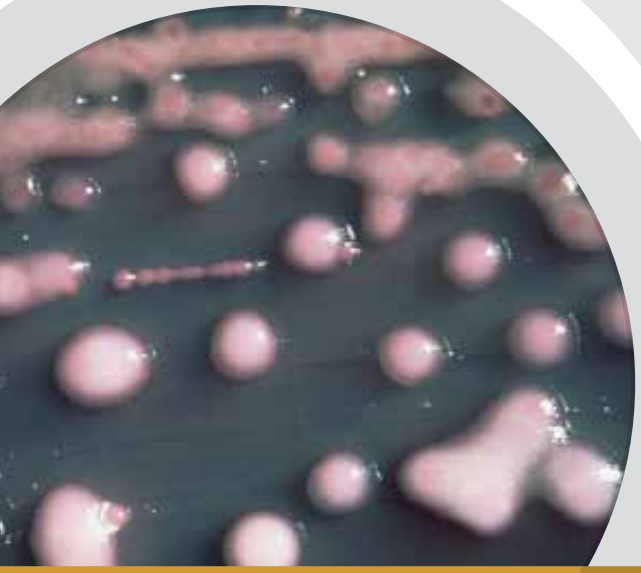

TECHNICAL DOCUMENT

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

Version 2.0

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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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Abbreviations

AST	Antimicrobial susceptibility testing
CPE	Carbapenemase-producing Enterobacteriaceae
CRE	Carbapenem-resistant Enterobacteriaceae
ColRE	Colistin-resistant Enterobacteriaceae
E. coli	*Escherichia coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EuSCAPE	European survey on carbapenemase-producing Enterobacteriaceae
K. pneumoniae	*Klebsiella pneumoniae*
MDR	Multidrug resistant
MLST	Multilocus sequence typing
NUTS	Nomenclature of territorial units for statistics
PCR	Polymerase chain reaction
WGS	Whole genome sequencing

Executive summary

This updated ECDC study protocol describes the technical requirements for implementing future EU-level genomic-based surveillance of carbapenem-resistant Enterobacteriaceae (CRE) and/or colistin-resistant Enterobacteriaceae (ColRE). 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- and/or colistin-resistant *Escherichia coli (E. coli)* and *Klebsiella pneumoniae* (*K. pneumonia)* is based on the model of the European survey on carbapenemase-producing Enterobacteriaceae (EuSCAPE) project. The proposed surveillance design is a structured and periodically repeated pan-European multicentre molecular epidemiological survey of the prevalence and distribution at the regional, national and European levels of CRE and/or ColRE 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 population dynamics of high-risk CRE and/or ColRE clones, and/or transmissible resistance/genetic elements of critical public health importance in Europe, in order to inform risk assessment, prevention and control policies.

Secondary objectives include identifying epidemiological risk factors for infection or colonisation with CRE and/or ColRE and supporting EU Member States in developing technical capabilities and proficiency in genomic-based surveillance and risk assessment of multidrug-resistant pathogens.

An additional public health benefit 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 few 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). 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 based on phenotypic testing, which does not allow for understanding the transmission dynamics of resistance genes in the human population needed for implementing targeted control measures. Only genomic-level investigations and epidemiological data can 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 spread of carbapenemase-producing Enterobacteriaceae (CPE) and build laboratory capacity for the diagnosis and surveillance of CPE in Europe [5-7]. The data collected showed that on average, 1.3 patients per 10 000 hospital admissions had carbapenemase-producing *K. pneumoniae* or *E. coli* isolated from a clinical specimen in European hospitals [7]. In the same study, an increasing number of European countries reported interregional hospital spreads of CPE or an endemic situation[7]. The EuSCAPE project demonstrated the feasibility of conducting integrated epidemiological and microbiological sentinel multicentre structured surveys and collecting comparable quality-assessed data suitable for EU-level analysis.

In 2014, ECDC held two expert consultations on the development of a molecular strategy for MDR pathogens, resulting in an ECDC strategy for the molecular surveillance of CPE 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 (Table 1). In consultation with national technical coordinators and national focal points for antimicrobial resistance during a meeting in November 2017, this strategy was extended for protocol version 2.0 to include all CRE in addition to CPE.

Table 1. EU-level public health objective, risk assessment benefits and potential risk management implications of molecular typing of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae

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 CRE and/or 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.

Source: ECDC [8]

** High-risk clones/plasmids: ecologically successful clonal types carrying chromosomal resistance determinants or plasmid-borne resistance genes associated with high or increasing population prevalence across surveys and/or displaying extensive or expanding geographical distribution (interregional spread or international spread) and/or association with multiple hospital outbreaks reported in literature.*

Using the guidance of the CPE molecular surveillance strategy and building on the experience of the EuSCAPE project, this ECDC study protocol was developed and updated to describe the technical requirements for implementing EU-level genomic-based surveillance of CRE and/or ColRE.

