OPERATIONAL SUPPORT

Protocol for genomic surveillance of carbapenem-resistant Enterobacterales 2025 (CRE25 survey)

1 Introduction

Carbapenem-resistant Enterobacterales (CRE) pose a significant threat to patients and healthcare systems in European Union/European Economic Area (EU/EEA) countries as outlined in the third update of ECDC's rapid risk assessment on the topic [1]. Since the survey of carbapenem- and/or colistin-resistant Enterobacterales (CCRE survey) conducted in 2019, the following developments indicate that the epidemiological situation in the EU/EEA continues to deteriorate:

- an increase in the incidence of carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections;
- convergence of virulence and resistance in *K. pneumoniae*;
- newly-emerging Enterobacterales species carrying carbapenemase genes;
- plasmid-mediated spread of carbapenemase genes;
- increasing detection of isolates of high-risk lineages of *Escherichia coli* carrying carbapenemase genes with a risk of spread in the community [1].

To obtain an overview of the spread of high-risk lineages of *K. pneumoniae* and *E. coli* to target infection prevention and control (IPC) measures, a new European-wide survey of CRE is planned for the period 1 October 2025–31 December 2025 (CRE25 survey). For the CRE25 survey, National Reference Laboratories (NRLs) from 37 countries participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) are invited to submit whole genome sequencing (WGS) data to ECDC, accompanied by standardised epidemiological and microbiological metadata for up to 40 isolates per Nomenclature of Territorial Units for Statistics level 2 (NUTS-2) region according to the inclusion criteria outlined in this protocol. For NRLs without sufficient WGS capacity, central sequencing support will be provided.

1.1 Objectives of the CRE25 survey

The primary EU-level public health objective of the CRE25 survey is to determine the occurrence, geographical distribution and population dynamics of high-risk CRE lineages and related transmissible genetic elements of critical public health importance within healthcare settings in Europe, to inform risk assessment and control policies. A secondary objective is to support participating countries in developing technical capability and proficiency for genomic-based surveillance and for conducting risk assessments of multidrug-resistant pathogens with epidemic potential.

Suggested citation: European Centre for Disease Prevention and Control, 2025. Protocol for genomic surveillance of carbapenem-resistant Enterobacterales 2025 (CRE25 survey). October 2025. Stockholm; ECDC; 2025.

ISBN 978-92-9498-826-3; doi: 10.2900/8146846; Catalogue number TQ-01-25-056-EN-N

Table 1. European-level public health objective, risk assessment benefits and potential risk management implications of genomic surveillance of carbapenem-resistant Enterobacterales

European-level public health objective	European-level risk assessment benefits	Potential risk management implications
Monitoring of high-risk lineages of carbapenem-resistant Enterobacterales and related transmissible genetic elements of critical public health importance in Europe	Detection and genotypic identification of high-risk* lineages/plasmids Monitoring time trends in the frequency of particular genotypes in the population Identification of geographical areas associated with spread of specific high-risk lineages Detection/delineation of cross-region and cross-border dissemination of high-risk lineages/plasmids	Evaluation, refinement and/or revision of local, regional and national IPC programmes Contribution to the impact evaluation of hospital and community antimicrobial policies and stewardship programmes Better targeting of resources to populations at higher risk of acquiring carbapenem-resistant Enterobacterales, geographical areas and dissemination pathways

** High-risk lineages/plasmids: ecologically successful clonal lineages 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 (inter-regional or international spread) and/or association with multiple hospital outbreaks reported in the literature.*

1.2 Lessons learned from the survey of carbapenem- and/or colistin-resistant Enterobacterales (CCRE survey)

The CCRE survey 2019 was the second European genomic survey of Enterobacterales following the European Survey of Carbapenemase-Producing Enterobacterales (EuSCAPE) conducted in 2013-2014 and using the same protocol with only few modifications [2,3]. The final *K. pneumoniae* data set of the CCRE survey included 2 973 *K. pneumoniae* species complex (SC) isolates received from 36 countries. The *E. coli* data set was less representative with 548 isolates from 32 countries. The main reason for this difference was the higher prevalence of carbapenem-resistant *K. pneumoniae* SC than of carbapenem-resistant *E. coli* in participating countries. Another limitation of the CCRE survey was the long duration between isolate collection and results, which was partially due to the COVID-19 pandemic. Nevertheless, the CCRE survey was first to detect the emergence of *K. pneumoniae* sequence type (ST)39 as a new high-risk lineage and of *E. coli* ST167, ST361, ST405, ST410 and ST648 carrying $bla_{\text{NDM-5}}$ due to the absence of any other comprehensive genomic surveillance at the European level [4-7]. Several opportunities for process improvement were identified in the CCRE survey. One such area was hospital recruitment, which had to be completed before the start of isolate collection. While 527 hospitals had been recruited for the survey, only 346 (65.7%) of these submitted isolates. Long delays also occurred during confirmatory testing at the NRLs, during the shipment process of isolates from the NRLs to the strain collection, and while awaiting central WGS results.

An important question regarding the protocol of future EURGen-Net surveillance had already been answered by the EURGen-Net Operational Contact Points (OCPs) at a network meeting in 2022. Many of the 39 participating OCPs had voted to continue with structured surveys (n=17, 44%), followed by the options to move to comprehensive WGS-based surveillance (n=13, 33%) or to move to intermittent/sentinel surveillance (n=6, 15%). Importantly, only a minority of OCPs (n=3, 8%) considered it sufficient for ECDC to focus mainly on outbreak and emerging resistance investigations based on country reporting in EpiPulse. To follow the OCP vote, but also adapt to increasing national WGS capacity and address the challenges identified during the CCRE survey, a simplified and faster survey model which could facilitate transitioning to future comprehensive surveillance is proposed for the CRE25 survey and is described below.

