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TECHNICAL DOCUMENT

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

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This report of the European Centre for Disease Prevention and Control (ECDC) was coordinated by Therese Westrell (ECDC Food- and Waterborne Diseases and Zoonoses Programme).

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Contents

Abbreviations

AMC	Amoxicillin +clavulanic acid
AMP	Ampicillin
AMR	Antimicrobial resistance
AMX	Amoxicillin
AST	Antimicrobial susceptibility testing
AZM	Azithromycin
CAZ	Ceftazidime
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
COL	Colistin
CRO	Ceftriaxone
CTX	Cefotaxime
EC	European Commission
ECOFF	Epidemiological Cut-OFF value
EFSA	The European Food Safety Authority
ESBL	Extended-Spectrum Beta-Lactamase
ETP	Ertapenem
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
EURL	European Union Reference Laboratory
FLR	Florfenicol
FWD-Net	Food- and Waterborne Diseases and Zoonoses Network
GEN	Gentamicin
IPM	Imipenem
IZD	Inhibition Zone Diameter
NAL	Nalidixic acid
NPHRL	National Public Health Reference Laboratory
MEM	Meropenem
MIC	Minimum Inhibitory Concentration
pAmpC	Plasmid-encoded Ambler class C β-lactamases
SIR	Susceptible, intermediate, resistant
SMX	Sulfamethoxazole
SXT	Trimethoprim + sulfamethoxazole (co-trimoxazole)
TESSy	The European Surveillance System
TCY	Tetracycline
TGC	Tigecycline
TMP	Trimethoprim

Executive summary

This protocol for harmonised monitoring of antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* from human isolates aims to increase the quality and comparability of AMR data collected at the EU level from different Member States. As such, it is primarily targeted to the National Public Health Reference Laboratories to guide the susceptibility testing needed for EU surveillance and the reporting to ECDC. It also provides guidance on how to improve the comparison of results with the AMR monitoring performed in isolates from animals and food products. The protocol was developed by ECDC in close co-operation with representatives of the Food- and Waterborne Diseases and Zoonoses (FWD) network and supports the implementation of the Commission Action Plan on antimicrobial resistance.

Surveillance objectives for monitoring of antimicrobial resistance in human clinical isolates of *Salmonella* and *Campylobacter* at the EU level were agreed within the FWD network. Based on these, a priority list was set of antimicrobial agents to monitor for surveillance purposes. The list comprises ten antimicrobial substances for *Salmonella* and three for *Campylobacter*. For laboratory measurement of antimicrobial activity the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method is recommended. Additional detail is given regarding methods for detection and confirmation of two specific resistance phenotypes of particular concern – extended-spectrum beta-lactamase (ESBL) producers and carbapenemase producers.

Member States are encouraged to submit results of susceptibility testing as 'quantitative' values (minimum inhibitory concentration in mg/L or zone diameter in mm) to facilitate comparison of data over time, and to allow comparison with quantitative AMR data from animal and food isolates that takes account of epidemiological cut-off values for the relevant bacterial species. It is also possible to continue reporting of the interpretation of the susceptibility testing i.e. susceptible, intermediate or resistant, either separately with the case-based data or together with the isolate-based quantitative values. The reporting of interpreted values through the case-based data will however be phased out in the coming years.

1. Background

The European Centre for Disease Prevention and Control (ECDC) has a mandate to gather and analyse data and information on emerging public health threats and developments for the purpose of protecting public health in the European Community [1]. The collection of data related to antimicrobial resistance (AMR) is included as part of the European Surveillance System (TESSy) through several networks:

- **EARS-Net** collects data on AMR in eight bacterial pathogens from invasive human infections (*Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter* spp.);
- **HAI-Net** collects data on AMR in selected pathogens associated with healthcare-associated infections;
- **ESAC-Net** collects data on the consumption of antimicrobial agents in humans;
- **FWD-Net** collects data on AMR in *Salmonella* spp., *Campylobacter* spp. and Shiga toxin/verocytotoxin-producing *Escherichia coli* (STEC/VTEC).