Study objectives

The primary EU-level public health objective is to determine the occurrence, geographic distribution and population dynamics within the healthcare setting of high-risk CRE and/or ColRE 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 epidemiological risk factors for infection or colonisation with CRE and/or ColRE at bacterial clonal and sub-genomic level; and
- Support EU Member States in developing technical capabilities and proficiency in genomic-based surveillance and risk assessments of multidrug-resistant pathogens associated with epidemic potential.

Study protocol

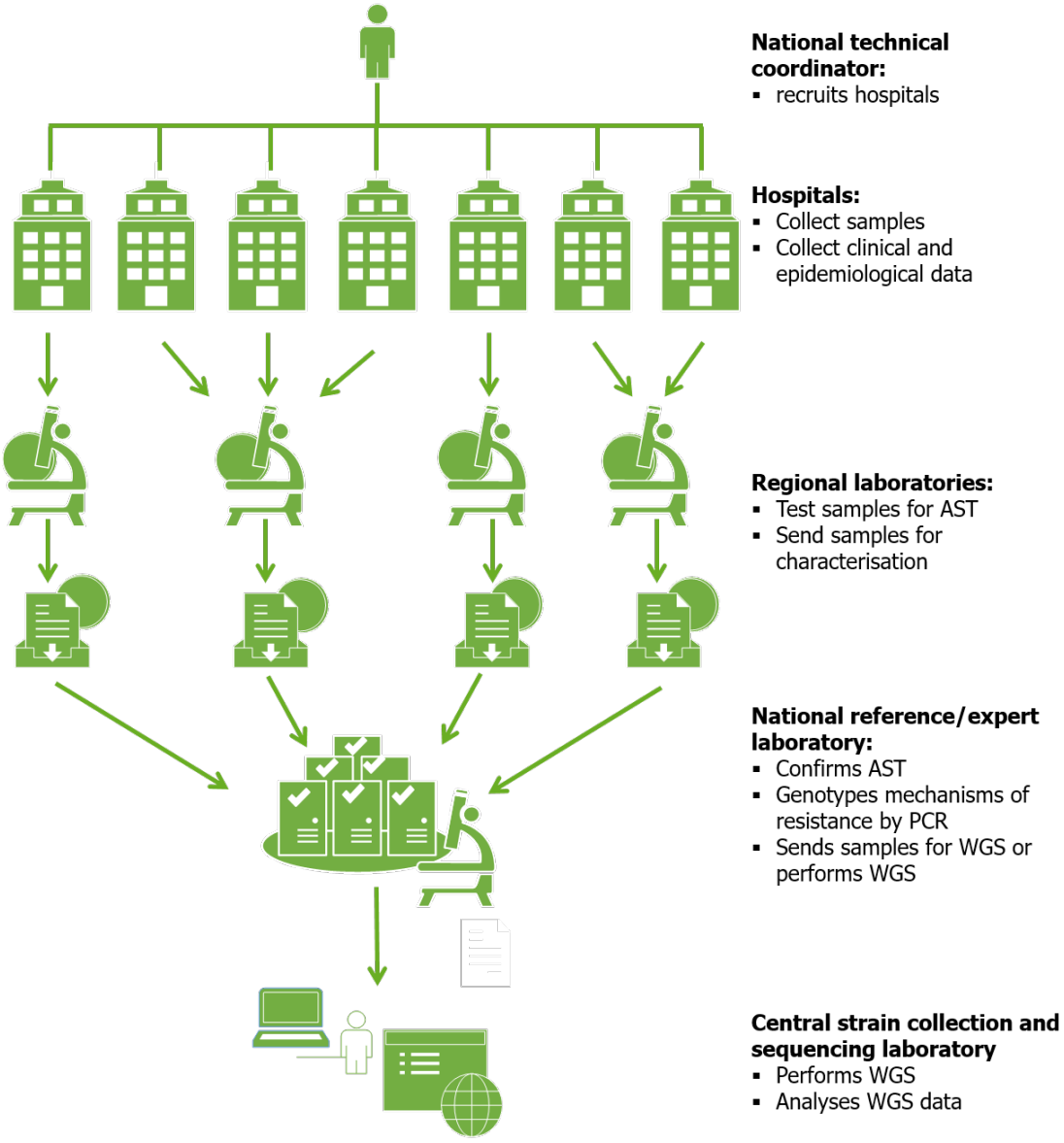
Study and surveillance design

The study design follows the EuSCAPE project model [7] and structured survey protocol. A stepwise workflow is performed in each country (Figure 1).

