Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

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Model protocol for national surveillance of AMR in human *Salmonella* and *Campylobacter* infections

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1. BACKGROUND

Since 2005, all European Union (EU) Member States (MS) are obliged to collect relevant and comparable data on *Salmonella* and *Campylobacter* infections in humans, food-related outbreaks, and the occurrence of resistance to antimicrobials (AMR) relevant for the treatment of human infections with these bacteria [1].

According to the EU case definitions, the laboratory criteria for confirmed human cases of *Salmonella* and *Campylobacter* enteritis relies on either isolation of *Salmonella* and *Campylobacter* or detection of their nucleic acid in a clinical specimen. Furthermore, *Salmonella and Campylobacter* antimicrobial susceptibility testing should be carried out on a representative subset of isolates [2] following the methods and criteria established by ECDC as specified in the EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates [3]. The results of antimicrobial susceptibility tests must be reported to European Centre for Disease Prevention and Control (ECDC). In 2020, the majority of countries provided AMR surveillance data for *Salmonella* (20 countries) and *Campylobacter* (18 countries) to ECDC [4] whereas approx. a third of the countries did not perform surveillance of AMR. The data reported to ECDC differed in respect to number of isolates tested for AMR and not all countries reported results for all the antimicrobials in the harmonised panel [4]. Currently, no guideline exists for the selection of a representative subset of isolates for national or EU level surveillance of AMR.

To support the implementation of the EU protocol for harmonised monitoring of AMR in human *Salmonella* and *Campylobacter* isolates [3], each country should develop and implement a protocol for the national surveillance of AMR in these organisms. To guide the NRL in each country to create an appropriate protocol for the national surveillance, the FWD AMR-RefLabCap project has developed a model protocol, which includes models for setting up representative national sampling schemes as indicated in the EU case definitions for human *Salmonella* and *Campylobacter* infections [2].

2. INTRODUCTION AND SCOPE

This model protocol provides suggestions for how the laboratories that function as public health national reference laboratories (NRLs) on antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* can set up the national surveillance of AMR in their countries in line with the EU case definitions. The EU case definitions define that antimicrobial susceptibility testing (AST) should be carried out on a representative subset of isolates of *Salmonella* and *Campylobacter* from human infections. Furthermore, the EU protocol for harmonised monitoring (including the suggested update of this protocol, encompassing the use of WGS) provides guidance on the AMR testing methodology. Thus, this model protocol covers the procedures beginning from when isolates are obtained at primary diagnostic laboratories to the actual testing performed according to the EU protocol, including suggestions for setting up representative national sampling schemes.

3. SURVEILLANCE OBJECTIVES

The national surveillance objectives for antimicrobial resistance in *Salmonella* and *Campylobacter* should preferable be in line with the EU surveillance objectives [3]:

a) To monitor, in human clinical isolates, trends in the occurrence of resistance to antimicrobial agents relevant for treatment of human *Salmonella* and *Campylobacter* infections, including comparison with food/animal isolates
b) To monitor, in human clinical isolates, trends in the occurrence of resistance to other antimicrobial agents of public and animal health importance, including comparison with food/animal isolates
c) To monitor, in human clinical isolates, the prevalence of ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC) and carbapenemase phenotypes
d) To use antimicrobial resistance patterns to characterise human clinical isolates, i.e. as an epidemiological marker, to support identification of outbreaks and related cases
e) To identify and monitor, in human clinical isolates, genetic determinants of resistance that are important for public health e.g. to aid recognition of epidemic cross-border spread of multi-drug resistant *Salmonella* strains
f) To monitor, in human clinical isolates, trends in the occurrence of resistance to antimicrobial agents that may be needed for future therapeutic use.

Specific national surveillance objectives other than above should be considered.

4. NATIONAL NETWORK OF LABORATORIES

Local/regional clinical microbiology laboratories are an essential part of the national public health system. The main obligation of these laboratories is to perform primary diagnostic testing, characterisation and antimicrobial susceptibility testing with a focus on patient management and preventive services.

If not already in place, the NRL on AMR in *Salmonella* and *Campylobacter* should establish and coordinate a national (sentinel) network of clinical laboratories that can support the national surveillance of AMR in these pathogens. Optimally, such networks should consist of local/regional laboratories representing the whole country. However, if not possible to establish such a comprehensive network, the involvement of all or a selection of hospital/regional laboratories would be sufficient. The local/regional laboratories should collaborate with the NRL and other relevant public health bodies and contribute with isolates and/or AMR data on *Salmonella* and *Campylobacter* for surveillance purposes.

