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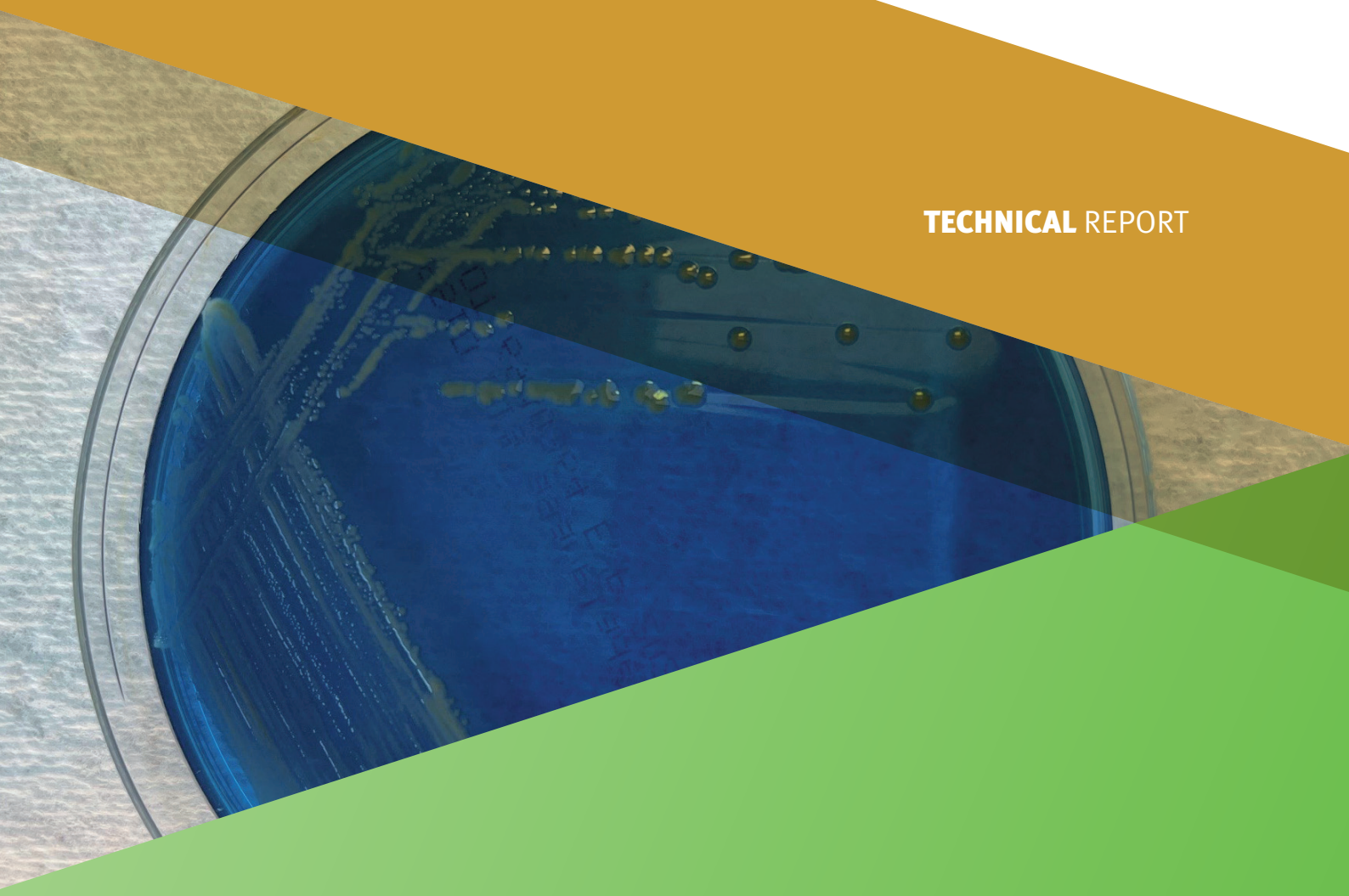

TECHNICAL REPORT

Laboratory manual for the ECDC genomic-based survey of carbapenem-resistant *Acinetobacter baumannii*

Version 1.2

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This technical report is a laboratory manual that accompanies the 'Survey protocol for the ECDC genomic-based surveillance of carbapenem-resistant *Acinetobacter baumannii* in Europe' [1]. The survey protocol contains a detailed description of the survey, objectives and sampling frame.

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This technical report was sent for consultation to the 'ECDC Expert Group for microbiological support to the ECDC CRAb survey', and subsequently sent for national comments, to ECDC National Focal Points for Antimicrobial and Operational Contact Points for Antimicrobial-resistant isolates. The scope and purpose of the Expert Group, and the recruitment process for group membership, are presented in Annex 1.

