

TECHNICAL REPORT

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

Version 1.0

ECDC TECHNICAL REPORT

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

Version 1.0



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):

Barbara Albiger, Alma Brolund, Sara Byfors, Christian G. Giske, Gunnar Kahlmeter, Anke Kohlenberg, Marc Struelens and Karin Tegmark Wisell.

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).

Suggested citation: European Centre for Disease Prevention and Control. Expert consensus protocol on colistin resistance detection and characterisation for the survey of carbapenem and/or colistin-resistant Enterobacteriaceae – Version 1.0. Stockholm: ECDC; 2019.

Stockholm, January 2019

ISBN: 978-92-9498-300-8

DOI: 10.2900/388839

Catalogue number: TQ-02-19-015-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 colistin susceptibility/non-susceptibility 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

AST	Antimicrobial susceptibility testing
BMD	Broth microdilution
CCRE	Carbapenem- and/or colistin-resistant Enterobacteriaceae
CPE	Carbapenemase-producing Enterobacteriaceae
CRE	Carbapenem-resistant Enterobacteriaceae
<i>E. coli</i>	<i>Escherichia coli</i>
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
<i>K. pneumoniae</i>	<i>Klebsiella pneumoniae</i>
LPS	Lipopolysaccharide
MIC	Minimum inhibitory concentration
NRL	National reference or expert laboratory
PCR	Polymerase chain reaction
WGS	Whole genome sequencing

Background

Colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* are increasing globally. Of particular concern is the rapidly increasing occurrence of colistin resistance amongst carbapenem-resistant Enterobacteriaceae (CRE). Colistin resistance in CRE has traditionally been encountered in hospital settings. Historically, the mechanisms of colistin resistance were described as chromosomal, but plasmid-mediated (mobile) colistin resistance conferred by the *mcr* gene was reported for the first time in 2015. Faecal carriage of *mcr*-positive Enterobacteriaceae has been reported in up to 30% of humans in community settings in China, with the *mcr* gene associated with successful plasmid families [1,2]. Plasmid-mediated co-resistance to both colistin and carbapenems has been described for *mcr* and the *bla*_{NDM}, *bla*_{KPC}, *bla*_{IMP} and *bla*_{OXA-48} carbapenemase genes. This is of significant concern since the potential for colistin and carbapenem resistance genes to disseminate jointly increases considerably if they are associated on mobile genetic elements.

Colistin is active against most members of the Enterobacteriaceae family and common non-fermentative Gram-negative bacteria [3]. It is a cationic lipopeptide antibiotic and acts by disrupting the inner and outer bacterial cell membranes through binding to the negatively charged lipopolysaccharide (LPS). The *mcr* gene encodes for production of a phosphoethanolamine transferase. This enzyme binds to lipid A and adds a phosphoethanolamine group to the core component of the LPS, thus rendering the outer bacterial cell membrane less anionic and impairing the interaction with the cationic colistin. Several chromosomally mediated colistin resistance mechanisms have also been described.

The European Centre for Disease Prevention and Control (ECDC) has developed a strategy for molecular surveillance of carbapenemase-producing Enterobacteriaceae [4]. This strategy together with the experience from the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) project [5] 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 healthcare settings 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 colistin resistance detection and characterisation was jointly developed by the EURGenCCRE consortium, the scientific advisory board for EURGen-Net and ECDC to agree upon the best available strategy for phenotypic and genotypic colistin-resistance detection and confirmation to be used for the CCRE survey. A separate expert consensus protocol was also developed for carbapenem resistance detection and characterisation, as well as a laboratory manual with detailed methodological information for characterisation of CCRE isolates.

The authors of this expert consensus protocol support and recommend the application of 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 [6]. A description of the workflow for collecting isolates for inclusion in the CCRE survey is described in the following paragraphs.

