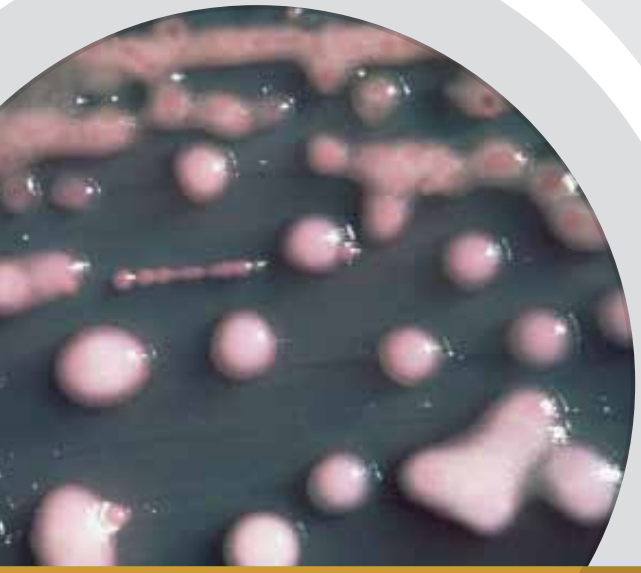

TECHNICAL DOCUMENT

ECDC study protocol for genomic-based surveillance of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* at the EU level

Version 1.1

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This protocol of the European Centre for Disease Prevention and Control (ECDC) was coordinated by the Microbiology Coordination Section, the Antimicrobial Resistance and Healthcare-Associated Infections (ARHAI) Disease Programme and the Molecular Surveillance Operations Group.

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Contents

Abbreviations

AST	Antimicrobial susceptibility testing
CPE	Carbapenemase-producing *Enterobacteriaceae*
ColRE	Colistin-ressitant *Enterobacteriaceae*
E. coli	*Escherichia coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EuSCAPE	European Survey on Carbapenemase-producing *Enterobacteriaceae*
K. pneumonia	*Klebsiella pneumoniae*
MDR	Multidrug-resistant
MLST	Multi Locus Sequence Typing
NUTS	Nomenclature of Territorial Units for Statistics
PCR	Polymerase chain reaction
WGS	Whole genome sequencing

Executive summary

This ECDC study protocol describes the technical requirements for implementing future EU-level genomic-based surveillance of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae.* It is meant to guide the consolidation of ECDC activities in relation to molecular typing of multidrug-resistant pathogens, and to focus the development of genomic typing enhanced surveillance. It builds upon and synthesises evidence and the opinion of experts in Member States and at ECDC, compiled since 2014.

The EU-wide whole genome sequencing-based surveillance of carbapenem-resistant and/or colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* is based on the model of the European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) project. The proposed surveillance design is a structured, periodically repeated pan-European multi-centre molecular epidemiological survey of the prevalence and distribution at regional, national and European level of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* by genotype among patients seeking hospital care.

The primary public health objective of this type of EU-level surveillance is to determine the occurrence, geographic distribution and the population dynamics of high-risk carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* clones, and/or transmissible resistance/genetic elements of critical public health importance in Europe, in order to inform risk assessment, prevention and control policies.

The secondary objectives are:

- to identify the epidemiological risk factors for infection or colonisation with carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* at bacterial, clonal and sub-genomic level
- to support EU Member States in developing technical capability and proficiency for genomic-based surveillance and risk assessment of multidrug-resistant pathogens with epidemic potential.

An additional public health added-value at the national level is that such highly discriminatory typing should facilitate the analysis of transmission chains and source identification of emerging clones, facilitating targeted interventions and improving understanding of the determinants of healthcare-associated infections.

Background

The past decades have witnessed the rapid emergence of multidrug-resistant (MDR) gram-negative bacteria [1]. This increasing resistance originates from multiple mechanisms including the acquisition of resistance genes carried by mobile genetic elements (e.g. plasmids). The global epidemics of extended-spectrum beta-lactamases (ESBLs), carbapenemases and of the recently discovered *mcr-1*-mediated colistin resistance are typical examples of these plasmid-mediated resistance epidemics [2,3].