Directive 2003/99/EC requires Member States to monitor and report comparable data on AMR in zoonoses and zoonotic agents in food-producing animals and food [2]. This directive is supplemented by the monitoring of AMR in human isolates conducted in accordance with Decision 1082/2013/EU [3], and Commission Implementing Decision 2012/506/EU [4]. To promote data comparison, monitoring should take place on a harmonised basis so that evaluation of trends and sources of AMR in zoonotic agents within the European Union would be possible.

In this regard, ECDC has been collecting interpreted results from antimicrobial susceptibility testing (AST) as part of the case-based data collection for *Salmonella* and *Campylobacter* (but also STEC/VTEC). In the course of detailed analysis of the data and comparison with those collected from animal and food isolates in the first joint EFSA-ECDC 'European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food' published in 2011, several problematic issues were identified [5]. The methods of measuring antimicrobial activity, and origin of the data submitted, varied markedly between countries. In several countries, the national public health reference laboratories (NPHRLs) measured antimicrobial activity on only a fraction of the isolates and the remaining were tested by hospital or local laboratories in which the methods used were not reported to the NPHRL. The guidelines used for the interpretation of the measurements also varied between countries and also within countries for different antimicrobials, with both international and national guidelines used. Direct comparisons between AMR data from humans and animal and food isolates were hampered because of the use of different test methods and different interpretive criteria. Antimicrobial susceptibility testing performed on human isolates in a clinical setting would for example be interpreted with clinical breakpoints for assessing treatment options.

In contrast, animal isolates originate from monitoring programmes on healthy animals and subsequently, both animal and food isolates are generally interpreted based on epidemiological cut-off (ECOFF) values. Due to the differences described above there was a need for harmonisation of AMR monitoring.

In 2011, the European Commission (EC) launched its Commission Action Plan on antimicrobial resistance [6]. The objectives of the Action Plan are to combat the rising threat of AMR, to reduce and prevent the spread of AMR and to preserve the ability to treat microbial infections. Twelve action points were proposed and two of them, action point nine and ten, deal with strenghtening of surveillance systems on AMR and antimicrobial consumption in human and animal medicine, respectively. In particular, action point ten highlights the need to 'review the monitoring of AMR in zoonotic bacteria and/or indicators' and 'with the support of the relevant EU agencies, establish harmonisation between human and veterinary surveillance to allow comparison of data'.

As the Decision 2007/407/EC on harmonised monitoring of antimicrobial resistance in *Salmonella* in poultry and pigs [7] expired at the end of 2012, the EC requested the European Food Safety Authority (EFSA) to prepare new specifications for AMR monitoring, which would be used to revise the legislation. In 2012, EFSA published its 'Technical specifications for harmonised monitoring and reporting of antimicrobial resistance in *Salmonella* spp., *Campylobacter* spp. and indicator *Escherichia coli* and *Enterococcus* spp. transmitted through food' [8]. The new specifications included a revised list of antimicrobials to monitor, updated ECOFF values, minimum inhibitory concentration (MIC) ranges to be tested, and specific monitoring of extended-spectrum-beta-lactamase-producing bacteria. Based on these specifications, the EC prepared a Commission Implementing Decision on harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [9] which entered into force 1 January 2014.