Definitions

Colistin non-susceptibility and detection, confirmation and differentiation of colistin-resistant and/or *mcr*-positive *E. coli* and *K. pneumoniae* for inclusion in CCRE survey

Colistin-non-susceptible Enterobacteriaceae are defined as isolates with a colistin minimum inhibitory concentration (MIC) >2 mg/L and/or with detection of an *mcr* gene.

Local clinical microbiology laboratories

As specified in the ECDC study protocol for genomic-based surveillance of CCRE at the EU level [7], participating local clinical microbiology laboratories can also include *E. coli* or *K. pneumoniae* isolates with phenotypic resistance to colistin or detection of an *mcr* gene in the CCRE survey. It is recommended that colistin susceptibility testing is done in accordance with EUCAST guidelines [6], i.e. determination of MIC with broth microdilution (BMD), followed by interpretation using EUCAST clinical breakpoints. If other colistin susceptibility testing or resistance detection

methods are used, they should be described by each participating local clinical microbiology laboratory. Detection of the *mcr* gene can be performed with any validated method. The method used and results from the performed antimicrobial susceptibility testing as well as hospital and patient data as specified in the ECDC study protocol [7] should be entered by local clinical microbiology laboratories in 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 the local clinical microbiology laboratories, the results from tests performed at the NRL (mandatory and voluntary, Figure 1) should be entered in the database for the CCRE survey. Detection of colistin resistance mechanisms will be confirmed by whole genome sequencing (WGS) performed centrally. WGS results will be reported back to the NRL of each country.

Mandatory confirmation of colistin susceptibility/non-susceptibility for CCRE survey

According to the ECDC study protocol for genomic-based surveillance of CCRE at the EU level [7], the NRL should confirm colistin susceptibility or non-susceptibility of all isolates. This applies to the isolates selected based on their carbapenem resistance as well as colistin-resistant isolates included on a voluntary basis. This confirmation is crucial for quality assurance and obtaining the best possible interpretability of the isolate collection and subsequent analysis. The colistin susceptibility/non-susceptibility of all isolates should be tested by determination of MIC with BMD. Importantly, no surrogate method for determination of MIC can be used. EUCAST breakpoints should be applied for the interpretation of BMD MIC results.

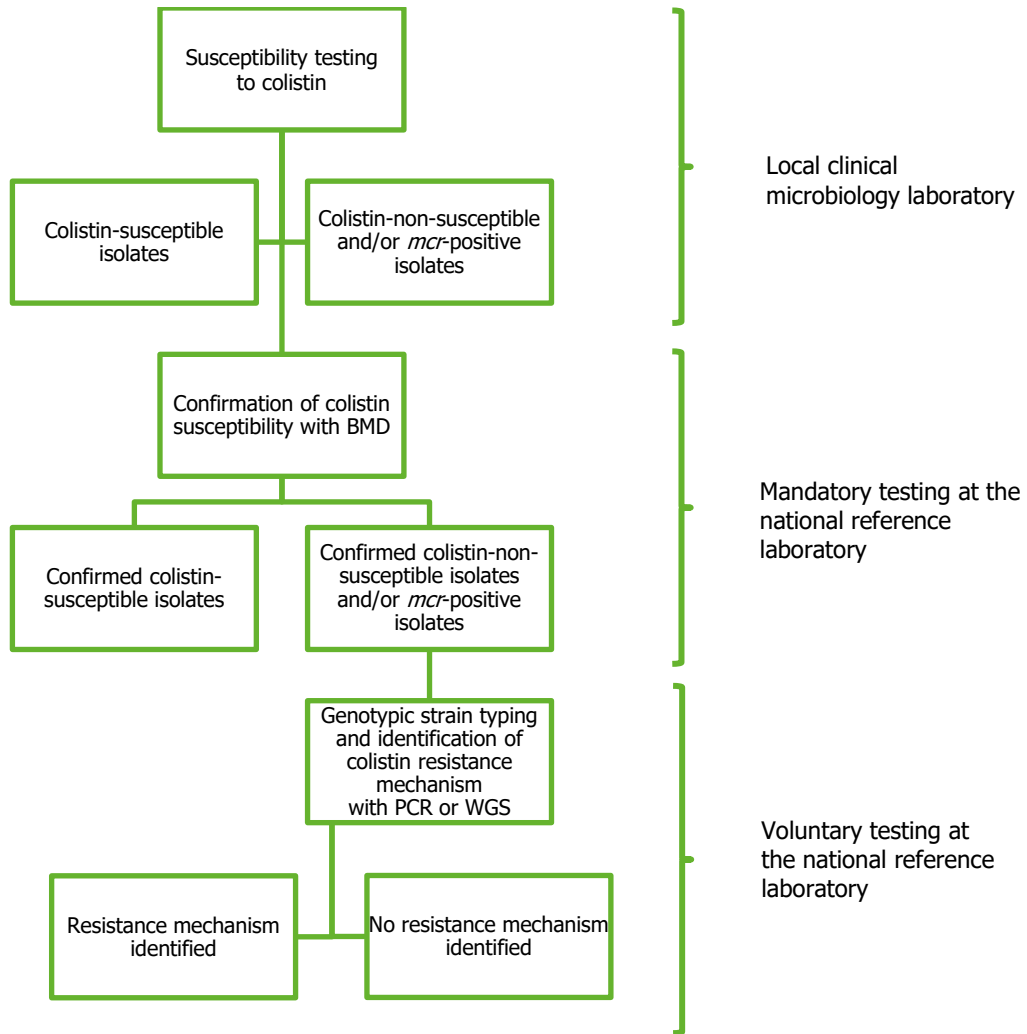
Voluntary phenotypic and genotypic confirmation and differentiation