The proposed surveillance design is a structured and periodically repeated pan-European multicentre molecular epidemiological survey of the prevalence and distribution at the regional level of CRE and/or ColRE by genotype among patients seeking hospital care.

These surveys are expected to take place every three years, or more frequently as indicated by the epidemiological situation, and subject to resource availability at ECDC and national and regional levels.

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



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

Study inclusion criteria

National technical coordinator and national reference / expert laboratory

Each participating country selects a national technical coordinator and national reference/expert laboratory with strong expertise in molecular characterisation of CRE and ColRE. The coordinator has the central role of organising the national workflow from recruiting participating hospitals and associated laboratories to supervising national sample and data collection (Figure 1). This person is preferably located within the national reference/expert laboratory that is responsible for further characterising the collected isolates.

Hospitals and regional laboratories

Participating hospitals and their associated laboratories are selected with the aim of covering the full extent of the healthcare referral network across the national territory and mapping the inter-hospital dissemination perimeter and 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, sample site selection is based on the Nomenclature of territorial units for statistics (NUTS)-2 regions, the broad geodemographic territorial subunits of Member States. At least one sentinel acute care hospital site per NUTS-2 region with a population of at least 250 000 inhabitants located on the European mainland should be 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 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 and colistin resistance of Enterobacteriaceae, including antimicrobial susceptibility testing (AST) and genotyping of carbapenemase genes and colistin resistance genes, should be selected. Only one national reference/expert laboratory per country should be recruited.

Patient population

Target patient populations are recruited at the acute care hospital level:

- **Outpatients**: patients not hospitalised or those not staying overnight in hospital (day care) at the time of sampling
- **Inpatients:** patients hospitalised or staying overnight in the hospital at the time of sampling.

Bacterial species

For the first survey, the target bacterial species proposed for inclusion are *K. pneumoniae* and *E. 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 *K. pneumoniae* or *E. coli* infection or colonisation is an individual patient infected and/or colonised with at least one of the following:

- An isolate of carbapenem-resistant *K. pneumoniae;* or
- An isolate of carbapenem-resistant *E. coli*.

Colistin-resistant but carbapenem-susceptible isolates can also be collected according to the following definition:

- An isolate of *K. pneumoniae* or *E. coli* with confirmed phenotypic colistin resistance and/or detection of an *mcr* gene.

Comparator patient definition

A comparator patient is an individual patient colonised and/or infected with carbapenem-susceptible *K. pneumoniae* or *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 fewer than six months are asked to record and report the actual sampling period.

Biological samples

Non-duplicate bacterial isolates from both non-duplicate patients meeting the case definition and non-duplicate comparator patients meeting the comparator definition are accepted. Preferably, isolates from clinical specimens collected for diagnostic purposes (e.g. blood, urine, sputum and wound secretions) should be included.

If there is a high likelihood based on local epidemiology that the hospital will not be able to collect 10 carbapenem-resistant isolates from clinical samples (e.g., low prevalence country or region), rectal or faecal samples for screening for CPE or ColRE rectal carriage can also be included, but need to be marked as screening samples so that they can be analysed separately from clinical samples.

Sample size

Cases: The first 10 non-duplicate consecutive isolates of carbapenem non-susceptible *K. pneumoniae* or *E. coli* isolated from clinical samples from individual consecutive patients[1].

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.

Optional addition: Colistin-resistant but carbapenem-susceptible *K. pneumoniae* or *E. coli* isolates can optionally be collected in addition to the above 20 isolates per hospital without the need for comparator isolates.

Clinical and epidemiological data

Each collected isolate is accompanied by the respective microbiological, clinical and epidemiological data and/or whole genome sequencing (WGS) 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 European Committee on Antimicrobial Susceptibility Testing (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 in the separate laboratory manual for this study. 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 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).