2 Protocol changes to improve timeliness and representativeness of results

Potential elements that could facilitate faster and more comprehensive surveillance in comparison to the CCRE survey protocol are:

- to only focus on carbapenem-R/I isolates;
- to independently collect *K. pneumoniae* and *E. coli* isolates;
- to replace hospital recruitment with isolate recruitment;
- to shorten the collection time for the survey to three months;
- to pilot uploading isolates with minimal metadata for interim analyses.

It is important to note that the full effect of these protocol changes on timeliness will depend on the switch from central WGS to national WGS data. As described above, the shipments and related batching of isolates required for central WGS will always result in delays, even if the process is accelerated to a certain extent compared to the CCRE survey. Several EU/EEA countries have good WGS capacity at the NRL and routinely perform WGS of all or a subset of received CRE isolates. It is expected that these countries can generate and upload timely national WGS data for early interim analysis. Central WGS will continue to be available as a backup option for countries without sufficient capacity.

2.1 Focus on carbapenem-R/I Enterobacterales isolates

As per the CCRE survey protocol, participating hospital laboratories collected the next carbapenem-susceptible isolate of the same species for each included *K. pneumoniae* or *E. coli* isolate in the categories carbapenem-resistant or carbapenem-susceptible increased exposure (carbapenem-R/I). These carbapenem-susceptible isolates were useful as background population to improve understanding of the acquisition of resistance genes, resistance development of high-risk lineages over time and the distribution of hypervirulent *K. pneumoniae* isolates. However, the carbapenem-susceptible isolates were of more value for research into the emergence of resistance than for public health and control of CRE. It will therefore be difficult to justify the continued use of national and ECDC resources for WGS of carbapenem-susceptible Enterobacterales isolates. Furthermore, the inclusion of carbapenem-susceptible isolates would require hospital recruitment, as susceptible isolates are not usually submitted by clinical laboratories to NRLs. This would be an obstacle for a faster isolate inclusion process (see section 2.3). Finally, the additional focus of the CCRE survey on colistin resistance no longer seems justified due to the approval in recent years of several new antimicrobial agents with activity for CRE.

At the EURGen-Net meeting on 25 September 2024 the proposal to focus the CRE25 survey on the collection of carbapenem-R/I *K. pneumoniae* and *E. coli* isolates only received a positive vote from 46/50 (92%) participants, while four (8%) participants were not in favour of this change.

2.2 Independent collection of *Klebsiella pneumoniae* and *Escherichia coli* isolates

For the CCRE survey, ten carbapenem-R/I *K. pneumoniae* or *E. coli* isolates from individual consecutive patients had to be collected per NUTS-2 region. Due to a much higher prevalence of carbapenem resistance in *K. pneumoniae*, carbapenem-R/I *K. pneumoniae* isolates outnumbered carbapenem-R/I *E. coli* isolates by more than 5:1 in the final data set. While there are early genomic signs of the increasing spread of high-risk lineages of carbapenemase-producing *E. coli* (documented by the EURGen-Net investigations of the increase of *E. coli* carrying $bla_{\text{NDM-5}}$ and *E. coli* ST131 carrying carbapenemase genes [7,8]), their overall prevalence continues to be low. It is therefore likely that for the CRE25 survey, the *E. coli* dataset would still not be representative, if the same sampling strategy is kept.

Therefore, it was proposed at the EURGen-Net meeting in 2024 that for the CRE25 survey, carbapenem-resistant *E. coli* and *K. pneumoniae* isolates should be collected independently (ten isolates of each). This was agreed upon by 46 out of 48 (96%) participants, while two (4%) participants chose the option 'not sure'.

2.3 Replacement of hospital recruitment with isolate recruitment

The CCRE survey protocol required recruiting at least one hospital per NUTS-2 region for comprehensive geographical coverage [3]. However, about one third of the recruited hospitals did not provide isolates for the CCRE survey for different reasons. Once the CCRE survey had started, it was no longer possible to change or add hospitals, meaning the drop-out of hospitals frequently resulted in 'loss of NUTS-2 regions' in the final data set.

Even for the more representative *K. pneumoniae* data set, only 227 (69.4%) of 327 NUTS-2 regions in participating countries were covered with at least one *K. pneumoniae* isolate [4].

The proposed solution is to "recruit isolates" arriving at the NRL instead of recruiting hospitals. Many NRLs routinely receive CRE isolates from clinical laboratories for confirmatory testing. These isolates could be submitted to the CRE25 survey by NUTS-2 region. For this inclusion process it would not be relevant if ten isolates arrive from only one hospital or various hospitals within the same NUTS-2 region. It could also potentially allow for better coverage and faster completion and avoid reliance on a single hospital per NUTS-2 region. A disadvantage could be that the survey no longer provides hospital-based results and there is reduced comparability with previous surveys. However, the objective of the CRE25 survey is to track high-risk lineages at the European level and not to investigate hospital outbreaks, for which ten isolates per hospital would also be insufficient. In addition, the new protocol has flexibility, meaning if a country still prefers recruiting hospitals or if there are NUTS-2 regions from which the NRL does not receive any isolates on a routine basis, the NRL could still recruit one or more hospitals from these NUTS-2 regions to send isolates for inclusion.

After presentation of the isolate-based recruitment process at the EURGen-Net meeting, 39 (85%) of 46 votes were in favour of the proposed change towards isolate recruitment at the NRL, five (11%) against and two (4%) participants were 'not sure'.