NRLs are encouraged to proceed with the detection of colistin resistance mechanisms in carbapenem- and/or colistin-resistant isolates. This can be done with various molecular methods.

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



BMD: broth microdilution

PCR: polymerase chain reaction

WGS: whole genome sequencing.

References

1. Shen Y, Zhou H, Xu J, Wang Y, Zhang Q, Walsh TR, et al. Anthropogenic and environmental factors associated with high incidence of *mcr-1* carriage in humans across China. *Nat Microbiol*. 2018 Sep;3(9):1054-1062.
2. Zhong LL, Phan HTT, Shen C, Vihta KD, Sheppard AE, Huang X, et al. High Rates of Human Fecal Carriage of *mcr-1*-Positive Multidrug-Resistant Enterobacteriaceae Emerge in China in Association With Successful Plasmid Families. *Clin Infect Dis*. 2018 Feb 10;66(5):676-685.
3. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin Microbiol Rev*. 2017 Apr;30(2):557-596.
4. European Centre for Disease Prevention and Control. ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness – Version 2.1, 2016-19. Stockholm: ECDC; 2016. Available from: <http://ecdc.europa.eu/publications-data/ecdc-roadmap-integration-molecular-typing-and-genomic-typing-european-level>.
5. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*. 2017 Feb;17(2):153-163.
6. 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, July 2017. Växjö: EUCAST; 2017. Available from: http://www.eucast.org/resistance_mechanisms.
7. European Centre for Disease Prevention and Control. ECDC study protocol for genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level – Version 2.0. Stockholm: ECDC; 2018. Available from: <http://ecdc.europa.eu/publications-data/ecdc-study-protocol-genomic-based-surveillance-carbapenem-resistant-andor>.

**European Centre for Disease
Prevention and Control (ECDC)**

Address:
Gustav III:s boulevard 40, SE-169 73 Solna,
Sweden

Tel. +46 858601000
Fax +46 858601001
www.ecdc.europa.eu

An agency of the European Union
www.europa.eu

Subscribe to our publications
www.ecdc.europa.eu/en/publications

Contact us
publications@ecdc.europa.eu

Follow us on Twitter
[@ECDC_EU](https://twitter.com/ECDC_EU)

Like our Facebook page
www.facebook.com/ECDC.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.
www.ecdc.europa.eu/en/aboutus/transparency

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>).



■ Publications Office