PRESENTATION OF THE UPDATED EU PROTOCOL FOR HARMONISED MONITORING OF AMR AND THE DRAFT MODEL PROTOCOL FOR NATIONAL SURVEILLANCE

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ON-LINE WORKSHOP

3 NOVEMBER 2022

FWD AMR DOCUMENTS IN RELATION TO AMR SURVEILLANCE

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- **EU Protocol for harmonised monitoring of AMR in Salmonella and Campylobacter**
  - **Suggested updates to include genomic determinants**
- **Protocol for WGS based analysis of AMR in Salmonella and Campylobacter (FWD AMR-RefLabCap project)**
- **Model protocol for national surveillance of AMR in Salmonella and Campylobacter (FWD AMR-RefLabCap project)**

WGS PROTOCOL

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www.fwdamr-reflabcap.eu/resources/protocols-and-guidelines

Proposed protocol for whole genome sequencing-based analysis for detection and tracing of epidemic clones of antimicrobial resistant *Salmonella* and *Campylobacter*

- to be used for national surveillance and integrated outbreak investigations by NRLs for public health

8 July 2022

FWD AMR· RefLabCap

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EU PROTOCOL + SUGGESTED UPDATES

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TECHNICAL DOCUMENT

EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates

June 2016

ECDC NORMAL

European Commission

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

SC 2019 74 09

Deliverable T2.4

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

Version n°: 3

19 October 2022

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DTU

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HTTPS://WWW.ECDC.EUROPA.EU/EN/PUBLICATIONS-DATA/EU-PROTOCOL-HARMONISED-MONITORING-ANTIMICROBIAL-RESISTANCE-HUMAN-SALMONELLA-AND-0

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European Centre for Disease Prevention and Control
An agency of the European Union

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> EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates – June 2016

< Analysis and guidance

EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates – June 2016

Guidance
7 Jun 2016
Cite:

Translate this page

This protocol for harmonised monitoring of antimicrobial resistance in Salmonella and Campylobacter from human isolates was updated from the March 2014 version. While the revised version introduces a number of new antimicrobials and resistance breakpoints, its overall objectives – to increase the quality and comparability of EU/EEA antimicrobial resistance data – remain unchanged.

The Protocol is targeted at the national public health reference laboratories to guide the susceptibility testing needed for EU surveillance and the reporting to ECDC.

Note that annex 1 and 2 were updated in August 2021 and are available below

Download

- EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates, June 2016 - EN - [PDF-928.92 KB]
- EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates - Annexes August 2021 - EN - [PDF-100.96 KB]
- EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates, March 2014 - EN - [PDF-1.2 MB]

ECDC HARMONIZED PROTOCOL - CONTENTS

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Contents

Tables and figures

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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Protocol – A ECDC document

Plans for revisions ?

FWD-AMR revision approach:

- **All sections were reviewed – and suggestions for changes were made for each section**
- **Minor overall editorial changes suggested**
- **Suggested new/updated references when appropriate**
- **Suggested to include guidance on how to fulfil the surveillance objectives**
- **On-boarded DNA based methods, (PCR and sequenced based)**

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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2 EU surveillance objectives

The proposed surveillance objectives for antimicrobial resistance in zoonotic bacteria, specifically *Salmonella* spp. and *Campylobacter* spp. are:

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.

Suggestion: Provide input on how to fulfill the surveillance objectives

"To fulfil these surveillance objectives at the national level, each country should consider how to achieve appropriate national data. The number of *Salmonella* and *Campylobacter* isolates from humans that are tested for surveillance purposes as well as the representativeness of these isolates should be considered. **The model protocol for national surveillance of AMR in *Salmonella* and *Campylobacter*, which will be developed by the FWD AMR-RefLabCap project, should be used for setting up such representative sampling and testing."**

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3 Panel of antimicrobials to be tested

- The list of antimicrobials and their prioritization should be reviewed

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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Annex 1. EUCAST clinical breakpoints and epidemiological cut-off values for the priority list of antimicrobials to be tested for *Salmonella enterica* as of 31 August 2021