5. ISOLATES FOR AMR TESTING

The most comprehensive AMR surveillance for *Salmonella* and *Campylobacter* from human infections would include culturing all *Salmonella* and *Campylobacter* positive samples and carrying out further characterisation, including AMR testing, of all isolates. This is an expensive and time-consuming approach and rarely possible for the national surveillance of AMR in *Salmonella* and *Campylobacter*. In that case, the national surveillance should be based on a carefully selected subset of isolates that is as representative as possible.

5.1. Surveillance of infections

To support the selection of a representative subset of isolates for the AMR surveillance, data on the cases in the country should be available as background information for the NRLs for *Salmonella* and *Campylobacter*. This will often require close collaboration between the national authority of public health and the NRLs in order to collect the relevant data from clinical/public health laboratories and for linking case data with laboratory data at an individual case/isolate level. Data on sample type, isolation date, geography, age group, gender, hospitalisation, travel history, etc. should preferably be available. Likewise, basic microbiological data should be available, e.g. species of *Campylobacter* isolates and serotype of *Salmonella* isolates.

5.2. Selecting a representative subset

Optimally, a substantial and representative proportion of the laboratory-confirmed human *Salmonella* and *Campylobacter* cases in the country should be included in the national surveillance of AMR.

5.2.1. Number of isolates for AMR-testing

If the NRLs do not have the capacity and capability to test isolates from all or a nearly all cases, it is recommended that *Salmonella* and *Campylobacter* isolates representing a smaller proportion (e.g., 5-10%) of the nationally reported cases should be characterised and AMR-tested. The proportion of cases to be included in the surveillance should be dependent on the total number of *Salmonella* and *Campylobacter* cases in the country (the fewer the cases, the higher proportion should be included). Even a small representive subset of isolates (e.g., 100-200 isolates), will give valid information on the overall situation in the country including the overall trends if routine surveillance is conducted.

5.2.2. Selection criteria

The subset should as far as possible be selected to representatively cover the characteristics of the cases and the isolates. The following suggested criteria may be used for setting up surveillance and/or for evaluating the representativeness of the subset included in the current surveillance. It may be an active decision to set up a surveillance that is not fully representative based on all criteria, e.g. because a bias towards severe infections is wanted or because some criteria, which are difficult to fulfill, are assessed to have minor influence on the overall surveillance results.

Geography. Consider how well the whole country is represented (all regions or a relevant selection of regions) and if known geographical differences are represented, e.g. rural and urban areas

Time periods/season. Consider how well the whole year is covered and/or the known seasonal differences

Case data. Consider the representativeness of the population served by the laboratories providing isolates and/or data for the surveillance

Infections. Consider the representativeness of hospitalisation, sample type (e.g. invasive infections), and other indicators of severity of infection

Isolate characteristics/outbreaks. Consider the representiveness of the isolates included in the surveillance, e.g. the relative distribution *of Salmonella* serotypes included in the AMR surveillance compared to the distribution of serotypes among the reported cases in the country; and likewise the relative distribution of *Campylobacter* species compared to the species distribution among all reported cases. In addition, it would be relevant to ensure that isolates representing known outbreaks are included, but not overrepresented, in the surveillance.

It may not be possible to set up a fully representative surveillance in a short time, but it is useful to consider each point and assess how the chosen/available subset of isolates influences the value of the AMR surveillance. Based on this assessment, further development over time should be considered to continuously improve the surveillance. In any case, the representativeness and the size of the subset used for national surveillance of AMR should be evaluated at regular intervals to assess if the chosen setup is still the most suitable under the given circumstances. Such a retrospective assessment of the representativeness of isolates may be done on an annual basis.

5.3. Referral of isolates and/or data collection

The NRLs and local/regional laboratories involved in the surveillance of AMR should agree on the isolate selection and frequency of referral to the NRL. For example, clinical laboratories may submit a specified number or proportion of isolates per week, per month, or per quarter. In the optimal situation, additional isolates may be submitted and further characterised, e.g. in outbreak situations, if certain *Salmonella* serovars and *Campylobacter* species with certain AMR characteristics emerge, or if a certain strain causes severe disease outcome. Such a surveillance system will ensure the detection of rare and/or emerging resistant *Salmonella* and *Campylobacter* clones.
If local/regional laboratories have the capacity for further characterisation, including AMR-testing at the required quality level for surveillance, the AMR data should be collected and included in the national surveillance. In that case, the NRL should ensure that the applied methodology is according to the EU protocol for harmonised monitoring of AMR in *Salmonella* and *Campylobacter* and is performed at a sufficient quality level for inclusion in the national surveillance, e.g. by ensuring that the laboratories have internal quality assurance procedures in place and preferable also participate in relevant EQA schemes.

If culture-independent diagnostic tests are used in the laboratories performing the primary diagnostics, it is important to ensure culturing (a subset) of positive samples for further characterisation. If this cannot be done by the diagnostic laboratories, it might be necessary to submit samples to the NRL or other laboratories that can do the culturing to obtain isolates for AMR testing.