ECDC initiated activities on harmonisation of AMR surveillance for zoonotic bacteria in human infections in 2012. The first activity was a session during the joint meeting of the Food- and Waterborne Diseases and Zoonoses (FWD) network's *Salmonella* and *Campylobacter* experts and the national AMR focal points in Copenhagen in March 2012, when the FWD experts had initial discussions on the antimicrobial panels and antimicrobial resistance monitoring needs for *Salmonella* and *Campylobacter* respectively. This meeting was followed by an expert workshop at ECDC in May 2012 on the objectives and needs for an EU protocol for harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* infections in humans with representatives from EFSA, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), FWD-Net, EARS-Net, the European Union Reference Laboratory (EURL) for *Campylobacter* and the EURL for antimicrobial resistance, as well as external scientific experts. The first draft of the EU protocol was reviewed and discussed in a second expert workshop with AMR experts from the FWD-Net, and a representative from EUCAST at the Danish Technical University, Denmark, in April 2013. This was followed by discussions with the EURL for antimicrobial resistance network and thereafter presented at the joint EFSA-ECDC networks meeting later in April 2013. The FWD network was consulted on the final draft of the EU protocol in October 2013 and amendments were made in February 2014 before publication on the ECDC website.

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.

3. Panel of antimicrobials to be tested

In order to obtain comparable AMR surveillance data, NPHRLs are encouraged to include a specific set of antimicrobials for their routine susceptibility testing of *Salmonella* spp. and *Campylobacter* spp. isolates. The set of antimicrobials below was selected in order to reflect the importance both for human and veterinary medicine, and relevance for AMR monitoring, as specified in the surveillance objectives in section 2. They should also allow for comparable analysis between animal, food and human data (see section 9).

Tables 1 and 2 present the antimicrobials to be included for reporting to the EU level for *Salmonella* spp and *Campylobacter* spp, respectively. The surveillance objectives that are relevant for each antimicrobial are highlighted. Both tables also list optional antimicrobials that can either replace some of the first priority antimicrobials or are options for future monitoring, when enough data are collected for EUCAST interpretive criteria to be set.

For confirmation of suspect ESBL-producing and suspect carbapenemase-producing *Salmonella*, second level testing is recommended (see section 9). If there is enough space (on 96-well plate or petri dish), additional antimicrobials used for confirmation and classification of ESBL-producing *Salmonella* can be included in the first level testing.

Table 1. List of antimicrobials to be tested for human *Salmonella* spp. isolates

Class	Name (abbreviation*)	Surveillance objectives	Comments
First priority			
Aminoglycosides	Gentamicin (GEN)	b, d	
Aminopenicillins	Ampicillin (AMP)	a, b, d	
Amphenicols	Chloramphenicol (CHL)	a, d	
Carbapenems	Meropenem (MEM)	a, b, c, d, e	EUCAST recommend meropenem as it offers the best compromise between sensitivity and specificity in terms of detecting carbapenemase-producers
Cephalosporins	Cefotaxime (CTX)	a, b, c, d, e	May be insensitive for detection of ceftazidimase-type ESBLs
	Ceftazidime (CAZ)	a, b, c, d, e	Added to increase sensitivity of screening for full range of ESBL with diverse substrate specificities
Dihydrofolate reductase inhibitors	Trimethoprim (TMP)	d	Value as an epidemiological marker.
Quinolones	Ciprofloxacin (CIP)	a, b, c, d, e	Preferably test with broad MIC range, see section 0. Nalidixic acid (NAL) is recommended in addition to testing ciprofloxacin for laboratories using disk diffusion as the ciprofloxacin disk diffusion is not sensitive to detection of low levels of resistance.
Sulphonamides	Sulfamethoxazole (SMX)	d	Value as an epidemiological marker. Produce reliable data in EQAs.
Tetracyclines	Tetracycline (TCY)	b, d	Used both in veterinary and human medicine.
Optional			
Aminopenicillins	Amoxicillin (AMX)		Alternative for testing and reporting if AMP not tested.
Carbapenems	Ertapenem (ETP)		Many human laboratories test for ertapenem so should be possible to report.
Cephalosporins	Ceftriaxone (CRO)	a, b, c, d, e	Alternative for cefotaxime with disk diffusion method as has similar spectrum of activity.
Combination drugs	Trimethoprim + sulfamethoxazole (co-trimoxazole) (SXT)		No need to test if the substances are tested separately.
Macrolides	Azithromycin (AZM)	f	Include when ECOFF values are available. Encourage Member States to send their data to EUCAST for determination of ECOFF. May be considered as a last resort drug for invasive salmonellosis.
Polymyxins	Colistin (COL)	b	Last resort drug in human medicine and used in animal medicine. Its chemical properties however cause unreliable results with dilution and render it impossible to test with disk diffusion. NB. Any laboratory that wants to report an isolate as resistant to colistin must get the result confirmed at a reference laboratory that is up to date with the latest method developments for testing of colistin.
Quinolones	Nalidixic acid (NAL)		Mainly for quality assurance purposes, but recommended in addition to ciprofloxacin for laboratories using disk diffusion as the ciprofloxacin disk diffusion is not sensitive to detection of low levels of resistance.
Tetracyclines	Tigecycline (TGC)	f	Include when ECOFF values are available. Encourage Member States to send their data to EUCAST for the determination of ECOFF.