Antimicrobial	Criteria based on MIC dilution (mg/L)			Recommended concentration range[1] (mg/L) (number of wells)	Criteria based on disk diffusion (mm)			Disk load (µg)
	S≤	R>	NWT>		S≥	R<	NWT<	
First priority								
Ampicillin (AMP)	8.0	8.0	4.0	1-32 (6)	14	14	18	10
Azithromycin (AZM)	ND	ND	16	2-64 (6)	ND	ND	12	15
Cefotaxime (CTX)	1.0	2.0 (1.0)[2]	0.5	0.25-4 (5), 0.25-64 (9)[3]	20	17 (21)[2]	20	5
Ceftazidime (CAZ)	1.0[2]	4.0 (1.0)[2]	2.0	0.25-8 (6), 0.25-128 (10)[3]	22[3]	19	20	10
Chlorampenicol (CHL)	8.0	8.0	16.0	8-64 (4)	17	17	19	30
Ciprofloxacin (CIP)	0.06	0.06	0.064	0.015-8 (10)	NA	NA	NA	NA
Colistin (COL)	2.0	2.0	NA	1-16 (5)	NA	NA	NA	NA
Gentamicin (GEN)	2.0	2.0	2.0	0.5-16 (6)	17	17	17	10
Meropenem (MEM)	2.0	8.0	0.06 (0.125)[2]	0.03-16 (10)	22	16	27 (28)[2]	10
Pefloxacin	NA	NA	NA	NA	24	24	24	5
Sulfamethoxazole (SMX)	ND	ND	ND	8-512 (7)	ND	ND	ND	100
Tetracycline (TCY)	ND	ND	8.0	2-32 (5)	ND	ND	17	30
Tigecycline (TGC)	ND	ND	ND	0.25-8 (6)	ND	ND	16	15
Trimethoprim (TMP)	4.0	4.0	2.0	0.25-16 (7)	15	15	23	5
Second level testing ESBL-producers								
Cefepime (FEP)	1.0	4.0	ND		27	24	ND	30
Cefoxitin (FOX)	ND	ND	8.0[2]	0.5-64 (8)	19	19[2]	21	30
Optional								
Amoxicillin (AMX)	8.0	8.0	4.0		ND	ND	ND	10
Ceftriaxone (CRO)	1.0	2.0 (1.0)[2]	0.25		25	22 (23)[2]	ND	30
Ertapenem (ETP)	0.5	0.5	ND (0.125)[2]	0.015-2 (8)	25	25[3]	ND	10
Nalidixic acid (NAL)	ND	ND	8.0	4-64 (5)	ND	ND	16	30
Trimethoprim-sulfamethoxazole (SXT)	2.0	4.0	ND		14	11	22	1.25-23.75

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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Annex 2. EUCAST clinical breakpoints and epidemiological cut-off values for the priority list of antimicrobials to be tested for *Campylobacter jejuni* and *C. coli* as of 31 August 2021

Antimicrobial	Criteria based on MIC dilution (mg/L)			Recommended concentration range[1] (mg/L) (number of wells)	Criteria based on disk diffusion (mm)			Disk load (µg)
	S≤	R>	NWT >		S≥	R<	NWT<	
First priority								
Ciprofloxacin (CIP)	0.001	0.5	0.5	0.12-32 (9)	50	26	26	5
Erythromycin (ERY) *C. jejuni*	4.0	4.0	4.0	1-512 (10)	20	20	22	15
Erythromycin (ERY) *C. coli*	8.0	8.0	8.0	1-512 (10)	24	24	24	15
Gentamicin (GEN)	ND	ND	1.0	0.25-16 (7)	ND	ND	20	10
Tetracycline (TCY) *C. jejuni*	2.0	2.0	1.0	0.5-64 (8)	30	30	30	30
Tetracycline (TCY) *C. coli*	2.0	2.0	2.0	0.5-64 (8)	30	30	30	30
Optional								
Amoxicillin + clavulanic acid (AMC)	ND	ND	ND		ND	ND	ND	30
Azithromycin (AZM) *C. jejuni*	ND	ND	0.25		ND	ND	ND	
Azithromycin (AZM) *C. coli*	ND	ND	0.5		ND	ND	ND	
Ertapenem (ETP)	ND	ND	ND	0.125-4 (6)[1]	ND	ND	ND	
Imipenem (IMP)	ND	ND	ND		ND	ND	ND	
Meropenem (MEM)	ND	ND	ND		ND	ND	ND	10

