

Title: Metrology to enable accurate antimicrobial resistance identification and surveillance with a One Health Approach

Abstract

Antimicrobial resistance (AMR) threatens the health of humans, animals, and environment and is predicted to be the main cause of human death by 2050. The European Commission had identified AMR as one of the top three priority health threats highlighting the need for EU One Health action against AMR. Given the interdependencies between the farming and medical practices a One Health approach, addressing AMR across human, animal, and environmental settings, is vital to monitor current practices. Nevertheless, the metrology to underpin traceable and reproducible AMR measurements at the One Health level is almost completely absent because the lack of metrological sound AMR surveillance tests undermining interventions and countermeasures. Therefore, a metrology infrastructure needs to be developed to support the accuracy of analytical methods deployed in the identification and surveillance of AMR surveillance across medical, agricultural and environmental sectors.

Keywords

Antimicrobial resistance (AMR), AMR surveillance, One Health Approach, artificial intelligence (AI), digital PCR (dPCR), next generation sequencing (NGS), mobile genetic elements (MGEs), animal health, human health, environmental health

Background to the Metrological Challenges

Antimicrobial resistance (AMR) is recognised as one of the 10 major global threats to human health. Drug-resistant microorganisms, which can spread across humans, animals, and the environment, undermine our ability to treat infections effectively and in Europe, AMR caused by bacteria represents one of the highest healthcare burdens. The European Commission and the Member States identified AMR as one of the top three priority health threats and highlights the need for EU One Health action against AMR. The increasing global threat of antimicrobial-resistant bacteria needs the development of robust and reliable AMR surveillance systems across human, animal, and environmental settings. This means that antimicrobial-resistant bacterial surveillance now extends beyond hospitals and healthcare settings. Environmental monitoring is crucial to detect bacteria in wastewater, rivers and agricultural settings (e.g. wastewater, animal and human food), providing early signals of bacterial resistance genes. Monitoring relevant antimicrobial-resistant bacteria in food supply chain, and including livestock is important given the significant use of antibiotics in agriculture. One Health action acknowledges that human and animal health as well as the environmental health are interconnected, recognising that diseases can be transmitted from humans to animals and vice versa and must therefore be considered together.

To avoid an era of untreatable infections, it is imperative to accelerate the development of a framework of reference measurement procedures, which does not currently exist, to improve the comparability and accuracy of AMR surveillance test results. Existing methods used for antimicrobial-resistant bacterial surveillance and transmission studies lack standardisation, leading to discrepancies in measurement accuracy and comparability across monitoring laboratories. This limits the ability to monitor antimicrobial-resistant bacteria trends accurately to mitigate its spread.

Establishing appropriate reference materials (e.g. bacterial DNA and/or whole cells) and methods would allow for the traceability to the International System of Units (SI) and standardisation of a wide range of associated surveillance testing, from molecular biomarkers to phenotypic assessments of microbial resistance,

Matrix-Assisted Laser Desorption/Ionization time of flight (MALDI-TOF) and imaging techniques. Furthermore, evaluating the measurement uncertainty determination of the bioinformatics applied to AMR bacterial genes, mobile genetic elements (MGEs) data and understanding the source of errors (e.g. by training AI models) is essential. Previous projects (EMRP project HLT08 INFECT-MET, and EMPIR projects 15HLT07 AntiMicroResist and 18HLT03 SEPTIMET) were focused on the development of genomic standards and quality assurance for monitoring infectious diseases and antimicrobial resistant microorganisms in human samples highlighting the lack for other settings such as the environment, and agricultural samples.

Alternative methods are starting to be used for AMR surveillance monitoring such as matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS)-based, Raman spectrometry, flow cytometry, and 2D/3D quantitative phase imaging microscopy or hyperspectral imaging. These methods enable varied assessment of bacterial populations and support complementary methods for quantification, viability and antibiotic susceptibility testing for implementation of AMR surveillance systems. However, metrological principles need to be built for these phenotypic, molecular and optical methods. Moreover, AI may lead to a new direction in antimicrobial detection. The availability of large datasets from multiple sources may make it more effective to use AI techniques to identify interesting insights into AMR genes/MGEs data (e.g. new genes, mutations). Additionally, AI can be applied to images with significant potential for the use in the antimicrobial resistant bacteria monitoring.

Therefore, quality control and reference materials and methods to support quality assurance of molecular, phenotypic, and imaging methods routinely used in antimicrobial-resistant bacteria surveillance laboratories is required. Further requirements include guidelines for measurement uncertainty determination using artificial intelligence applied to AMR genes/MGEs binary data as well as computational microscopy data supporting standardisation of AI methods, with potential application in antimicrobial-resistant bacteria surveillance. These improvements will help on better harmonisation and standardisation of surveillance data, reducing inconsistencies in data collection and making data more reproducible and reliable. The engagement of stakeholders in understanding the importance of antibiotic-resistant bacteria and MGEs is essential for the long-term success of antimicrobial-resistant bacteria surveillance and control.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The proposal shall focus on the traceable measurement and characterisation of accurate antimicrobial resistance identification and surveillance with a One Health Approach.