** Abbreviations/antibiotic codes as used in EARS-Net and based on WHONET 5.3*

Table 2. List of antimicrobials to be tested for human *Campylobacter* spp. isolates

Class	Name (abbreviation*)	Surveillance objectives	Comments
First priority			
Macrolides	Erythromycin (ERY)	a, b	
Quinolones	Ciprofloxacin (CIP)	a, b	
Tetracyclines	Tetracycline (TCY)	a, b	
Optional			
Aminoglycosides	Gentamicin (GEN)	a, b	Include for invasive disease monitoring when MIC values are available. Encourage Member States to send their data (MIC) to EUCAST for determination of MIC ECOFFs.
Carbapenems	Meropenem (MEM) Ertapenem (ETP) Imipenem (IPM)	a, c	Include for invasive disease monitoring when MIC values are available. Encourage MSs to send their data (MIC) to EUCAST for the determination of ECOFFs. CLSI criteria exists. Both testing method and related quality control range are needed for disk diffusion.
Combination drug	Amoxicillin + clavulanic acid (AMC)		Currently no standardised method available.
Macrolides	Azithromycin (AZM)	f	Not included at this stage. Option for future.

** Abbreviations/antibiotic codes as used in EARS-Net and based on WHONET 5.3*

4. Methods to test for susceptibility

Disk diffusion is the most widely used method for measurement of antimicrobial activity against *Salmonella* (inhibition zone diameters (IZD) expressed in mm) in routine clinical laboratories since it is inexpensive and relatively easy to perform. Dilution methods, where the minimum inhibitory concentration (MIC) is determined (value expressed in mg/L), is a more accurate measurement than disk diffusion and is therefore considered the gold standard for AST. There is however a good to excellent correlation between the values obtained in mm and in mg/L.

Micro-broth dilution is recommended as the preferred testing method for monitoring purposes. However, validated methods of gradient strip diffusion or disk diffusion according to EUCAST protocols are also accepted. ECDC supports EUCAST methods, including interpretation criteria, for AST for *Salmonella* and *Campylobacter* isolates. The methods are available for free on the EUCAST website[1] and an overview of the relevant methods is provided below. Please note that some EUCAST documents are translated into other languages[2].

Media preparation: Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 3.0, April 2012[3].

Dilution method: EUCAST recommends the International Organization for Standards (ISO) reference methods ISO 20776–1:2006 and ISO 20776–2:2007 for MIC determination of non-fastidious and fastidious organisms.

Concentration ranges to test for micro-broth dilution: The concentration ranges to be tested for each antimicrobial should include a span large enough to encompass both the clinical breakpoints and the ECOFF-values, to facilitate comparison with the animal and food data. At the same time, the space available on the 96-well plates must be taken into consideration for cost-efficient testing. The ranges to be included for each of the first priority antimicrobials are therefore proposed to be harmonised with the antimicrobial drug concentration ranges to be tested in food and animal monitoring [9], also listed in Annex 3. Plate compositions for these have been designed by the EU Reference Laboratory for antimicrobial resistance and are now commercially available.