The members of the Expert Group were Silva Tafaj (Microbiology Department, University Hospital 'Shefqet Ndroqi', Tirana, Albania); Anette Marie Kühle Hammerum (National Reference Laboratory for Antimicrobial Resistance, Statens Serum Institut, Copenhagen, Denmark); Sotirios Tsiodras (Department of Medicine, Attikon University Hospital, Athens, Greece); Antoni P.A. Hendrickx (Center for Infectious Disease Control, Diagnostics and Laboratory Surveillance (IDS), National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands); Ørjan Samuelsen (Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, University Hospital of North Norway, Tromsø, Norway); Dorota Żabicka (National Reference Centre for Susceptibility Testing, National Medicines Institute, Warsaw, Poland); Vera Manageiro (National Reference Laboratory of Antibiotic Resistance and Healthcare Associated Infections, National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal); Ana Rita Rebelo (EARS-Net EQA (ECDC contractor) and EURGen-RefLabCap; National Food Institute, Technical Univeristy of Denmark, Copenhagen, Denmark); Thierry Naas (ESGARS; Assistance Publique–Hôpitaux de Paris (AP-HP), Paris, France); Elmine Alp Meşe (ESGCIP and EUCIC; University of Verona, Italy & Faculty of Medicine, Ankara Yıldırım Beyazıt University, Türkiye); Christian Giske (EUCAST; Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden).

Erratum: In Figure 3, the categorisation of EUCAST clinical breakpoints was updated on 2 October 2024. There is also a new section, 'Submitting bacterial samples for quality verification', for national teams.

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
ATC	Anatomical Therapeutic Chemical classification
CFU	Colony-forming units
CLSI	Clinical and Laboratory Standards Institute
CRAb	Carbapenem-resistant *Acinetobacter baumannii*
CSAb	Carbapenem-susceptible *Acinetobacter baumannii*
DNA	Deoxyribonucleic acid
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EuSCAPE	European survey on carbapenemase-producing Enterobacteriaceae
IDSA	Infection Diseases Society of America
LIMS	Laboratory information system
LOINC	Logical Observation Identifier Names and Codes
MIC	Minimum inhibitory concentration
MH	Mueller-Hinton
NCBI	National Center for Biotechnology Information
NHSN	National Healthcare Safety Network
PCR	Polymerase chain reaction
PHIC VADS	Public Health Information Network Vocabulary Access and Distribution System
UMLS	United Medical Language System
US CDC	United States Centers for Disease Control and Prevention

Intended audience

The intended audience includes those who may implement, or participate in, an ECDC survey to collect carbapenem-resistant *Acinetobacter baumannii* (CRAb) specimens and patient metadata from acute care hospitals, especially those working in clinical laboratories, including reference laboratories.

Brief background and overview

The detailed description of the survey is provided in the ECDC technical document that accompanies this laboratory manual, i.e. the 'ECDC survey protocol for genomic-based surveillance of carbapenem-resistant *Acinetobacter baumannii* at the European level' [1]. It includes a full description of the survey methodology, inclusion/exclusion criteria, sampling frame, metadata, and the practical steps to execute the survey.

Aims and objectives

For convenience, the full text of the survey's aim and public health objectives are specified here.

The **aim** is to conduct a survey of CRAb identified in clinical laboratories in European countries, to acquire a snapshot of circulating strains for the purposes of genomic surveillance; and to support national activities to collect a representative sample of CRAb isolates, to support national CRAb infection prevention and control efforts.

The **primary objective** is to describe the occurrence and geographic distribution of CRAb strains, and/or transmissible resistance/genetic elements of critical public health importance within CRAb strains, among patients in acute care hospitals in Europe, to inform prevention and control activities.

The **secondary objectives** are:

- To support EU/EEA countries, Western Balkan countries, and Türkiye in developing technical capabilities and proficiency in genomic-based surveillance and risk assessments of CRAb, to facilitate their identification of transmission chains, to enable targeted infection control interventions.
- To estimate the cumulative incidence of CRAb infections in participating hospitals during the survey period, to provide additional contextual information for the genomic results.
- To identify epidemiological factors for infection (or colonisation) with CRAb at clonal and sub-genomic level, to inform CRAb preparedness, prevention and control activities.

Overview of study design

The responsibilities for the National Survey Coordinator include coordination of the survey, nationally; recruitment of laboratories and hospitals; collating and reporting metadata; and ensuring the quality of biological material and metadata submitted for this survey [1].

Laboratories are eligible to participate if they provide clinical laboratory services for acute care hospital(s), have the capability to isolate species within the *Acinetobacter calcoaceticus/baumannii* complex (NCBI:txid909768) from a clinical sample, and routinely test isolates of *A. baumannii* for susceptibility against any of the commonly available carbapenems (doripenem, imipenem, or meropenem).

Laboratories should select non-duplicate isolates from non-duplicate patients, obtained from acute care hospital(s) designated by the National Survey Coordinator, during a six-month survey period between 10/2024 and 06/2025. The isolates should be selected according to species, phenotypic susceptibility to carbapenems, and sample type.