Classical surveillance of antimicrobial resistance is currently based on phenotypical testing, which does not allow understanding of transmission dynamics of resistance genes in the human population needed for the implementation of targeted control measures. Only genomic level investigations combined with epidemiological data will provide information with sufficient resolution on the distribution of resistance genes by space, time and person, enabling the reconstruction of transmission chains and source identification [4].

In 2012, ECDC launched the European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) project to gain insights into the occurrence, epidemiology and the spread of carbapenemase-producing *Enterobacteriaceae,* and to build laboratory capacity for diagnosis and surveillance of carbapenemase-producing Enterobacteriaceae (CPE) in Europe [5-7]. The data collected showed that on average, 1.3 patients per 10 000 hospital admissions have carbapenemase-producing *Klebsiella pneumoniae* or *Escherichia coli* isolated from a clinical specimen in European hospitals [7]. In the same study, an increasing number of European countries reported interregional hospital spread of CPE or an endemic situation [7]. The EuSCAPE project demonstrated the feasibility of conducting integrated epidemiological and microbiological sentinel multi-centre structured surveys, and of collecting comparable quality-assessed data suitable for EU-level analyses.

In 2014, ECDC held two expert consultations on the development of a molecular strategy for MDR pathogens, resulting in an ECDC strategy for molecular surveillance of carbapenemase-producing *Enterobacteriaceae*[i], supported by the ECDC Advisory Forum. This strategy defined the EU-level public health objective, as well as the EU-level risk assessment benefits and potential risk management implications of the integrated analysis of epidemiological and microbiological data on CPE (Table 1).

Table 1. EU-level public health objective, risk assessment benefits and potential risk management implications of molecular typing of carbapenemase-producing *Enterobacteriaceae* and colistin-resistant *Enterobacteriaceae* [8]

EU-level public health objective	EU-level risk assessment benefits	Potential risk management implications
To collect and analyse information about the occurrence and dynamics of high-risk CPE and colistin-resistant *Enterobacteriaceae* (ColRE) clones, and/or transmissible resistance/ genetic elements of critical public health importance in Europe.	Detection and genotypic identification of high-risk* clones/plasmids Monitoring time trends in the frequency of particular genotypes in the population, and identification of high-risk population groups Identification of geographical areas associated with spread of specific high-risk clones Detection/delineation of cross-region and cross-border dissemination of high-risk clones/plasmids	Evaluation, refinement and/or revision of local, regional and national infection control and prevention programmes. Contribution to the impact evaluation of hospital and community antibiotic policies and stewardship programmes Better targeting of resources to high-risk populations, geographical areas and dissemination pathways

**High-risk CPE clones/plasmids: ecologically successful clonal types carrying plasmid-borne carbapenemases or carbapenemase-encoding plasmids associated with high or increasing population prevalence across surveys, and/or displaying extensive or expanding geographical distribution (inter-regional spread or international spread) and/or association with multiple hospital outbreaks reported in the literature.*

Using the guidance of the carbapenemase-producing *Enterobacteriaceae* molecular surveillance strategy, and building on the experience of the EuSCAPE project, this ECDC study protocol was developed to describe the technical requirements for implementing the EU-level genomic-based surveillance of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae.*

[i] AF40/05 the carbapenemase-producing *Enterobacteriaceae* molecular surveillance strategy.

Objectives of the study

The primary EU-level public health objective is to determine the occurrence, geographic distribution and population dynamics within the healthcare setting of high-risk carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* clones, and/or transmissible resistance/genetic elements of critical public health importance in Europe, in order to inform risk assessment and control policies.

The secondary objectives are:

- to identify the epidemiological risk factors for infection or colonisation with carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* at bacterial clonal and sub-genomic level
- to support Member States in developing technical capability and proficiency for genomic-based surveillance and risk assessment of multidrug-resistant pathogens associated with epidemic potential.