2.4 Shortening of isolate collection period to three months

Shortening the isolate and data collection period to three months (compared to the six-month collection period in the CCRE survey) could also gain time. This change could be supported by the fact that about two thirds of isolates were already collected in the first three months of the CCRE survey. In addition, CRE prevalence has increased since 2019 and the new isolate recruitment process outlined under section 2.3 allows faster and more comprehensive collection of isolates. However, the shortened period could result in a lower number of isolates, especially from countries with a low prevalence of CRE and reduce comparability with EuSCAPE and CCRE survey results.

Of 46 votes, 30 (65%) votes were in favour of the proposed reduction on the collection period, eight (17%) were against, and eight (17%) were 'not sure'. In addition to a higher number of votes against this change than for other proposed changes to the protocol, the EURGen-Net OCPs posted several comments with concerns that a three-month collection period may not be long enough to achieve a full collection of isolates, especially for carbapenem-R/I *E. coli*. The initial plan was therefore to keep the isolate collection period at six months, however, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) informed ECDC about a likely change in carbapenem clinical breakpoints from January 2026 onwards. A change in the carbapenem breakpoints, which are essential for inclusion of isolates into the CRE25 survey during the survey period would compromise isolate collection and analysis. It was therefore decided to keep the proposed restriction of the collection period to three months from 1 October to 31 December 2025.

2.5 Interim analyses for early signal detection

Experience from the CCRE survey and EURGen-Net outbreak investigations have shown that genomic data are of high value for early signal detection, even when epidemiological data are incomplete. In addition, even with the large and standardised metadata set of the CCRE survey, the epidemiological results have often been inconclusive. It is therefore advisable to prioritise rapid genomic analysis and allow WGS data upload with minimal metadata (sampling date and country) followed by piloting of interim analyses for early signal detection. This does not preclude efforts to gather more complete epidemiological data for a more detailed final analysis. Of 40 votes received for this question, all were in favour of allowing WGS data upload with minimal metadata and piloting interim analysis.

2.6 Use of screening vs clinical breakpoints

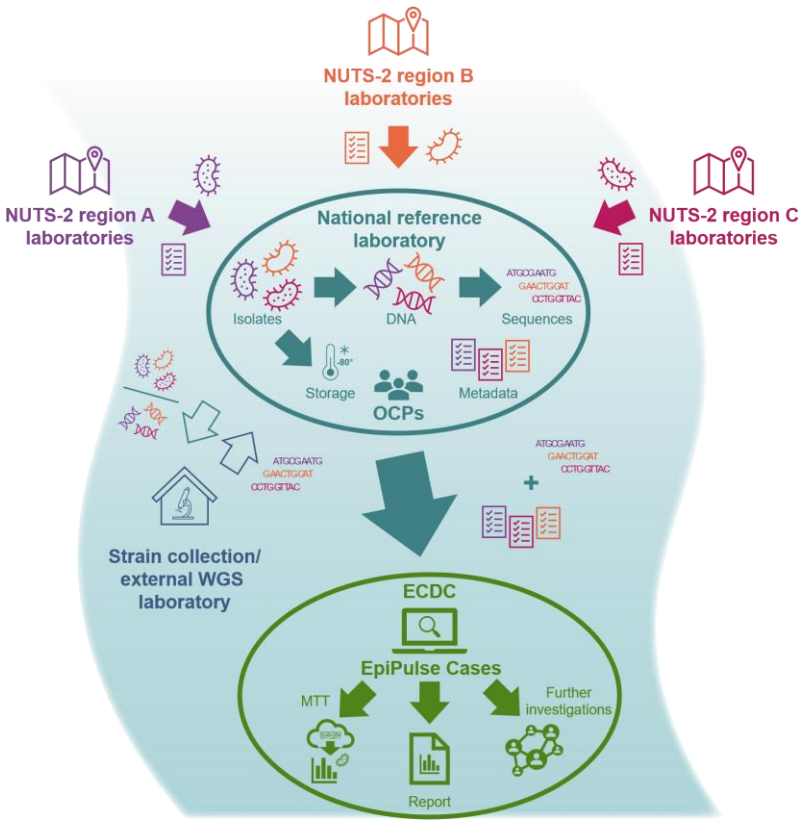
A long-standing discussion that took place during the CCRE survey preparation in 2019 and again for the CRE25 survey is the use of EUCAST clinical breakpoints [9] versus EUCAST 'screening breakpoints' [10] for inclusion of isolates into the survey. It is undisputed that EUCAST screening breakpoints are the adequate method for comprehensive detection of carbapenemase-producing Enterobacterales isolates. The use of clinical breakpoints will result in under-detection of isolates producing carbapenemases that confer low level carbapenem resistance such as OXA-48. However, in 2025, screening breakpoints are not comprehensively implemented in all countries invited to participate in the CRE25 survey. Insistence on screening breakpoints may result in a loss of participating laboratories or even countries. The compromise for the CRE25 survey is that the main collection will again be performed using clinical breakpoints with the same inclusion criteria as for EuSCAPE and the CCRE survey [3,11]. In addition, there will be a voluntary additional collection based on screening breakpoints for countries where these are sufficiently implemented as outlined in further detail below.

3 Survey protocol

3.1 Survey and surveillance design

The CRE25 survey is based on the models from EUSCAPE [2] and the CCRE survey [3] with the above-mentioned modifications to enable faster completion. The proposed surveillance design is a structured, European multi-centre molecular epidemiological survey of the distribution of CRE by genotype among patients seeking medical care in 2025 as depicted in Figure 1. Future surveys and their frequency will be agreed with the EURGen-Net OCPs in 2026.