Laboratories should identify species that that they consider to be *'A. baumannii'* according to their standard practices, or alternatively, the speciation result should not exclude that the species meets the 'gold standard species definition' (for the purposes of this survey) of *A. baumannii*, i.e. NCBI:txid470 [2]. The section 'Identification of *eligible* Acinetobacter species' provides information to support them in their selection.

The survey protocol provides algorithms to assist laboratories selection samples, with preference for carbapenem-resistant *A. baumannii* (CRAb) from a diagnostic sample [1]. Alternatively, permitted sample types are (in order of preference) CRAb from a screening sample, carbapenem-susceptible *A. baumannii* (CSAb) from a diagnostic sample, or CSAb from a screening sample. The preferred methodology for antimicrobial susceptibility testing (AST) is provided in the section 'Phenotypic carbapenem susceptibility testing to select eligible isolates (EUCAST guidelines)'.

Metadata should be collected, using standard forms, from patients and hospitals that supplied an eligible sample that was identified by the participating laboratory to support completion of the survey objectives [1]. Using these forms, local and reference laboratories may report phenotypic AST results produced for standard clinical practice. The section 'Reporting available results from additional AST, by local and regional laboratories' provides further information.

The study design envisages the National Survey Coordinator selecting 10 strains from each participating hospital for inclusion in a European dataset, with WGS performed at a central laboratory to generate FASTQ files. The survey protocol contains a model Material Transfer Agreement for this purpose. Submitting laboratories will also receive these data from their isolates within about eight weeks of submission and retain ownership of the data.

Bioinformatic analysis at ECDC, will, when combined with the submitted metadata, permit completion of the survey aims and objectives. In 2025, this activity may include phenotypic AST, performed on a subset of isolates, to confirm antimicrobial resistance (AMR) results suggested by the bioinformatic (genomic) analyses.

Throughout, the survey methodology seeks to utilise existing local and national practices as this ECDC activity does not include provision of laboratory equipment, staff or training.

Identification of eligible *Acinetobacter* species

Background and purpose

The overall aims and objectives of the ECDC genomic-based survey can be achieved if participating clinical laboratories submit strains from acute care hospitals that they consider to be *'A. baumannii'* according to their standard laboratory practices. Ultimately, the epidemiology of species within the *Acinetobacter calcoaceticus/baumannii* complex are broadly similar. During analysis, ECDC will describe the phylogenetic distribution of the submitted strains.

The ability of clinical laboratories to identify *'A.' baumannii'* depends on their laboratory methodologies and taxonomic nomenclature. In Europe, methodologies such as MALDI-TOF can discriminate *A. baumannii* from other species within the genus Acinetobacter and the *Acinetobacter calcoaceticus/baumannii* complex (NCBI:txid909768), e.g. *A. calcoaceticus*, *A. pittii*, *A. nosocomialis*. However, technologies with this discriminatory power are not available in all laboratories eligible to participate in this ECDC survey. Therefore, this section of the laboratory manual provides information to help laboratories include or exclude strains, according to the common taxonomic nomenclatures.

Gold standard species definition for this survey

The 'gold standard species definition' of *A. baumannii* for this survey has the NCBI taxonomy code NCBI:txid470 (Bouvet and Grimont, 1986 [2]). In subsequent surveys, other species from the genus Acinetobacter may be included, depending on changes in the epidemiological situation.

Instructions

Participating laboratories should compare Table 1 to the documentation available for their laboratory methodology, to identify species as close as possible to the 'gold standard species definition', within the constraints of their laboratory methodology. The table below is for reference. Inherently, most of the codes will be unobtainable in most laboratories.

- For example, if a laboratory system exports data using the WHONET nomenclature, and it displays *Acinetobacter baumannii* (code: B_ACNTB_BMNN) then that strain is eligible. Similarly, if a laboratory system exports SNOMED nomenclature, and it displays *Acinetobacter baumannii* (code: 91288006), then that strain is eligible.

Exclusion criteria

Exclude strains that cannot be *Acinetobacter baumannii*, according to the methodologies available during standard clinical practice, using best local practices. This can be achieved even if the methodology does not use any of the nomenclature systems listed in Table 1.

- For example, if a standard laboratory methodology in a local laboratory can discriminate that a strain is within the *Acinetobacter calcoaceticus/baumannii* complex, but the methodology cannot discriminate the species within this group, then:
  - a strain is eligible for inclusion if the methodology identifies that the strain is from the *Acinetobacter calcoaceticus/baumannii* complex, because its species has the potential to be the 'gold standard' species definition'.
  - a strain should be excluded from the study if the methodology identifies that it is not in the *Acinetobacter calcoaceticus/baumannii* complex, because the species cannot meet the 'gold standard' species definition'.

Table 1. Reference table presenting the codes that most closely match *Acinetobacter baumannii* within common nomenclature databases and systems.