Study protocol

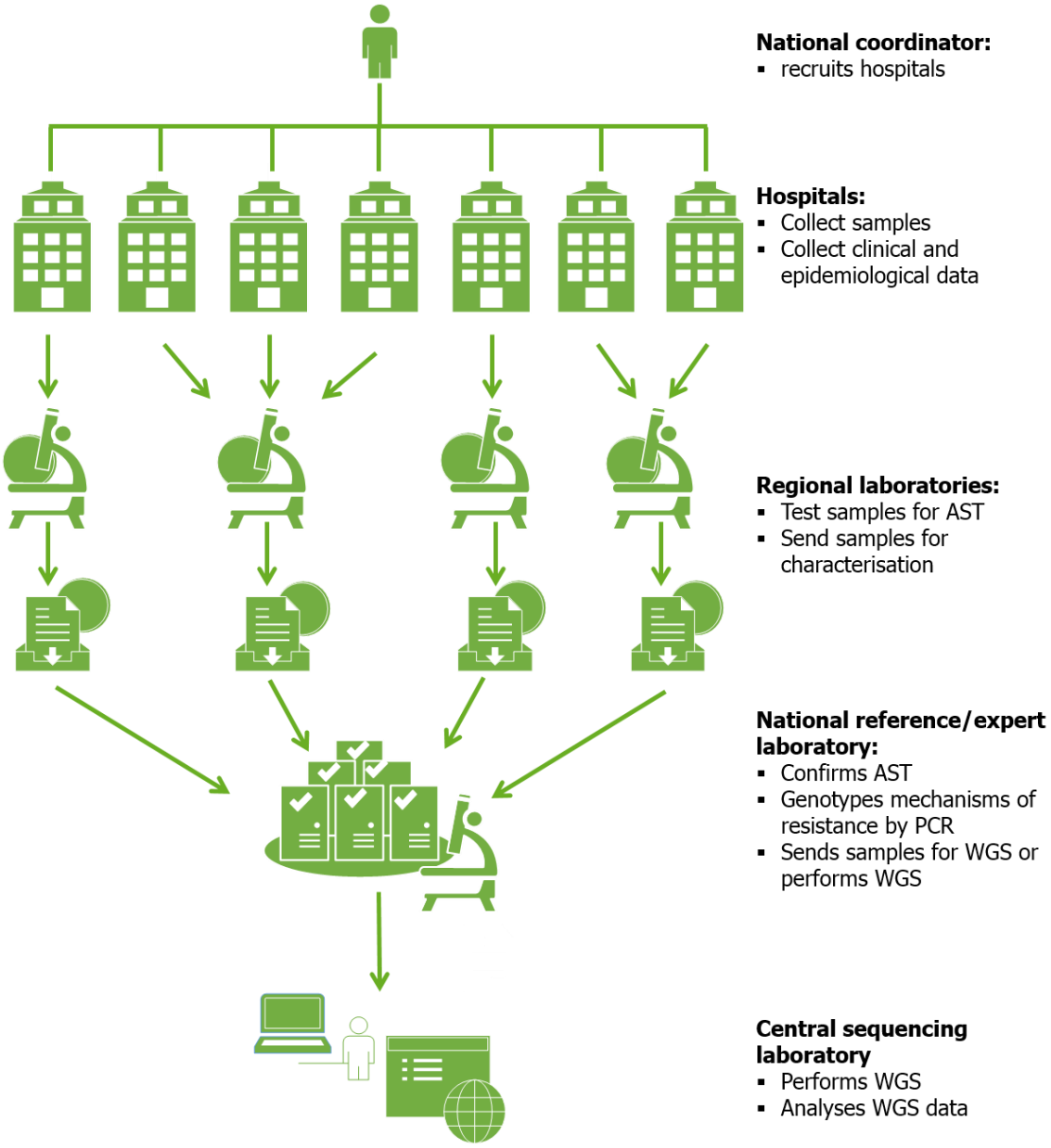
Study and surveillance design

The study design follows the EUSCAPE project model (Figure 1) and the EuSCAPE structured survey protocol: a stepwise workflow through the structured survey performed on a country by country basis [7].

The proposed surveillance design is a structured, periodically repeated pan-European multi-centre molecular epidemiological survey of the prevalence and distribution of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae* by genotype among patients seeking hospital care at regional level.