6. AMR TESTING AND REPORTING

The phenotypic and/or genotypic testing of AMR in isolates of *Salmonella* and *Campylobacter* should be done in accordance with the guidelines in the EU protocol for harmonised monitoring of AMR [3]. Likewise, the reporting of data to ECDC for the EU surveillance is described in this protocol.

If not already in place, a national system for capturing and analysing the AMR data for national surveillance purposes should be implemented.

To guide the NRLs, the following recommendations have been developed in the FWD AMR-RefLabCap project regarding the minimum and optimal requirements of the characterisation of *Salmonella* and *Campylobacter* isolates at the reference level for the national surveillance of AMR in these pathogens.

6.1. Reference diagnostics and characterisation of *Salmonella*

6.1.1. Minimum requirements

The NRL should have the capacity and capability to isolate S*almonella* from clinical samples for reference purposes and/or to support routine diagnostics, and be able to determine serotypes that are common within the country and at the EU level [5] with appropriate phenotypic and/or genotypic methods. The NRL should also have appropriate methods in place to perform phenotypic or genotypic antimicrobial susceptibility testing according to the EU protocol for harmonised monitoring of AMR [3].

6.1.2. Optimal requirements

Optimally, the NRL should have the capacity and capability to isolate *Salmonella* from clinical samples and to perform either phenotypic or genotypic identification of any *Salmonella* serovar. The NRL should also have implemented genotypic AMR and cluster detection based on WGS [6] while maintaining the capacity for phenotypic antimicrobial susceptibility testing (AST) (Table 1). If only WGS-based AMR prediction is performed, it is recommended that I) bioinformatics pipelines and databases used to detect resistance genes/point mutations are validated against phenotypic AST test, and that II) a defined proportion of isolates or selected isolates are periodically tested phenotypically to ensure detection of novel resistance mechanisms and the inclusion of these in the applied database.

Table 1. Recommended minimum and optimal requirements for reference diagnostics and characterisation of Salmonella

Requirements	Serotyping	Antimicrobial resistance	Cluster detection
Minimum	Phenotypic or genotypic: common serovars	Phenotypic AST or genotypic AMR prediction	Not applicable*
Optimal	Phenotypic or genotypic: all serovars	Phenotypic AST and WGS-based AMR prediction**	WGS-based (e.g. cgMLST, wgMLST, SNP***)

* if the NRL has not yet implemented any method for cluster detection, we recommend implementation of WGS-based cluster detection

** a defined proportion of isolates or selected isolates are periodically tested phenotypically to ensure detection of novel resistance mechanisms

*** cgMLST – core genome Multilocus Sequence Typing, wgMLST whole genome Multilocus Sequence Typing, SNP – Single Nucleotide Polymorphism

6.2. Reference diagnostics and characterisation of *Campylobacter*

6.2.1. Minimum requirements

The NRL should have the capacity and capability to isolate *Campylobacter* from clinical samples for reference purposes and/or to support routine diagnostics as well as appropriate methods for either phenotypic or genotypic identification of the common species of *Campylobacter* (Table 2). The NRL should also have appropriate methods to

perform phenotypic or genotypic antimicrobial susceptibility testing according to the EU protocol for harmonised monitoring of AMR [3].

6.2.2. Optimal requirements

The NRL should have implemented appropriate methods for isolation of *Campylobacter* from clinical samples and for phenotypic or genotypic detection/characterisation of *Campylobacter* at the species level. In addition, the implementation of WGS-based genotypic AMR prediction and cluster detection by WGS-based techniques is recommended [6] while maintaining the capacity for phenotypic AST (Table 2). If only WGS-based AMR prediction is performed, it is recommended that I) bioinformatics pipelines and databases used to detect resistance genes/point mutations are validated against phenotypic AST test, and that II) a defined proportion of isolates or selected isolates are periodically tested phenotypically to ensure detection of novel resistance mechanisms.

Table 2. Recommended minimum and optimal requirements for reference diagnostics and characterisation of Campylobacter

Requirements	Species	Antimicrobial resistance	Cluster detection
Minimum	Phenotypic or genotypic: *C. jejuni, C. coli*	Phenotypic AST or genotypic AMR prediction	Not applicable*
Optimal	Phenotypic or genotypic: all species	Phenotypic AST and WGS-based AMR prediction**	WGS-based (e.g., cgMLST, wgMLST, SNP***)

* if the NRL has not yet implemented any method for cluster detection, we recommend implementation of WGS-based cluster detection

** a defined proportion of isolates or selected isolates are periodically tested phenotypically to ensure detection of novel resistance mechanisms

*** cgMLST – core genome Multilocus Sequence Typing, wgMLST whole genome Multilocus Sequence Typing, SNP – Single Nucleotide Polymorphism

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