regulations for Officials and Conditions of Employment of Other Servants of the European Union and the ECDC Independence Policy, ECDC staff members shall not, in the performance of their duties, deal with a matter in which, directly or indirectly, they have any personal interest such as to impair their independence. Declarations of interest must be received from any prospective contractor(s) before any contract can be awarded.


HOW TO OBTAIN EU PUBLICATIONS

Free publications:

• one copy:
  via EU Bookshop (http://bookshop.europa.eu);

• more than one copy or posters/maps:
  from the European Union’s representations (http://ec.europa.eu/represent_en.htm);
  from the delegations in non-EU countries (http://eeas.europa.eu/delegations/index_en.htm);
  by contacting the Europe Direct service (http://europa.eu/europedirect/index_en.htm) or calling 00 800 6 7 8 9 10 11 (freephone number from anywhere in the EU) (*).

(*) The information given is free, as are most calls (though some operators, phone boxes or hotels may charge you).

Priced publications:

• via EU Bookshop (http://bookshop.europa.eu),