The frequency of these surveys is foreseen to be every third year, or more frequently as indicated by the epidemiological situation, and subject to resource availability at ECDC, and the national and regional levels.

Figure 1. National workflow for participation in the EU-level genomic-based surveillance of carbapenem-resistant and/or colistin-resistant *Enterobacteriaceae.*



AST: antimicrobial susceptiblility testing; PCR: polymerase chain reaction; WGS: whole genome sequencing

Study inclusion criteria

National coordinator and national reference/expert laboratory

Each participating country selects a national coordinator and a national reference/expert laboratory with strong expertise in molecular characterisation of carbapenemase-producing and colistin-resistant *Enterobacteriaceae.* The national coordinator has the central role of coordinating the national workflow from recruiting the participating hospitals and associated laboratories, to supervising the national sample and data collection (Figure 1). He or she is preferably located within the national reference/expert laboratory, which has the responsibility to further characterise the collected isolates.

Hospitals and regional laboratories

Participating hospitals and their associated laboratories are selected with the aim to cover the full extent of the healthcare referral network across the national territory, to map the inter-hospital dissemination perimeter and the spatial structure. The selected hospitals should offer acute care services as part of the national healthcare system.

Selection of hospitals

Since there is no European healthcare referral network map, the selection of the sampling sites is based on the Nomenclature of Territorial Units for Statistics (NUTS)-2 regions, the broad geo-demographic territorial sub-units of Member States. At least one sentinel acute care hospital site per NUTS-2 region with a population of at least 250 000 inhabitants and located on the European mainland is included [9]. For NUTS-2 regions with a population of more than 2.5 million inhabitants, an additional hospital should be recruited for this survey if possible.

Selection of laboratories associated with hospitals included in the survey

Only laboratories that routinely test all clinical isolates of *Enterobacteriaceae* for susceptibility against any of the commonly available carbapenems (imipenem, meropenem, ertapenem, or doripenem) should be included.

Selection of national reference/expert laboratory

Only laboratories performing reference services related to carbapenem resistance and colistin resistance of *Enterobacteriaceae,* including antimicrobial susceptibility testing (AST) and genotyping of carbapenemase genes and colistin resistance genes should be selected.

Patient population

The target patient populations are recruited at the acute care hospital level:

- **outpatients**: patients not hospitalised or those staying in hospital for less than 24h (day care) at the time of clinical sampling
- **inpatients:** patients hospitalised for more than 24h at the time of clinical sampling.

Bacterial species

For the first survey, the target bacterial species proposed for inclusion are *Klebsiella pneumoniae* and *Escherichia coli.* In subsequent surveys, the species of *Enterobacteriaceae* included may require revision/extension depending on changes in the epidemiological situation.

Case definition

A case of carbapenem-resistant or colistin-resistant *K. pneumoniae,* or *E. coli* colonisation or infection, is an individual patient colonised and/or infected with at least one of the following isolates:

- an isolate of carbapenem-resistant *E. coli* or
- an isolate of carbapenem-resistant *K. pneumoniae* or
- an isolate of *E. coli* or *K. pneumoniae* with confirmed colistin resistance and/or detection of a *mcr* gene (e.g., *mcr*-1, *mcr*-2).

The confirmation of a case of carbapenem-resistant *Enterobacteriaceae* and/or colistin-resistant *Enterobacteriaceae* (ColRE) requires a three-step microbiological characterisation (Figure 2):

- Step 1: Detection of isolates showing non-susceptibility to any carbapenem (e.g. imipenem, meropenem, ertapenem, doripenem) or to colistin at the hospital level, detected by routine microbiological characterisation (i.e. species identification, AST according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) carbapenem and colistin testing guidelines and recommendations, phenotypic testing for carbapenemase production) [10,11];
- Step 2: Confirmation of the phenotypic carbapenem resistance at a national reference/expert laboratory level and molecular characterisation i.e. positive genotypic confirmation of presence of carbapenemase genes (e.g. by PCR or sequencing), or positive genotypic confirmation (e.g. by PCR or sequencing) of *mcr* colistin resistance genes;

- Step 3: Identification and confirmation of the resistance mechanisms and genetic background of all isolates regardless of detected phenotypic or molecular resistance, by whole genome sequencing at the national reference/expert laboratory level or at an appointed central sequencing facility.