Database/surveillance system	Code that matches *A. baumannii* most closely	Description of code within the taxonomy system	Version	Comments	Total codes in this system
NCBI Taxonomy	**470**	***Acinetobacter baumannii*** (Bouvet and Grimont, 1986 [2]). **Semantic tag: NCBI:txid470**	Date accessed: 23 February 2024	**Gold standard species definition for this survey.** Highest resolution, available to those with WGS of *Acinetobacter* spp..; can be reported to TESSy/EpiPulse Cases.	>5 million
SNOMED (CDC PHIN VADS)	91288006	CONCEPT_NAME = '*Acinetobacter baumannii* (organism)'	Version: 1 September 2020	Commonly used in European countries for clinical systems/LIMS. Use of SNOMED implies having a license.	~18 900
LOINC	LA24372-7	*Acinetobacter baumannii* isolated	Version 2.74	NIH system that provides universal codes and names to identify laboratory and other clinical observations	~100 000
UMLS	C202492	*Acinetobacter baumannii*	Version 24.01e	NIH system popular in the US that requires registration. It is a biomedical 'meta-thesaurus' that links synonymous names from >200 source vocabularies. It 'identifies useful relationships between concepts and preserves the meanings, concept names, and relationships from each vocabulary' .For example, LOINC 'LA24372-7' is mapped to UMS as 'CL930085', within C202492.	3.5 million concepts, 7 million codes, 15 million names.
WHONET	B_ACNTB_BMNN	*Acinetobacter baumannii*	v2023	Commonly used globally, for national and international surveillance activities, including EARS-Net and GLASS	~2 900
US CDC NHSN	ACBA	*Acinetobacter baumannii*	2024 version, updated 12-2023	NHSN Organism List, curated by the US CDC	2 278
WHOCARE ORG CODE	ACIBAU	ACINETOBACTER BAUMANNII	Most recent update: 2022	ECDC-curated aggregate list. Well-established: software version released in 1991 by WHO/EURO and distributed at a non-profit price [1,2].	991
WHOCARE ORG CODE (**REDUCED**)	ACIBAU	ACINETOBACTER BAUMANNII	Most recent update: 2016	Used in ECDC surveillance protocols for healthcare-associated infections, e.g. HAI-PPS, HAI-HALT, HAI-ICU and HAI-SSI [3].	153
EARS-Net (insufficient for this survey)	ACISPP	*Acinetobacter* species	Metadata Set 53	ECDC-coordinated surveillance of eight key bacterial species.	8

Key: LIMS – laboratory information systems; LOINC – Logical Observation Identifier Names and Codes; PHIC VADS – Public Health Information Network Vocabulary Access and Distribution System; NCBI – National Center for Biotechnology Information; NHSN – National Healthcare Safety Network; UMLS – United Medical Language System; US CDC – United States Centers for Disease Control and Prevention;

[1] – SSI. Health Policy. 1991; 19(2–3):257-259; [2] – Mertens R, et al. Infection Control & Hospital Epidemiology. 1994;15(9):574-580.

Phenotypic carbapenem susceptibility testing to select eligible isolates (EUCAST guidelines)

This survey recommends using the latest EUCAST guidelines for AST of bacteria. For a complete list of breakpoints, consult the EUCAST breakpoint table: http://www.eucast.org/clinical_breakpoints [4]. The latest recommendations for AST, and warnings, can be found on the EUCAST website: https://www.eucast.org/ast_of_bacteria [5]. For convenience, this document contains the EUCAST protocol version 14.0 (published on 1 January 2024) for broth microdilution (Protocol 1; recommended) and disk diffusion (Protocol 2).

If national guidelines include E-tests for carbapenem susceptibility testing as part of the hospital diagnostic pathway, the national reference laboratory(s) should confirm the results using one of the EUCAST-approved methods outlined below.

Protocol 1: carbapenem susceptibility testing by broth microdilution (EUCAST)

For more information, consult the EUCAST recommendation for media preparation [5]: http://www.eucast.org/ast_of_bacteria/media_preparation.

Minimum inhibitory concentration (MIC) determination (broth microdilution according to ISO standard 20776-1) Medium: Mueller-Hinton broth.

Inoculum: $5x10^5$ colony-forming units (CFU)/mL. Incubation: sealed panels, air, 35±1º C, 18±2h.

Reading: unless otherwise stated, read minimum inhibitory concentrations (MICs) at the lowest concentration of the agent that completely inhibits visible growth.

Quality control: *Pseudomonas aeruginosa* ATCC 27853.

Table 2. EUCAST clinical breakpoints for carbapenems among *Acinetobacter* species

Carbapenems*	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Doripenem	0.0001	2	10	50	22
Imipenem	2	4	10	24	21
Meropenem	2	8	10	21	15
Meropenem (meningitis)	2	2	10	21	21

Source: EUCAST clinical breakpoint table v14.0. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf

**: certain isolates that produce carbapenemase are categorised as susceptible with these breakpoints and should be reported as tested, i.e. the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility.*

Protocol 2: carbapenem susceptibility testing using Kirby-Bauer disk diffusion (EUCAST)

If national guidelines include E-tests for carbapenem susceptibility testing as part of the hospital diagnostic pathway, the national reference laboratory(s) should confirm the results using one of the EUCAST-approved methods.