3 Panel of antimicrobials to be tested

- The list of antimicrobials could mirror the antimicrobials that are used on the food and animal side according to Commission Implementing Decision (EU) 2020/1729.
  - Amikacin for *Salmonella*
  - Chloramphenicol (and ertapenem) for *Campylobacter*

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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6 Genotyping for further identification of resistance mechanisms

- New title and new contents that covers the use of DNA based methods - "**Whole genome sequencing and other DNA based molecular methods for identification of resistance markers and characterisation of resistant clones**"
- The WGS protocol from the FWD AMR-RefLabCap project (www.fwdamr-reflabcap.eu/resources/protocols-and-guidelines) should be referenced

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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7 Interpretive criteria

- The epidemiological and clinical breakpoints should be updated.
- The new EUCAST definitions of clinically susceptible, intermediate or resistant (SIR) should be included, see https://www.eucast.org/newsiandr/

8 Reporting format

- The text should be updated so that it presents the current ECDC guidelines for uploading of results.
- A new section describing the reporting of genetic data (WGS/genes/point mutations) to the ECDC surveillance system should be added.

PROPOSALS FOR REVISION OF ECDC PROTOCOL

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FURTHER SUGGESTIONS

The current edition is complex and covers a range of different areas, from overall surveillance objectives to detailed technical guidance on how to perform and report results

Simplify the process of updating the EU protocol, by increasing the use of annexes, e.g. to cover the technical laboratory guidance that needs frequent updates

MODEL PROTOCOL FOR AMR SURVEILLANCE

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

SC 2019 74 09

Deliverable T2.4

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MODEL PROTOCOL FOR AMR SURVEILLANCE

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- Proposed model protocol covers the procedures beginning from when isolates are obtained at the primary diagnostic laboratories to the actual testing performed according to the EU protocol
- The proposed model protocol does not tell how to do surveillance but gives input on aspects for consideration
- The proposed model is currently in consultation in the network
- Deadline for feedback – November 7th 2022

MODEL PROTOCOL FOR AMR SURVEILLANCE

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Background – legal basis

- Countries are obliged to collect relevant and comparable data on Salmonella and Campylobacter infections in humans, food-related outbreaks, and the occurrence of resistance

- Antimicrobial susceptibility testing (AST) should be carried out on a representative subset of isolates of Salmonella and Campylobacter from human infections

NATIONAL NETWORK OF LABORATORIES…

- Local/regional laboratories perform primary diagnostic testing, characterisation and antimicrobial susceptibility testing with a focus on patient management and preventive services
- The NRL on AMR in *Salmonella* and *Campylobacter* should coordinate a national (sentinel) network of laboratories that can support the national surveillance of AMR
- Optimally, such networks should consist of local/regional laboratories representing the whole country.
- If not possible selected representative laboratories should be involved

ISOLATES FOR AMR TESTING…

The most comprehensive AMR surveillance of isolates from human infections would include culturing all *Salmonella* and *Campylobacter* positive samples and carrying out further characterisation, including AMR testing

But in most cases a national surveillance can be based on a carefully selected subsets of isolates that are as representative as possible

When selecting isolates consider

- Geography
- Time periods/season
- Case data (representativeness of the population served by the laboratories)
- Type of infections - hospitalization, sample type e.g. invasive infections, and other indicators of severity of infection

Number of isolates for AMR-testing

If the NRL cannot test isolates from all or a nearly all cases, it is recommended that isolates representing a smaller proportion (e.g., 5-10%) of the nationally reported cases should be characterized and AMR-tested

The proportion of cases should be dependent on the total number of cases in the country (the fewer the cases, the higher proportion should be included).

Even a small representative subset of isolates (e.g., 100-200 isolates), will give valid information on the overall situation including the overall trends if routine surveillance is conducted

Referral of isolates and/or data collection

The NRLs and local/regional laboratories should agree on the isolate selection and frequency of referral to the NRL

If local/regional laboratories have the capacity for further characterisation, including AMR-testing at the required quality level for surveillance, the AMR data can be collected and included in the national surveillance

If culture-independent diagnostic tests are used in the primary diagnostics, it is important to ensure culturing (a subset) of positive samples for further characterisation.

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

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

AMR data should be reported to the ECDC

Characterisation of isolates

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.

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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

MODEL PROTOCOL FOR AMR SURVEILLANCE

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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

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Questions

Coffee break