Disk diffusion method: EUCAST disk diffusion method. Version 3.0, April 2012[4].

[1] European Committee on Antimicrobial Susceptibility Testing: http://www.eucast.org/antimicrobial_susceptibility_testing/

[2]European Committee on Antimicrobial Susceptibility Testing. Documents in other languages available here: http://www.eucast.org/translations/

[3]European Committee on Antimicrobial Susceptibility Testing. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Media_preparation_v_3.0_EUCAST_AST.pdf.

[4] European Committee on Antimicrobial Susceptibility Testing. Disk Diffusion Method, version 3. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Manual_v_3.0_EUCAST_Disk_Test.pdf.

5. Detection and confirmation of ESBL-, acquired AmpC, and carbapenemase-producing *Salmonella* spp.

Screening for extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* spp. is important as the ESBL-enzymes hydrolyse and thus inactivate extended-spectrum cephalosporins which are used for treatment of severe *Salmonella* infections, particularly in children [10]. In severe infections due to ESBL-producing bacteria, carbapenems are then one of a very limited number of options for treatment, and therefore also screening of carbapenemase-producing *Salmonella* spp. is vital.

The EUCAST subcommittee recommendations should be followed for identification and screening of these types of enzymes in human *Salmonella* spp. isolates. The main content of the 'EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance' [11] is briefly described below and summarised in Figure 1.

Screening, confirmation and differentiation of carbapenemase-producing *Salmonella* spp.

Out of the three carbapenems mentioned in the guidelines, meropenem is considered to give the best compromise between sensitivity and specificity in terms of detecting carbapenemase-producers. Screening results of carbapenemase-producing *Salmonella* spp should be reported quantitatively and not as interpreted value, as carbapenemase-producing *Enterobacteriaceae* often have MIC-values below the clinical breakpoint.

The classical phenotypic methods remain the recommended methods for confirmation of carbapenemase-production for laboratories without special expertise in β-lactamase detection. An algorithm is presented which differentiates between metallo-β-lactamases, class A carbapenemases, class D carbapenemases and non-carbapenemases (ESBL and/or AmpC plus porin loss). This is done through synergy tests with meropenem and different inhibitors or additional antimicrobial agents [11]. As the synergy test with the combination disk method takes 18 hours, the EUCAST guidelines also mention more rapid alternatives of which the Carba NP test is the only one with published evidence beyond the centre where it was developed.

Screening and confirmation of ESBL-producing *Salmonella* spp., including detection of pAmpC