For more information, consult the EUCAST Disc Diffusion Manual [6]:
http://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology

Preparation of media:

Prepare Mueller-Hinton (MH) agar according to manufacturer's instructions. The medium should have a level depth of 4 mm ± 0.5 mm (approximately 25 mL in a 90-mm circular plate, 31 mL in a 100-mm circular plate, 71 mL in a 150-mm circular plate, 40 mL in a 100-mm square plate). The surface of the agar should be dry before use. Storage and drying conditions determine whether plates require drying and the length of time needed to dry the surface of the agar. Do not over-dry plates.

Preparation of inoculum:

Use the direct colony suspension method to make a suspension of the organism in saline to the density of a McFarland 0.5 turbidity standard, approximately corresponding to 1–2 x10^8 CFU/mL for *E. coli*. Make the suspension from overnight growth on a non-selective medium. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant and suspend the colonies in saline with a sterile loop or cotton swab. Standardise the inoculum suspension to the density of a McFarland 0.5 standard.

Inoculation of agar plates:

Optimally, use the adjusted inoculum suspension within 15 minutes of preparation. The suspension must always be used within 60 minutes of preparation. Dip a sterile cotton swab into the suspension and remove the excess fluid by turning the swab against the inside of the container. It is important to remove excess fluid from the swab to avoid over-inoculation of plates, particularly for Gram-negative organisms. Spread the inoculum evenly over the entire surface of the plate by swabbing in three directions or using an automatic plate rotator. Apply disks within 15 minutes.

Application of antimicrobial disks:

Apply disks firmly to the surface of the inoculated and dried agar plate. The contact with the agar must be close and even. Disks must not be moved once they have been applied to plates as diffusion of antimicrobial agents from disks is very rapid. The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks depends on the organism and the selection of disks. Normally 6 and 12 disks are the maximum possible number on a 90- and 150-mm circular plate respectively.

Incubation of plates:

Invert plates and incubate them within 15 minutes of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition. Stacking plates in the incubator affects results owing to uneven heating of plates. The efficiency of incubators varies and therefore the control of incubation, including appropriate numbers of plates in stacks, should be determined as part of the laboratory's quality assurance programme.

Examination of plates after incubation:

A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. The growth should be evenly distributed over the plate to achieve uniformly circular (non-jagged) inhibition zones. If individual colonies can be seen, the inoculum is too light and the test must be repeated. Check that inhibition zones are within quality control limits.

Measurement of zones and interpretation of susceptibility:

For all agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. Read un-supplemented plates from the back with reflected light and the plate held above a dark background. Read supplemented plates from the front with the lid removed and reflected light.

Do not use transmitted light (plate held up to light) or a magnifying glass unless otherwise stated. Measure the diameters of zones of inhibition to the nearest millimetre with a ruler, calliper or automated zone reader. Interpret zone diameters by reference to breakpoint tables [3]: http://www.eucast.org/clinical_breakpoints. If templates are used for interpreting zone diameters, the plate is placed over the template and zones interpreted according to EUCAST.

This section of the protocol is based partly on the laboratory manual developed for the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE) [1].

Submitting isolates for this survey

Storage of original isolates

Ideally, bacterial samples should be stored for up to two years, following national best practices.

Submitting bacterial sample

The bacterial sample may be supplied as 2×1 ml overnight cultures (optical density ≥1 at 600 nm), expecting each culture to contain 8×10^8 cells on average) OR a pellet from equivalent cultures in 2 ml screw cap tubes; OR plated colonies on agar plates (≥10 colonies with a diameter ≥0.8 mm).

Shipment of materials

The packaging and shipment of isolates should comply with national and international shipment regulations for biohazardous material (packaging instructions P650, UN3373[7]).

Submitting bacterial samples for quality verification

National reference/expert laboratories are requested to submit bacterial samples to an ECDC contractor (CRBIP, Institut Pasteur, Paris, France) for quality verification, according to their bilateral Material Transfer Agreements. They may submit them in agar storage tubes, such as a standard 3ml tube. Suitable alternatives include equivalent agar storage tubes of similar format with sterile nutrient agar, TSA, or another non-selective medium. Additional technical details are available for 'National Survey Coordinators' and other ECDC 'Operational Contact Points' and 'National Focal Points', on request.

Reporting available results from additional AST, by local and regional laboratories

Rationale and purposes

In Europe, treatments for infections with *Acinetobacter baumannii*, and particularly CRAb infections, can include antimicrobial agents that do not have a EUCAST breakpoint, such as ampicillin-sulbactam [4,8]. Nonetheless, locally- and national-generated phenotypic AST results, from standard clinical practice, will be very valuable, to provide context for WGS results from a genomic-based survey.

Planned analyses

After initial analysis of the genomic data, ECDC may request a subset of isolates from national/expert reference laboratories for AST at a central reference laboratory, also guided by any voluntarily submitted phenotypic AST results. The objective of the central phenotypic AST will be to assess phenotypic susceptibility that is suggested by the genomic results.