Figure 1. Data collection and analysis workflow for participation in the EU-level genomic-based surveillance of carbapenem-resistant Enterobacterales



MTT: molecular typing tool; NUTS-2: nomenclature of territorial units for statistics level 2; OCP: Operational Contact Point; WGS: whole genome sequencing.

3.2 Inclusion criteria

3.2.1 National reference/expert laboratory

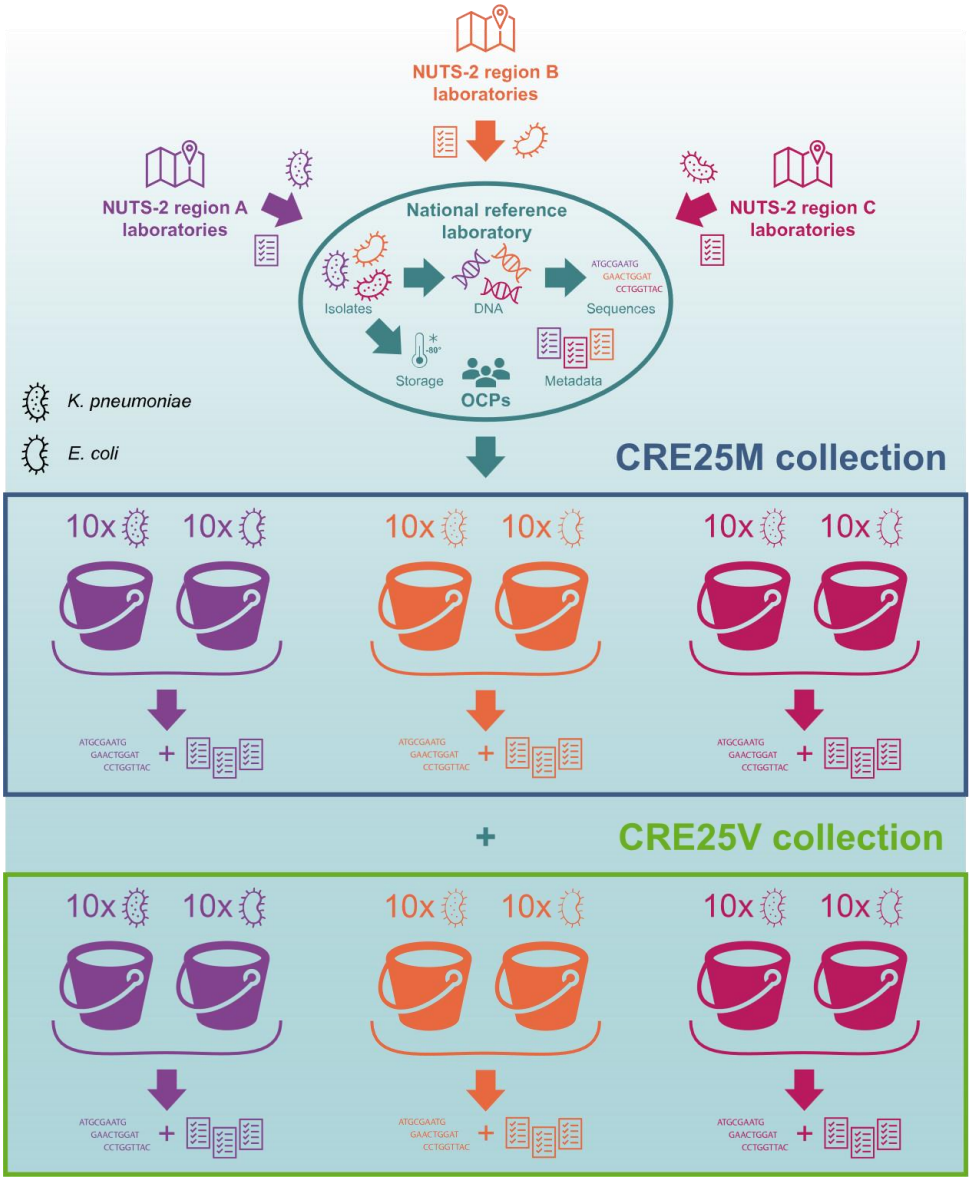
National isolate and data collection for the CRE25 survey will be coordinated by the NRLs and OCPs participating in EURGen-Net. Selection of additional National Technical Coordinators, as for the CCRE survey - when the EURGen-Net structure was not in place - is no longer required. The OCPs will be responsible for managing the national workflow and supervising national sample and data collection.

3.2.2 Hospitals and regional laboratories

Hospital recruitment is no longer required. Instead, *K. pneumoniae* and *E. coli* isolates arriving at the NRLs for reference testing during the collection period will be included until the maximum of 20 isolates fulfilling the inclusion criteria for the main collection and 20 isolates fulfilling the inclusion criteria for the voluntary collection by NUTS-2 region is reached (Figure 2). The isolates can originate from all types of healthcare facilities including acute care or specialised hospitals and community healthcare services. Isolates can be included from one or more healthcare facilities within each NUTS-2 region. In case the NRL does not receive sufficient isolates from healthcare facilities in a specific NUTS-2 region, isolates can be actively requested from hospitals in the

respective region. Information on NUTS-2 regions can be found at https://ec.europa.eu/eurostat/web/nuts. The most recent NUTS 2024 classification should be used.

Figure 2. Isolate recruitment workflow using the nomenclature of territorial units for statistics level 2 (NUTS-2)



CRE: carbapenem-resistant Enterobacterales; NUTS-2: nomenclature of territorial units for statistics level 2; OCP: Operational Contact Point.

3.2.3 Patient population

The target patient populations are recruited at healthcare facility level:

- outpatients: patients not hospitalised or hospitalised but not staying overnight in a hospital (day care) at the time of sampling;
- inpatients: patients hospitalised and staying overnight in a hospital at the time of sampling.