Comparator patient definition

A comparator patient is an individual patient colonised and/or infected with a carbapenem-susceptible *K. pneumoniae* and *E. coli.* Isolates from comparator patients are collected and included in the analysis to enable characterisation of the baseline genomic population structure.

Sample design

Sampling period

The sampling period is a maximum of six months per survey and the collection start date is defined before the launch of each survey. Countries collecting the required isolates in less than six months are asked to record and report the actual sampling period.

Biological samples

Non-duplicate bacterial isolates from both individual patients meeting the case definition, and individual comparator patients meeting the comparator definition are accepted. Isolates from any clinical specimens routinely collected for diagnostic purposes (e.g. blood, urine, sputum, wound secretion, etc.) can be included. Rectal or faecal samples for screening for CPE or ColRE rectal carriage are excluded, due to widely variable screening practice between hospitals.

Sample size

Cases: the first ten non-duplicate consecutive isolates of carbapenem non-susceptible *E. coli* or *K. pneumoniae* isolated from clinical samples from individual consecutive patients[ii]

Comparators: for each case identified according to the definition above, the first following carbapenem-susceptible isolate of the same species, from a clinical sample of an individual comparator patient up to a maximum ten isolates.

Clinical and epidemiological data

Each collected isolate is accompanied by the respective microbiological, clinical and epidemiological data and/or respective whole genome sequence data.

Sample flow

(Step 1) Sample identification and antimicrobial susceptibility testing

Consecutive presumptive cases and comparator patients are passively identified through routine culturing at the hospital level according to EUCAST detection guidance [10-12]. At the hospital level, each isolate collected is phenotypically tested and characterised by routine AST for carbapenem non-susceptibility, i.e. minimum inhibitory concentration of clinically relevant antibacterial agents (ISO standard microdilution test method or validated equivalent assay), as described by the EuSCAPE laboratory manual: identification and confirmation of carbapenemase-producing *Enterobacteriaceae* [7]. The locally-used susceptibility testing method(s) and interpretation guidelines are recorded for each participating hospital site.

The carbapenem non-susceptible isolates (Step 1 of the case definition), and the carbapenem susceptible isolates (from comparator patients) collected by the hospitals are sent to the national reference/expert laboratories for confirmation and further characterisation (Step 2 of the case definition).

(Step 2-3) Carbapenemase production and colistin resistance case confirmation

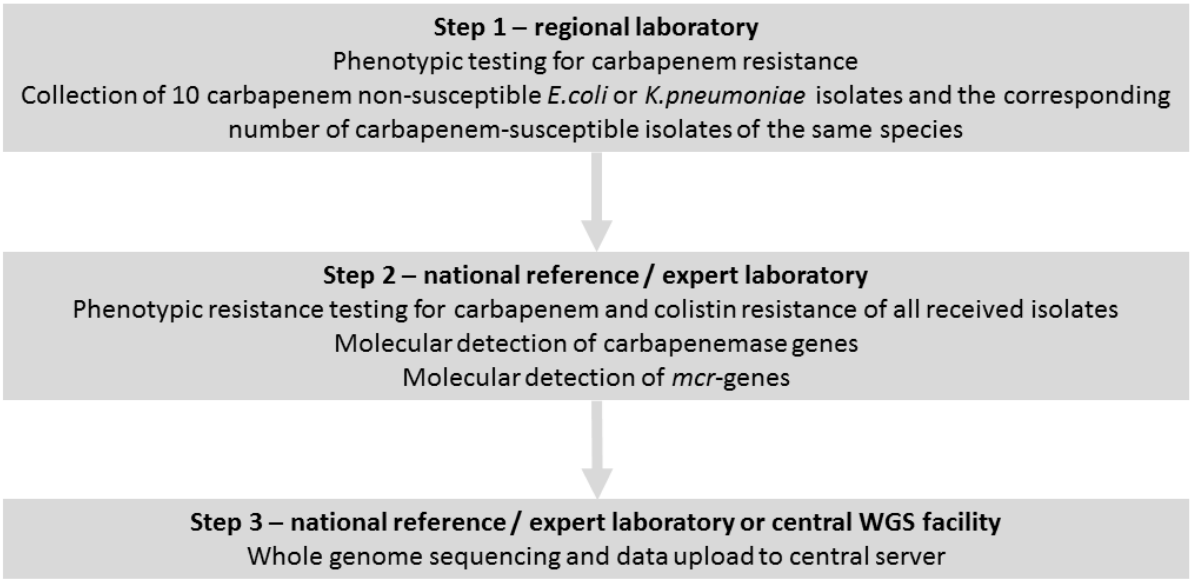
Confirmation and characterisation of case and comparator isolates is performed at the at national reference/expert laboratory level. It includes:

- phenotypic characterisation in accordance with EUCAST guidelines [10,11].
- genotypic characterisation of carbapenemase genes and colistin resistance genes:
  - by PCR or whole genome sequencing (WGS)* to identify family level for CPE
  - by PCR or WGS* to identify *mcr* genes (e.g., *mcr-1* and *mcr-2* family)
  - by WGS* to characterise other genetic resistance determinants.

[ii] As colistin susceptibility testing is not part of routine AST of *Enterobacteriaceae*, a case definition was chosen that focuses on carbapenem resistance. However, this way of sampling will not provide a representative sample of colistin-resistant *Enterobacteriaceae*.

All national reference/expert laboratories are requested to perform genotypic characterisation of carbapenemase genes by PCR. To ensure quality and comparability of data, all national reference/expert laboratories performing phenotypic and genotypic characterisation should participate in a pre-survey EQA exercise, i.e. MIC determination of colistin and genotypic confirmation of the presence of the *mcr* genes (e.g. *mcr-1* and *mcr-2*) presence by PCR [3,10,13,14].

Figure 2. Sample flow for phenotypic and molecular resistance testing



**All CPE/ColRE and susceptible isolates are sent to the appointed central sequencing laboratory for WGS analysis. If national capacity allows, isolates are sequenced at the national level using an agreed EU WGS protocol and compatible platform.*

Data collection

The data collection includes variables at the isolate level (microbiological data),the patient level (epidemiological and clinical data) and the hospital level (hospital data), and requires an active collection of laboratory and hospital information as well as information from medical records and/or clinician interview of the patient. If national capacity allows, WGS data are collected at national level, otherwise, they are collected centrally.

A unique identifier based on country code, year, hospital ID and case number is generated for each case/comparator patient filed in the system. This identifier is used to link case-based data to isolate-based information in the European Surveillance System during integrated analysis.

Isolate data

Microbiological data

- isolate unique identifier
- bacterial species
- sample collection date
- type of clinical specimen (e.g. urine, blood, lower respiratory tract specimen, wound, puncture fluid, abdominal soft tissue, catheter exit site, bone and joint specimen, cerebrospinal fluid, reproductive tract samples, other)
- routine antimicrobial susceptibility testing results and method(s) and interpretation guidelines used
  - Aminoglycosides: amikacin, tobramycin, gentamicin
  - Beta-lactams/penicillins: ampicillin
  - Beta-lactams/monobactams: aztreonam
  - Beta-lactams/carbapenems: ertapenem, imipenem, meropenem
  - Beta-lactams/cephalosporins: cefotaxime, cefepime, ceftazidime
  - Beta-lactam - beta-lactamase inhibitor combinations: amoxicillin-clavulanic acid, piperacillin-tazobactam
  - Fluoroquinolones: ciprofloxacin
  - Glycylcyclines: tigecycline
  - Polymyxins: colistin
  - Other: trimethoprim-sulfamethoxazole, fosfomycin
- mechanisms of resistance from the PCR results or WGS

Patient data

Demographic data

- age
- gender
- type of patient (e.g. in- or outpatient)
- type of unit/ward (e.g. intensive care unit, surgical, medical, etc.)
- date of hospitalisation

Epidemiological and clinical data

- colonisation or infection or undetermined clinical significance
- organ/system or location of infection/colonisation (skin and soft tissue, urinary tract, intra-abdominal, bloodstream, lower respiratory, etc.)
- hospital-acquired or community-onset:
  - **community-onset** if the sample is collected from an outpatient or from a hospitalised patient with acute care hospital stay shorter than 48h post-admission
  - **hospital-acquired** colonisation/infection if the sample is collected from an inpatient with acute care hospital stay longer than 48h post-admission.