Activities for local and reference laboratories

Options for countries to report additional AST results from local/reference laboratories

Participating local laboratories and reference laboratories are welcome to share additional AST results for the submitted strains that are generated for local/national purposes.

The ECDC genomic-based survey of CRAb does not request additional AST results beyond normal practice, and does not include the opportunity to reimburse AST.

If additional AST results are reported by local/reference laboratories, the order of preference to report such AST results to ECDC is:

Priority 1: AST results for antimicrobial agents used to treat for CRAb infections (for examples, see Table 4)

Priority 2: AST results for antimicrobial agents used to treat CSAb infections (for examples, see Table 3)

Priority 3: AST results for other antimicrobial agents.

Role of reference laboratories and National Survey Coordinators

National Survey Coordinators, in consultation with their national/expert reference laboratory, are welcome to update AST results submitted by local laboratories with AST results obtained by the national/expert reference laboratory, as per standard national practice.

For example, if the reference laboratory tests for an antimicrobial agent that was not tested at the local laboratory, those additional AST results can be added to the reported survey data. Also, if the local laboratory and reference laboratory obtain discordant AST results for the same antimicrobial agent, the National Survey Coordinator and reference laboratory may choose to report the AST result obtained by the reference laboratory.

In this ECDC survey, the metadata does not collect information on whether the AST result was generated by a local or reference laboratory, because that is beyond the scope of this activity.

How to report AST results

The form to collect specimen metadata 'FORM A' (Appendix 4 of the Survey Protocol) permits reporting of phenotypic AST results (Figure 1).

Antimicrobial agent: the permitted values of the metadata that can be reported to ECDC are ATC codes [9,10], and so it is preferable for laboratories to report ATC codes to the National Survey Coordinator.

If laboratories prefer to report local codes for the antimicrobial agent to the National Survey Coordinator, then the National Survey Coordinator should ensure that they are converted to ATC codes before upload to ECDC. The ATC codes for common treatments for infections with *Acinetobacter baumannii* are provided in Tables 3 and 4. Additionally, aztreonam-avibactam may be reported as 'J01DF51' [11,12].

National survey coordinator may wish to note that reporting by ATC code is commonly performed by their national colleagues that report antimicrobial consumption data to ECDC, for ESAC-Net [13]. If they are unsure who their national ESAC-Net colleagues are, National survey coordinators, and/or their ECDC National Focal Point for Antimicrobial Resistance, are welcome to contact ECDC.

AST guideline/breakpoint: indicate whether the local assessment of phenotypic resistance was performed using EUCAST guidelines (which is preferable); CLSI guidelines; another guideline, such as a local or national guideline, or whether no guideline was used or relevant.

AST method: participating laboratories should choose the option that most closely matches their AST methodology.

Figure 1. Collection of data regarding antimicrobial susceptibility testing on 'Form A: Specimen metadata'

Phenotypic antimicrobial susceptibility testing (at a minimum, report at least one carbapenem)

Antimicrobial agent *ATC code (preferably); or standard hospital code*	**AST guideline /breakpoint**	**AST Method**	**MIC** (mg/L), *if relevant*	**Disk diffusion zone diameter** (mm), *if relevant*
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		
	☐ EUCAST ☐ CLSI ☐ Other ☐ None/N.A.	☐ AUTOM ☐ BROTHDIL ☐ GRAD ☐ ZONE		

Key: MIC – minimum inhibitory concentration; AUTOM – Automated instrument method, BROTHDIL – Broth microdilution, GRAD – Antimicrobial gradient (E-test, etc), ZONE – Disc diffusion test; N.A. – not applicable. ATC – Anatomical Therapeutic Chemical classification, available from https://www.whocc.no/atc_ddd_index/

Table 3. ATC codes and clinical breakpoint guidelines for treatments for infections with *Acinetobacter* spp. that are included in EUCAST clinical breakpoint tables