3.2.4 Bacterial species

As for EuSCAPE and the CCRE survey, the target bacterial species are *K. pneumoniae* species complex (SC) and *E. coli.* A pilot study for *Enterobacter* spp. will be conducted separately with a separately developed protocol.

3.2.5 Case definition for the main CRE25 survey (CRE25M) collection

A case of carbapenem-R/I *K. pneumoniae* SC or carbapenem R/I- *E. coli* for the CRE25 survey main collection is an individual patient infected or colonised with an isolate of carbapenem-R/I *K. pneumoniae* SC or carbapenem-R/I *E. coli* according to EUCAST clinical breakpoints valid at the time of collection (version 15, valid from 2025-01-01 [9]) with:

- an MIC >0.5 mg/L or a disk diffusion zone <23 mm for ertapenem OR
- an MIC >2 mg/L or a disk diffusion zone <22 mm for imipenem OR
- an MIC >2mg/L or a disk diffusion zone of <22 mm for meropenem.

3.2.6 Case definition for the voluntary additional (CRE25V) collection

A case of carbapenemase-suspected *K. pneumoniae* SC or carbapenemase-suspected *E. coli* for the CRE25 survey additional voluntary collection is a patient infected or colonised with an isolate of *K. pneumoniae* SC or *E. coli* suspected to produce carbapenemases according to the EUCAST screening breakpoint for meropenem valid at the time of collection [10]:

- an MIC >0.125 mg/L or a disk diffusion zone of <28 mm for meropenem AND
- not eligible as carbapenem-R/I for the CRE25 main collection due to MICs or disk diffusion zone diameters being in the susceptible (S) range according to EUCAST clinical breakpoints for ALL tested carbapenems [9].

It is possible to participate in the voluntary collection, even if isolates are further selected by an additional confirmatory step for carbapenemase production or carriage of carbapenemase genes before WGS.

3.2.7 Comparator patient definition

Comparator isolates that are carbapenem-susceptible and not suspected of carrying carbapenemase genes will not be collected for the CRE25 survey.

3.3 Sample design

3.3.1 Sampling period

The sampling period is a maximum of three months from 1 October 2025–31 December 2025.

3.3.2 Biological samples

Non-duplicate bacterial isolates from persons meeting the case definition, are accepted. Preferably, isolates from clinical specimens collected for diagnostic purposes (e.g. blood, urine, sputum, wound secretion, etc.) should be included. However, isolates from rectal or faecal samples for CRE screening can also be included, but need to be marked as screening samples so that they can be analysed separately from clinical diagnostic samples.

3.3.3 Sample size

- **CRE25 main collection:** the first ten non-duplicate consecutive isolates of *K. pneumoniae* SC and the first ten non-duplicate consecutive isolates of *E. coli* categorised as I or R to one or several of the carbapenems per NUTS-2 region.
- **Comparators**: no longer included.
- **Additional CRE25 voluntary collection**: the first ten non-duplicate consecutive isolates of *K. pneumoniae* SC and the first ten non-duplicate consecutive isolates of *E. coli* suspected to be carbapenemase-producing based on the EUCAST screening breakpoint for meropenem [10], but not categorised as carbapenem-R/I to one or several of the carbapenems per NUTS-2 region.

The maximum resulting sample size is 40 isolates per NUTS-2 region including ten carbapenem-R/I *K. pneumoniae* SC isolates and ten carbapenem-R/I *E. coli* isolates for the main collection and ten carbapenemase-suspected *K. pneumoniae* SC isolates and ten carbapenemase-suspected *E. coli* isolates for the additional voluntary collection.

3.3.4 Metadata collection

Each collected isolate is accompanied by the respective microbiological, clinical and epidemiological data and WGS data as further outlined below.

3.4 Sample flow

Step 1: Species identification and antimicrobial susceptibility testing

Consecutive presumptive cases are passively identified through routine clinical microbiology procedures and referred to the NRL for reference testing. After species identification (*K. pneumoniae* SC or *E. coli*) and confirmation of isolates at the NRL as carbapenem-R/I according to the EUCAST clinical breakpoints for the main collection or as carbapenemase suspected according to the EUCAST screening breakpoint for meropenem for the voluntary collection, isolates will be registered with a unique identifier for the CRE25 survey (see 3.5.1 and 3.5.2). All registered isolates will have to be stored using an appropriate method to preserve their viability until conclusion of the analysis of the CRE25 survey.

Step 2: Whole genome sequencing

Performance of WGS of all isolates registered for the CRE25 survey will preferably be conducted at the NRL according to the established routine methodology. As part of capacity building activities, the European Reference Laboratory for Public Health for Antimicrobial Resistance (EURL-PH-AMR) can provide guidance and advice for generation of WGS data and analysis of national CRE25 data. If, despite EURL-PH-AMR support, national capacity is not sufficient, ECDC can provide central WGS services conducted by a contractor. To ensure quality and comparability of data, all NRLs performing phenotypic and genotypic characterisation are encouraged to participate in the phenotypic external quality assessment and genomic proficiency testing exercises conducted by the EURL-PH-AMR in 2025. If high quality long-read data can be generated (Oxford Nanopore with basecaller SUPv5.0.0 or later, or Pacific Biosciences HiFi reads) these are the preferred data to be submitted to ECDC. If only short reads are available (e.g. Illumina, Ion Torrent, or MGI) these should be provided. Provision of hybrid data (both long reads and short reads) is not required.