Healthcare exposure/referral history

- direct hospital transfer from:
  - another hospital in the same country
  - a hospital in another EU/EEA country (specify country)
  - a hospital in a non-EU/EEA country(specify country)
- previous hospitalisation within six months in:
  - same hospital
  - another hospital in the same country
  - a hospital in another EU/EEA country (specify country)
  - a hospital in a non-EU/EEA country (specify country)
  - unknown hospital
- previous residence in a long-term/elderly care facility (direct transfer or within six months):
  - in the same country
  - in another EU/EEA country (specify country)
  - in a non-EU/EEA country (specify country)
  - unknown long-term/elderly care facility

Travel history

- recent (past six months) travel history to another country other than country of hospitalisation (if yes, specify country).

Hospital data

Hospital form

- hospital unique identifier code (provided by the national coordinator)
- hospital location (NUTS-2 region name/code) and geolocation data
- estimate of population in the hospital catchment area
- actual sampling period
- number of occupied bed-days during sampling period
- total number of patient admissions during the actual sampling period
- total number of patients colonised and/or infected with *E. coli* during the actual sampling period
- total number of patients colonised and/or infected with *K. pneumoniae* during the actual sampling period
- practice of patient screening for CRE/CPE and colistin resistance.

Whole genome sequencing data

Whole genome sequencing data are either produced by an appointed central typing laboratory or collected from the national reference/expert laboratories for a centralised WGS analysis approach.

Data submission

The hospital, epidemiological and microbiological data are submitted directly or in a bulk to a web-based system, which is only accessible by selected staff in participating hospitals, the national coordinator and selected staff at national reference/expert laboratories as well as ECDC.

WGS data are deposited in a closed WGS workspace (to be defined at a later stage), open to a restricted group of users defined by the study coordinator and ECDC (e.g. national coordinators etc.).

Hospitals and regional laboratories

Each participating hospital submits isolate data, patient data and hospital data:

- Isolate data and patient data completed for each case/comparator patient from which a susceptible or non-susceptible isolate has been collected for further microbiological characterisation. Data on the mechanisms of resistance from the PCR results is collected by the national reference/expert laboratory.
- Hospital data - hospitals with more than one geographical site complete one hospital form per site.

National reference/expert laboratory

National reference/expert laboratories submit the complementary microbiological characterisation data, including data on the mechanisms of resistance from the PCR results and/or WGS data if available.

Central sequencing laboratory

Central sequencing laboratories provide the whole genome raw reads.

ECDC

All isolate, patient and hospital data are stored in a designated database. A unique identifier based on country code, year, hospital identifier and isolate identifier is generated for each case/comparator patient filed. This identifier is used to link isolate-based information to the genome data during integrated analysis.

Data management and analysis

The description of the analytical methodology for WGS analysis planned for identification of resistance determinants and clonal type of resistant organisms will not be specified in detail here.

Several different analytical approaches will support the WGS analysis, including phylogenetic analysis and resistome/virulence profiling, including analysis of the correlation between phenotypic resistance to carbapenem and colistin and known carbapenem and colistin genetic resistance markers, and the characterisation of the baseline genomic population structure.

Sequence-derived information, such as multi locus sequence typing (MLST) type, core genome MLST lineage and sub-lineage, predicted virulence determinants (virulome), and antimicrobial resistance determinants (resistome), will be retrieved through taxonomic and functional gene identification algorithms and nomenclature annotation (via public access bioinformatics platforms such as the Bacterial Isolate Genome Sequence Database at the Institut Pasteur http://bigsdb.web.pasteur.fr/klebsiella and the Comprehensive Antibiotic Research Database ; http://arpcard.mcmaster.ca).

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