Antimicrobial agent	ATC code	EUCAST clinical breakpoint [4]	Comments
Penicillins [1]			
Piperacillin	**J01CA12**	No	'Insufficient evidence' [4]
Ampicillin-sulbactam	**J01CR01**	No	'Insufficient evidence' [4]
Piperacillin-tazobactam	**J01CR05**	No	'Insufficient evidence' [4]
Ticarcillin-clavulanic acid	**J01CR03**	No	'Insufficient evidence' [4]
Cephalosporins			
Cefiderocol	**J01DI04**	No	'Insufficient evidence' and see 'Notes' [4]
Carbapenems			
Doripenem	**J01DH04**	Yes	
Imipenem	**J01DH51**	Yes	
Imipenem-relebactam	**J01DH56**	No	See 'Notes' [4]
Meropenem	**J01DH02**	Yes	
Meropenem-vaborbactam	**J01DH52**	No	See 'Notes' [4]
Fluoroquinolones			
Ciprofloxacin	**J01MA02**	Yes	
Delafloxacin	**J01MA23**	No	'Insufficient evidence' [4]
Levofloxacin	**J01MA12**	Yes	
Moxifloxacin	**J01MA14**	No	
Nalidixic acid (screen only)	**J01MB02**	NA (screen only)	
Aminoglycosides			
Amikacin	**J01GB06**	Yes	
Gentamicin	**J01GB03**	Yes	
Netilmicin	**J01GB07**	No	'Insufficient evidence' [4]
Tobramycin	**J01GB01**	Yes	
Tetracyclines			
Eravacycline	**J01AA13**	No	'Insufficient evidence' [4]
Minocycline	**J01AA08**	No	'Insufficient evidence' [4]
Tigecycline	**J01AA12**	No	'Insufficient evidence' [4]
Miscellaneous agents			
Colistin	**A07AA10**	Yes	See 'Notes' [4]
Fosfomycin iv	**J01XX01**	No	See 'Notes' - AST is discouraged [4]
Trimethoprim-sulfamethoxazole	**J01EE01**	Yes	See 'Notes' [4]

Source: EUCAST Clinical Breakpoints Table v. 14.0 [4]

[1] *Susceptibility testing of* Acinetobacter *spp. to penicillins is unreliable. In most instances,* Acinetobacter *spp. isolates are resistant to penicillins; ATC: Anatomical Therapeutic Chemical classification; NA: not applicable.*

Table 4. Treatments for CRAb specified in ESCMID or IDSA guidelines

Antimicrobial agent	ATC code	EUCAST breakpoint [a]	CLSI breakpoint [b]
Ampicillin-sulbactam	**J01CR01**	None; 'insufficient evidence' [4]	≤ 8/4 mg/L
Cefiderocol	**J01DI04**	None, but see 'Notes' [4]	≤ 4 mg/L [14]
Colistin	**A07AA10**	≤ 2 mg/L; see 'Notes' [4]	Intermediate ≤ 2 mg/L
Polymyxin B	**J01XB02**	None	Intermediate ≤ 2 mg/L
Minocycline	**J01AA08**	None; 'insufficient evidence' [4]	≤ 4 mg/L
Tigecycline	**J01AA12**	None	None

[a] EUCAST clinical breakpoints table v. 14.0 [4]; [b] CLSI interpretative criteria for susceptibility for 2023 [15], also published in 'Infectious Diseases Society of America Antimicrobial-Resistant Treatment Guidance: Gram-Negative Bacterial Infections. Infectious Diseases Society of America (IDSA) 2023; Version 3.0' (https://www.idsociety.org/practice-guideline/amr-guidance) [14]; ATC: Anatomical Therapeutic Chemical classification; CLSI: Clinical and Laboratory Standards Institute; IDSA: Infection Diseases Society of America. Note: on 21 March 2024, the EMA licensed aztreonam-avibactam, which will have the ATC Code J01DF51 (for more information, see https://www.ema.europa.eu/en/documents/smop-initial/chmp-summary-positive-opinion-emblaveo_en.pdf [11] and https://atcddd.fhi.no/lists_of__temporary_atc_ddds_and_alterations/new_atc_5th_levels/ [12])

Reporting antimicrobial agents prescribed to treat a reported episode of *Acinetobacter* infection

Overview

In this survey, hospitals (or laboratories) can choose the option of reporting the antimicrobial agent(s) that were prescribed to the patient who supplied an eligible isolate to treat their *Acinetobacter* infection. This information will support the completion of the second part of one of the secondary objectives: '*To identify epidemiological factors for infection (or colonisation) with CRAb at clonal and sub-genomic level, to inform CRAb preparedness, prevention and control activities.*' However, only the minimum information will be collected, in order to reduce the reporting burden for participants and to avoid collecting data for 'risk management' or research purposes, which are both outside of the mandate of ECDC.

Rationale and purpose

If an *Acinetobacter* isolate submitted for this survey is found to be resistant to an antimicrobial agent, this information alone is insufficient to discriminate how that resistance arose. The patient may have been infected with a resistant strain from, for example, an indirect transfer from a contaminated hospital environment. Alternatively, there may have been secondary (acquired) resistance arising within the patient that supplied the biological sample, due to exposure to an antimicrobial agent during treatment. This survey can provide some information to support that discrimination, by collecting data on the prescribed antimicrobial agents.

Planned analyses

The analyses will summarise data at European level, but not at country or hospital level. Specifically, the genotypic AMR profile of submitted strains, and, if available, phenotypic AST results for that strain, will be compared to any submitted antimicrobial use data for the patient that supplied the strain, to identify instances of AMR in the absence of reported exposure to the relevant antimicrobial agent(s).

For the purposes of this analysis, antimicrobial use results that are submitted for any one patient will be assumed to be complete. Conversely, if no antimicrobial use data are available for a patient, they will be excluded from this sub-analysis.