The specific objectives are

1. To establish a metrological framework to support high accuracy molecular quantification for the detection and measurement of antimicrobial-resistant bacterial genes/mutations and MGEs across environmental (e.g. wastewater), agricultural and clinical settings. This should include:
 - a. the development and characterisation of reference materials (e.g. bacterial DNA and/or whole cells) including different sources (e.g. wastewater, veterinary, food and human) and with biosafety level 1 or 2 to improve current reproducibility, comparability, and quality assurance of molecular approaches used to screen antibiotic-resistant bacteria and mobile elements carrying antimicrobial-resistance genes.
 - b. the development of higher order measurement methods (e.g. digital PCR (dPCR)) for quantification of antimicrobial-resistant bacteria genes (e.g. beta-lactamase genes), and mobile elements (e.g. integrons or transposons) looking for resistant conferring-mutations using the new developed reference materials with a targeted uncertainty of <20 %. The definition of mathematical sources of reduced specificity and inter-laboratory comparisons where uncertainty values will be calculated.
 - c. The assignation of SI-traceable values to current and new external quality assurance (EQA) materials to support existing or new EQA schemes, linking SI to international consensus values.
2. To develop methods and materials to support easily implemented high accuracy measurement procedures for environmental surveillance of antimicrobial resistant bacteria by evaluating error sources, uncertainty calculation, reproducibility and validation of the measurement methods used. This should include i) metagenomic screening of primary environmental specimens (e.g. wastewater) to identify and quantify antimicrobial-resistant bacterial genes, mobile genetic elements, using

quantitative next generation sequencing (NGS) and ii) phenotypic screening of bacterial isolates (e.g. culture and bioassays such as minimal inhibitory concentration method and disc diffusion).

3. To evaluate alternative methods to support quantification of antimicrobial-resistant bacteria and validation of materials, produced in objective 1 and 2. This should include: i) methods such as flow cytometry, computational microscopy, 2D and 3D quantitative phase imaging, Raman spectroscopy, hyperspectral imaging, and Matrix-Assisted Laser Desorption/Ionization time of flight (MALDI-TOF) for quality assurance and SI-traceability, linking molecular enumeration of resistance genes to bacterial cell counts, and ii) the assessment of analytical performance, sensitivity, specificity and reproducibility of the methods.
4. To develop guidelines for overall measurement uncertainty (MU) determination for AI in bioinformatics (e.g. antimicrobial-resistance bacterial genes, MGEs) and computational microscopy data (e.g. bacterial cell morphology) from objectives 1, 2 and 3 making use of the Guides to the Expression of Uncertainty in Measurement (GUM) framework where applicable. This should include the assessment of random and systematic errors and uncertainties influencing measurements such as short read lengths, mutation detection (for antibiotic-resistance bacterial gene, MGEs binary data), sensor noise, image acquisition, and statistical fluctuations for computational microscopy/imaging data.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology – CCQM, and European Metrology Network for Traceability in Laboratory Medicine – EMN TraceLabMed), standards developing organisations (ISO TC276 - Biotechnology, ISO TC334 - Reference materials, ISO TC212 WG4 - Microbiology & Molecular diagnostics, ISO TC194 - Biological and clinical evaluation of medical devices, CEN), other stakeholders (The Joint Committee for Traceability in Laboratory Medicine – JCTLM - database, the Clinical and Laboratory Standards Institute - CLSI, material manufacturers) and end users (e.g. surveillance testing manufacturers, manufacturers of medical and healthcare products, personalised medicine providers).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners, medical (academic) hospitals and industry is strongly recommended, both prior to and during methodology development. Where relevant, proposals are encouraged to build on, or seek collaboration with, existing projects and develop synergies with other relevant European, national or regional initiatives and funding programmes. In particular, links are encouraged with (i) the projects funded under earlier relevant topics of the Horizon Europe programme; or (ii) other relevant European Partnerships.

Proposers should establish the current state of the art and explain how their proposed project goes beyond this. In particular, proposers should outline the achievements of the EMRP project HLT08 INFECT-MET, and EMPIR projects 15HLT07 AntiMicroResist, and 18HLT03 SEPTIMET, and how the proposals will build on those.

Proposers should note that the programme funds the activity of researchers to develop the capability, not the required infrastructure and capital equipment, which must be provided from other sources.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 2.1 M€ and has defined an upper limit of 2.6 M€ for this proposal.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Any industrial beneficiaries that will receive significant benefit from the results of the proposed project are expected to be beneficiaries without receiving funding or associated partners.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the 'end user' community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your proposal's results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,

- Facilitate improved industrial capability, or improved quality of life for European citizens in terms of personal health, protection of the environment and the climate, or energy security,
- Transfer knowledge to the surveillance testing sector, clinical, environmental and agricultural sector.

You should detail other impacts of your proposed JRP as specified in the document “Guide 4: Writing Joint Research Projects (JRPs)”

You should also detail how your approach to realising the objectives will further the aim of the Metrology Partnership to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work.

Timescale

The project should be of up to 3 years duration.

Additional information

The links provided in this section are only correct at the time of publication up until the end of the Call year.

These references have been provided by EURAMET.

- [1]. EMN Traceability in Laboratory Medicine (TraceLabMed) Orientation paper 2025 Health
<https://metpart.eu/component/edocman/call-2025-orientation-emn-tlm-health/download.html?Itemid=0>
- [2]. EMN Mathmet Orientation paper 2025 Health
<https://metpart.eu/component/edocman/call-2025-orientation-emn-mathmet-all/download.html?Itemid=0>