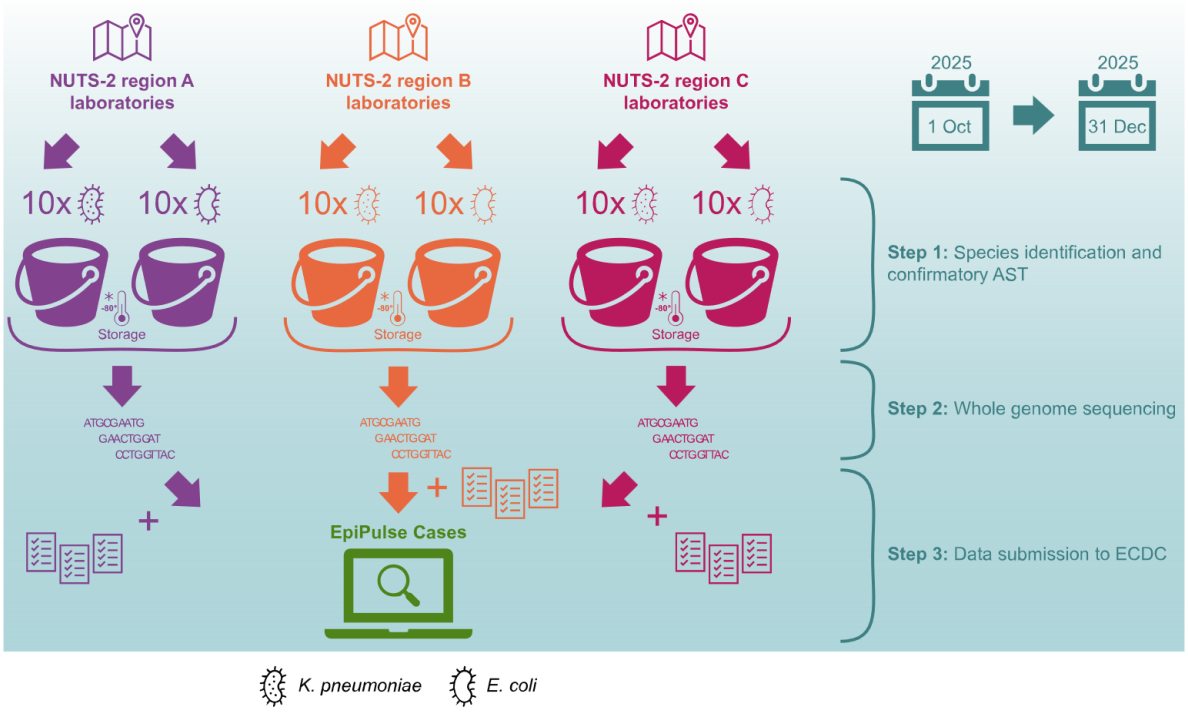
Step 3: Submission of data to ECDC

Metadata in the AMRISO and AMRISO$AST format together with WGS data will be submitted to ECDC via EpiPulse Cases. Data should be submitted as soon as available. Batching of isolates for data submission or delaying of WGS data upload until epidemiological metadata are complete should be avoided. More instructions on how to use EpiPulse Cases for data submission can be found under the Help section of the EpiPulse landing page or at the following links:

- EpiPulse Cases Guide
- EpiPulse Cases Visualised Guide

Once data have been submitted, they will automatically be processed by the ECDC Bioinformatics system. The analysis can generally be expected to be finished on the following working day after the submission. The progress of the analysis of your uploaded data can be tracked through the EpiPulse Molecular Typing Tool (MTT) by selecting the appropriate pathogen and selecting the *My Isolates Data* tab. The details of the included analyses are described in the analysis plan (see separate document). The entirety of the CRE25 survey dataset analysed so far can be displayed in the embedded Microreact tool by searching for the appropriate *Event code* (2025-ARH-00006, see 3.5.1) in the search and refine section of MTT.

Figure 3. Sample flow for the CRE25 survey



NUTS-2: nomenclature of territorial units for statistics level 2.

3.5 Data collection

The metadata collection includes variables at the isolate level (microbiological data) and the patient level (epidemiological and clinical data) as described in the AMRISO and AMRISO$AST subject specifications. Data will be transferred to ECDC through the EpiPulse Cases platform, which is only accessible by nominated users such as the OCPs for AMRISO as well as ECDC. The specifications of the AMRISO and AMRISO$AST subjects at the time of submission apply and can be found in the EpiPulse Cases metadata set. A list of variables to be collected for the CRE25 survey and related definitions can also be found below. The mandatory variables for a successful data submission to the EpiPulse Cases platform are highlighted in bold. In case of discrepancy, the AMRISO as well as AMRISO$AST subject specifications apply. Hospital denominator data will not be collected for the CRE25 survey. WGS data should be generated at national level and FASTQ files should also be submitted (i) directly via the EpiPulse Cases platform or (ii) indirectly by providing run identifiers from the public repositories such as European Nucleotide Archive (ENA) or Sequence Read Archive (SRA) (see 3.5.4). It is possible to instead submit assembled genomes as FASTA files, but raw data are preferable.

3.5.1 Survey identifier

To be able to identify CRE25 isolates within the large *K. pneumoniae* SC and *E. coli* datasets already in EpiPulse and to differentiate them from other ongoing investigations, it is important to allocate a unique CRE25 survey code to these isolates. This code (2025-ARH-00006) should be included in an ItemCode variable in the AMRISO subject.

3.5.2 Unique isolate identifier

For the CRE25 survey, ECDC accepts only pseudonymised codes and not national record identifiers which could allow identification of individual patients. Such pseudonymised isolate codes should be inserted into the field of NationalRecordId variable in the AMRISO subject as well as ParentNationalRecordId variable in the AMRISO$AST subject for AST results (see 3.5.4).

