TECHNICAL REPORT

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

Version 1.0

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### Abbreviations

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<th>Definition</th>
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<tr>
<td>AST</td>
<td>Antimicrobial susceptibility testing</td>
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<tr>
<td>BMD</td>
<td>Broth microdilution</td>
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<tr>
<td>CCRE</td>
<td>Carbapenem- and/or colistin-resistant Enterobacteriaceae</td>
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<tr>
<td>CPE</td>
<td>Carapenemase-producing Enterobacteriaceae</td>
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<tr>
<td>CRE</td>
<td>Carbapenem-resistant Enterobacteriaceae</td>
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<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
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<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
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<tr>
<td>EURGenCCRE</td>
<td>Genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level</td>
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<tr>
<td>EURGen-Net</td>
<td>European Antimicrobial Resistance Genes Surveillance Network</td>
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<tr>
<td>EuSCAPE</td>
<td>European Survey of Carbapenemase-Producing Enterobacteriaceae</td>
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<td>K. pneumoniae</td>
<td>Klebsiella pneumoniae</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<td>NRL</td>
<td>National reference or expert laboratory</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>WGS</td>
<td>Whole genome sequencing</td>
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</table>
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*<sub>TEM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub> and *bla*<sub>OXA-48</sub> 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 \textit{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

- **Susceptibility testing to colistin**
  - Colistin-susceptible isolates
  - Colistin-non-susceptible and/or mcr-positive isolates

- **Confirmation of colistin susceptibility with BMD**
  - Confirmed colistin-susceptible isolates
  - Confirmed colistin-non-susceptible isolates and/or mcr-positive isolates

- **Genotypic strain typing and identification of colistin resistance mechanism with PCR or WGS**
  - Resistance mechanism identified
  - No resistance mechanism identified

- **Local clinical microbiology laboratory**
- **Mandatory testing at the national reference laboratory**
- **Voluntary testing at the national reference laboratory**

*BMD: broth microdilution
PCR: polymerase chain reaction
WGS: whole genome sequencing.*
References


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