Precluded analyses

The metadata for this survey do not include data to support a retrospective analysis of whether patient management was appropriate, such as the dose, timing, or route of antimicrobial administration; for co-morbidities, such as co-infections; or indicators of infection prevention and control at the patient or ward level.

How to report antimicrobial use data

Hospitals may list all antimicrobial agents prescribed to the patient, subsequent to clinical suspicion or diagnosis of infection with an *Acinetobacter* spp., preferably using ATC codes (Figure 2) [9,10]. For convenience, the ATC codes of common treatments for *Acinetobacter* infections are listed in Tables 3 and 4.

As with the reporting of phenotypic AST results, the permitted values of metadata that can be reported to ECDC are ATC codes [9,10], so it is preferable for laboratories to report ATC codes to the National Survey Coordinator.

If laboratories prefer to report local codes for the antimicrobial agent to the National Survey Coordinator, then the National Survey Coordinator should ensure that they are converted to ATC codes before upload to ECDC. The ATC codes for common treatments for infections with *Acinetobacter baumannii* are provided in Tables 3 and 4. Additionally, aztreonam-avibactam may be reported as 'J01DF51' [11].

National survey coordinators may wish to note that reporting by ATC code is commonly performed by their national colleagues who report antimicrobial consumption data to ECDC, for ESAC-Net [13]. If they are unsure who their national ESAC-Net colleagues are, national survey coordinators and/or their ECDC National Focal Point for Antimicrobial Resistance are welcome to contact ECDC.

Figure 2. Collection of information regarding antimicrobial consumption for Acinetobacter infection on 'Form B: Patient metadata'

Antimicrobial agents prescribed/received following the clinical suspicion or diagnosis of Acinetobacter infection (LIST ALL)

Optional. Preferably report ATC codes, available from https://www.whocc.no/atc_ddd_index/. Alternatively, report local codes.
These data will generate European-level summary statistics; and not patient-, ward-, or hospital-level analyses.
Note: this survey obtains insufficient data to ascertain the appropriateness of individual patient care.

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Annex 1. ECDC CRAb survey Expert Group

The scope of the ECDC Expert Group for microbiological aspects of the CRAb survey was to provide advice to ECDC to: (a) support production of a laboratory manual to accompany the ECDC CRAb survey protocol; (b) provide minor input relevant to the survey protocol itself, to ensure consistency with the laboratory manual; and (C) provide input to draft analyses from the survey. The Expert Group was formed in February 2024.

In January 2024, ECDC sent an email invitation to register interest in the Expert Group to ECDC National Focal Points (NFPs) for Antimicrobial Resistance (AMR); NFPs for Microbiology; NFPs for AMR Observers; NFPs for Microbiology Observers; Operational Contact Points for Microbiology – Antimicrobial-resistant isolates (AMRISO); Contact Points for Operations (CPO) for Microbiology – AMRISO; EARS-Net Disease Network Coordination Committee Members and Observers; Participants from Western Balkan countries and Türkiye at the ECDC EURGen-Net network meeting (DPR179; 29–30 November 2023), National Coordinators in Coordinating Competent Bodies; and the National Correspondents in Western Balkan countries and Türkiye. The email specified the scope and purpose for the group, provided the ECDC selection criteria for membership, invited recipients to register their interest in the ECDC Expert Directory, and asked them to forward the email to their networks. To enable appropriate mitigation for potential conflicts of interest, ECDC reviewed the Declarations of Interest submitted by potential Expert Group members, in accordance with the ECDC policy on scientific integrity and independence.

The selected members of the Expert Group were: Silva Tafaj (Microbiology Department, University Hospital 'Shefqet Ndroqi', Tirana, Albania); Anette Marie Kühle Hammerum (National Reference Laboratory for Antimicrobial Resistance, Statens Serum Institut, Copenhagen, Denmark); Sotirios Tsiodras (Department of Medicine, Attikon University Hospital, Athens, Greece); Antoni P.A. Hendrickx (Center for Infectious Disease Control, Diagnostics and Laboratory Surveillance (IDS), National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands); Ørjan Samuelsen (Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, University Hospital of North Norway, Tromsø, Norway); Dorota Żabicka (National Reference Centre for Susceptibility Testing, National Medicines Institute, Warsaw, Poland); Vera Manageiro (National Reference Laboratory of Antibiotic Resistance and Healthcare Associated Infections, National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal); Ana Rita Rebelo (EARS-Net EQA (ECDC contractor) and EURGen-RefLabCap; National Food Institute, Technical Univeristy of Denmark, Copenhagen, Denmark); Thierry Naas (ESGARS; Assistance Publique–Hôpitaux de Paris (AP-HP), Paris, France); Elmine Alp Meşe (ESGCIP and EUCIC; University of Verona, Italy & Faculty of Medicine, Ankara Yıldırım Beyazıt University, Türkiye); Christian Giske (EUCAST; Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden).

At the time of publication, the Expert Group had held one meeting, the 'Virtual Expert Group meeting for the ECDC survey of CRAb 2024/2025', on 26 March 2024.

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