The unique identifier for each isolate submitted for the CRE25 survey should include the following elements:

- Survey code CRE25M for the main collection or CRE25V for the voluntary additional collection;
- NUTS-2 code of the region of origin of the isolate;
- Species KP for *K. pneumoniae* SC or EC for *E. coli*;
- Running number (01-10) for a maximum of 10 isolates per NUTS-2.

Example codes:

- CRE25M_SE11_KP01 for the first carbapenem-resistant *K. pneumoniae* SC isolate from Stockholm region included in the main collection;
- CRE25V_BE10_EC02 for the second carbapenemase-suspected *E. coli* isolate from Brussels region included in the voluntary collection.

3.5.3 General subject data

The generic variables should be filled out as follows:

- Health topic (HealthTopic): the code of the health topic that is being reported should be set as AMRISO
- Status (Status): the Status value is used to provide the functionality for a record within EpiPulse Cases database:
  - default value: NEW/UPDATE. If set to DELETE, the record with the specified NationalRecordId is deleted (invalidated) from EpiPulse Cases database, if it exists;
  - if set to NEW/UPDATE, the record is inserted into the database;
  - if the same NationalRecordId already exists for the same data source and subject code, then the current submitted record updates (replaces) the existing one.
- Data source (**DataSource**): the data source (surveillance system) that the record originates from; the DataSource value must be a special reference value from EpiPulse Cases metadata set and usually consists of two-letter country code followed by *-AMRISO*, e.g. SE-AMRISO for Sweden.
- Subject code (**SubjectCode**): this variable is a reporting model for a disease/health topic and identifies the reporting structure and format of a record (case based or aggregate reporting); for the CRE25 survey, in the appropriate templates, subject code should be set to:
  - AMRISO for the metadata reporting in the AMRISO subject template (Template_AMRISO.csv);
  - AMRISO$AST for the phenotypic AST data reporting in the AMRISO$AST subject template (Template_AMRISO$AST.csv).
- Reporting country (**ReportingCountry**): the country reporting the record (two-letter location code from the EpiPulse Cases metadata set).
- Isolate unique identifier (**NationalRecordId**): as described in 3.5.2, this variable will be populated by the CRE25 pseudonymised identifier, e.g. CRE25M_SE11_KP01.
- Date used for statistics (**DateUsedForStatistics**): the most epidemiologically relevant date for the isolate in the CRE25 survey is date of sampling if available; if not, use the date of receipt in the source laboratory, and if that is not available, the date of receipt in the NRL (yyyy-mm-dd).

3.5.4 Isolate data

Microbiological data

- Bacterial species (**Pathogen**): for CRE25 the following reference values are expected: KLEPNE – *K. pneumoniae* SC or ESCCOL – *E. coli;*
- Date of sampling (DateOfSampling): date the sample from which the isolate was derived was taken (yyyy-mm-dd);
- Date of receipt source laboratory (DateOfReceiptSourceLab): date of receipt in source laboratory, i.e. the laboratory the sample was first sent to (yyyy-mm-dd);
- Date of receipt reference laboratory (DateOfReceiptReferenceLab): if sample collection date is not available, date of receipt in NRL can be included (yyyy-mm-dd);
- Sample origin (SampleOrigin): source that the isolate was obtained from should always be set to human as non-human samples such as hospital environment samples are not collected for the CRE25 survey;
- Specimen source of human samples (SpecimenSource): clinical or screening sample;
- Type of human clinical specimen (Specimen): aspirate, blood, bone marrow, catheter exit site, cerebrospinal fluid, faeces, gastrointestinal tract, lower respiratory tract, reproductive tract, skin, soft tissue, urine, wound, other.

Antimicrobial susceptibility testing

- Antimicrobial susceptibility testing results and method(s) will be collected and interpreted based on EUCAST clinical breakpoints for the following antimicrobial agents (AntimicrobialAgent) using AMRISO$AST subject:
  - Aminoglycosides: amikacin (AMK), tobramycin (TOB), gentamicin (GEN);
  - Beta-lactams/monobactams: aztreonam (ATM);
  - Beta-lactams/cephalosporins: cefotaxime (CTX), ceftazidime (CAZ), cefiderocol (FDC);
  - Beta-lactams/carbapenems: ertapenem (ETP), imipenem (IPM), meropenem (MEM);
  - Beta-lactam - beta-lactamase inhibitor combinations: piperacillin-tazobactam (TZP), ceftazidime-avibactam (CZA), meropenem-vaborbactam (MEV), aztreonam-avibactam (AZA), imipenem-relebactam (IMR), ceftolozane-tazobactam (CZT);
  - Fluoroquinolones: ciprofloxacin (CIP), levofloxacin (LVX);
  - Tetracyclines: tigecycline (TGC, *E. coli* only);

  - Polymyxins: colistin (COL);
  - Other: trimethoprim-sulfamethoxazole (SXT), fosfomycin (FOS, *E. coli* only).

In case AST results cannot be provided for all antimicrobial agents listed above, the following prioritisation should be applied:

  - Priority 1: meropenem (if not possible, imipenem or ertapenem). Isolates that do not have an AST result for at least one carbapenem confirming their inclusion into the CRE25 main or voluntary collection will be excluded from the final analysis;
  - Priority 2: colistin, piperacillin-tazobactam, cefotaxime and ceftazidime, ciprofloxacin or levofloxacin, gentamicin (if not possible, the tobramycin and/or amikacin), ceftazidime-avibactam, meropenem-vaborbactam and aztreonam-avibactam, cefiderocol disk diffusion;
  - Priority 3: all other antibiotics listed above.