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

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

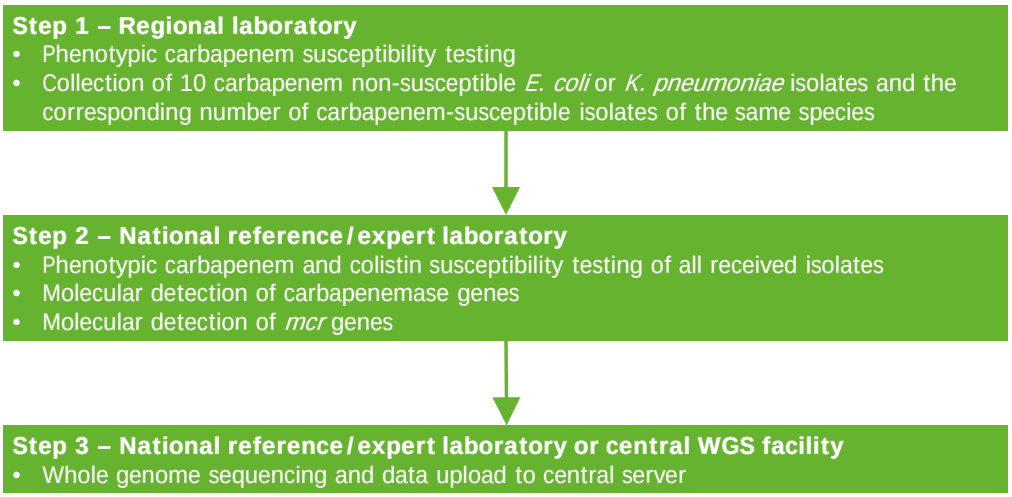
- Phenotypic characterisation in accordance with EUCAST guidelines [10,11].
- Genotypic characterisation of carbapenemase genes and colistin resistance genes according to the laboratory manual for this study (under revision) by:
  - Polymerase chain reaction (PCR) or WGS to identify family level for CPE
  - PCR or WGS to identify *mcr* genes
  - WGS to characterise other genetic resistance determinants.

All carbapenem non-susceptible and susceptible isolates will be subjected to WGS.

To ensure the 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 *mcr* genes by PCR [3,10,13,14].

[1] Since 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 ColRE.

Figure 2. Sample flow for phenotypic and molecular resistance testing



Data collection

Data collection includes variables at the isolate (microbiological data), patient (epidemiological and clinical data) and hospital levels (hospital data) and requires an active collection of laboratory and hospital information as well as information from medical records and/or clinician interviews. If national capacity allows, WGS data are collected at the 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 (TESSy) 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 specimens, wound swabs, aspirates, soft tissue samples, catheter exit site, bone and joint specimens, cerebrospinal fluid, reproductive tract samples, other)
- Routine antimicrobial susceptibility testing results and method(s) and interpretation guidelines used:
  - Aminoglycosides: amikacin, tobramycin, gentamicin
  - Beta-lactams/penicillin: 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, ceftazidime-avibactam
  - Fluoroquinolones: ciprofloxacin
  - Glycylcyclines: tigecycline
  - Polymyxins: colistin
  - Other: trimethoprim-sulfamethoxazole, fosfomycin
- Mechanisms of resistance from 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)
- Date of hospitalisation.

Epidemiological and clinical data

- Colonisation or infection or undetermined clinical significance
- Organ/system or location of infection/colonisation (e.g. skin and soft tissue, urinary tract, intra-abdominal, bloodstream, lower respiratory)
- Hospital-acquired or community-onset:
  - **Community-onset** if sample is collected from outpatient or hospitalised patient from acute care hospital stay less than 48 hours post-admission
  - **Hospital-acquired** colonisation/infection if sample is collected from inpatient from acute care hospital stay longer than 48 hours post-admission.

Healthcare exposure/referral history

- Direct hospital transfer from:
  - Another hospital in same country
  - Hospital in another EU/EEA country (specify country)
  - Hospital in non-EU/EEA country (specify country).
- Previous hospitalisation within six months in:
  - Same hospital
  - Another hospital in same country
  - Hospital in another EU/EEA country (specify country)
  - Hospital in non-EU/EEA country (specify country)
  - Unknown hospital.
- Previous residence in long-term/elderly care facility (direct transfer or within six months) in:
  - Same country
  - Another EU/EEA country (specify country)
  - 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 national technical coordinator)
- Hospital location (NUTS-2 region name/code) and geolocation data
- Estimate of population in hospital catchment area
- Actual sampling period
- Number of occupied bed days during sampling period
- Total number of patient admissions during actual sampling period
- Total number of patients colonised and/or infected with *K. pneumoniae* during actual sampling period
- Total number of patients colonised and/or infected with *E. coli* during actual sampling period
- Practice of patient screening for CRE/CPE and colistin resistance.

Whole genome sequencing data

WGS 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

Hospital, epidemiological and microbiological data are submitted directly or in bulk to a web-based system that is only accessible to selected staff in participating hospitals, the national technical 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) and open to a restricted group of users defined by the study coordinator and ECDC (e.g. national technical coordinators).

Hospitals and regional laboratories

Each participating hospital submits isolate, patient and hospital data:

- Isolate and patient data completed for each case/comparator patient from which susceptible or non-susceptible isolate is collected for further microbiological characterisation.
- 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 PCR results and/or WGS data if available.

Central sequencing laboratory

Central sequencing laboratories provide whole genome raw reads in a closed workspace accessible to participants.

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, with 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 multilocus 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 through public access bioinformatics platforms such as the Bacterial Isolate Genome Sequence Database at the Institut Pasteur (http://bigsdb.pasteur.fr/klebsiella) and the Comprehensive Antibiotic Research Database (http://card.mcmaster.ca).

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