- AST record identifier (**NationalRecordId**): unique identifier for each antimicrobial susceptibility test selected and generated by the country reporting the record
- Parent national record identifier (**ParentNationalRecordId**): the corresponding parent identifier for each record (i.e. CRE25 pseudonymised identifier described in 3.5.2 and corresponding to the specific isolate in AMRISO subject); a record with no corresponding parent identifier will not be added to EpiPulse Cases database
- AST method used for the antimicrobial agent (ASTMethod): automated instrument method, broth microdilution, antimicrobial gradient, disc diffusion test
- Minimum inhibitory concentration (MIC) sign (MICSusceptibilitySign):
  - < = Less than
  - <= = Less than or equal
  - = = Equal
  - > = Greater than
  - >= = Greater than or equal
- MIC value (MICValueAST): in mg/l; use '.' as decimal delimiter, e.g. 0.25
- Disk diffusion zone diameter sign (DDZDSusceptibilitySign):
  - < = Less than
  - <= = Less than or equal
  - = = Equal
  - > = Greater than
  - >= = Greater than or equal
- disk diffusion zone diameter value (DDZDValueAST): in mm

Whole genome sequencing

It is expected that WGS data are collected from the NRLs or produced by an appointed central laboratory for a centralised WGS analysis approach. The following variables will be collected:

- protocol used for sequencing, limited to the sequencing technology used and the read length (WGSProtocol):
  - ILLUMINA: Illumina short-read sequencing (<1000 bp);
  - IONTORRENT: Ion Torrent short-read sequencing (<1000 bp);
  - MGI: MGI short-read sequencing (<1000 bp);
  - ONT: Oxford Nanopore long-read sequencing (>1000 bp);
  - PACBIO: Pacific Biosciences long-read sequencing (>1000 bp).
- WGS accession identifier (WgsAccession): ENA or SRA run identifier, based on which, the sequence read data can be retrieved from the public domain. Starts with ERR or SRR, i.e. not the sample or experiment which start with ERS/ERX or SRS/SRX.
- If assemblies are provided instead of raw reads (not recommended), the assembly software used should be reported in WgsAssembler.

If WGS data for CRE25 isolates is provided to ECDC, it is not required to send any isolate to the strain collection. CRE25 isolates should be stored with a suitable method to preserve their viability until the end of the survey.

3.5.5 Patient data

Demographic data

- age (Age): age of patient in years at the date of sampling;
- gender (Gender): female, male or other;
- type of patient (PatientType): admission category of the patient, inpatient, outpatient or other;
- type of unit/ward (HospitalUnitType): hospital department at sample collection: emergency department, intensive care unit (ICU), infectious diseases ward, inpatient ward, internal medicine, obstetrics/gynecology, haematology/oncology, paediatrics/neonatal, paediatrics/neonatal ICU, primary health care, surgery, urology ward, other;

- healthcare facility NUTS-2 level location (HealthcareFacilityLocation): NUTS-2 region of the healthcare facility where the respective isolate is originating from;
- date of hospitalisation (DateOfHospitalisation): date of hospitalisation or outpatient visit (yyyy-mm-dd).

Epidemiological and clinical data

- clinical significance (ClinicalSignificance): colonisation or infection or undetermined/unknown clinical significance;
- organ/system or location of infection/colonisation (SiteOfInfection): intra-abdominal, bloodstream, lower respiratory tract, skin or soft tissue, urinary tract, other;
- hospital-acquired or community-onset (HospitalAcquiredSample):
  - Yes: hospital-acquired colonisation/infection if the sample is collected from an inpatient later than 48 h post-admission;
  - No: community-onset if the sample is collected from an outpatient or from a hospitalised patient earlier than 48 h post-admission.

Healthcare exposure/referral history

- direct hospital transfer (HospitalTransfer): direct transfer of patient from another hospital to the current hospital where patient is admitted:
  - Yes, same country: another hospital in the same country (specify country in CountryOfHospitalTransfer);
  - Yes, other country: a hospital in another country (specify country in CountryOfHospitalTransfer);
  - No.
- previous hospitalisation within six months before sampling date (PriorHospitalisation):
  - Yes, same country: another hospital in the same country (specify country in CountryOfPriorHospitalisation);
  - Yes, other country: a hospital in another country (specify country in CountryOfPriorHospitalisation);
  - No.
- previous residence in a long-term/elderly care facility, direct transfer or within six months before sampling date (PriorResidenceInLTCF):
  - Yes, same country (specify country in CountryOfPriorResidenceInLTCF);
  - Yes, other country (specify country in CountryOfPriorResidenceInLTCF);
  - No.

Travel history

- recent (past six months) travel history to another country prior to sample date (Travel):
  - Yes (specify country in TravelLocation);
  - No.

3.5.6 Data not collected for the CRE25 survey

The following variables will NOT be collected for CRE25 survey:

- AMRISO subject:
  - hospital identifier (HospitalId);
  - date of onset of disease (DateOfOnset);
  - date of discharge (DateOfDischarge);
  - date of death (DateOfDeath);
  - outcome of hospital stay (OutcomeHospital);
  - prescribed antimicrobial agent (PrescribedAntimicrobial);
  - sample identifier (SampleId);
  - laboratory code (LaboratoryCode);
  - WGS assembled genome (WgsAssembly);
  - WGS raw sequence reads (WgsRawReads).
- AMRISO$AST subject:
  - AST guideline (ReferenceGuidelinesSIR).

In addition, all variables of the AMRISODENOM subject are NOT collected for the CRE25 survey.

3.6 Data analysis

Data analysis will be described in the forthcoming analysis plan to be published at a later date.

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