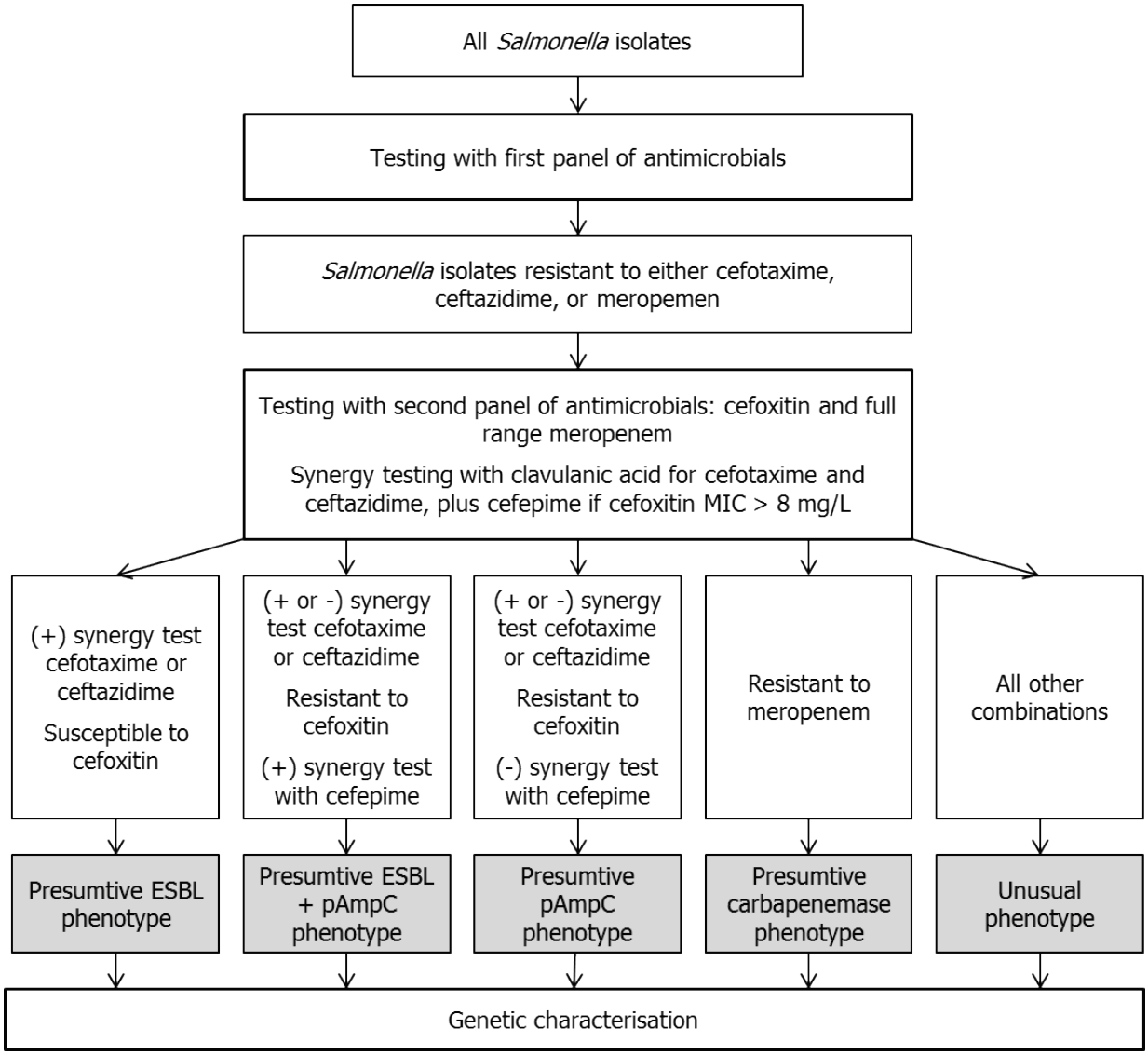
Detection of ESBL in *Enterobacteriaceae* is based on non-susceptibility to indicator oxyimino-cephalosporins. EUCAST recommend the screening to be done with both cefotaxime (alternatively ceftriaxone when using disk diffusion) and ceftazidime with a screening breakpoint of >1 mg/L.

If non-susceptibility to either cefotaxime (alternatively ceftriaxone) or ceftazidime is detected, phenotypic confirmation should follow. Any of four methods based on the inhibition of ESBL-activity by clavulanic acid are recommended for ESBL confirmation: a) the combination disk test, b) the double-disk approximation synergy test, c) the Etest ESBL or d) the broth microdilution test.

Isolates with high-level expression of AmpC β-lactamases can mask the simultaneous presence of ESBLs, resulting in an indeterminate test result or false-negative test results. An additional confirmation step with cefepime (which is not hydrolysed by AmpC β-lactamases) +/- clavulanic acid should therefore be included for isolates expressing high-levels of AmpC β-lactamases. Such isolates can be detected by testing for cefoxitin resistance, e.g. MIC >8 mg/L, as they usually are also resistant to cephamycins. Since AmpC-β-lactamase production is not naturally occurring in *Salmonella* spp., this test would reflect acquired AmpC through plasmids (pAmpC).

Based on the EUCAST recommendations and conclusions from the ECDC expert workshops, the proposed procedure to identify and confirm ESBL-, pAmpC- and carbapenemase-producing *Salmonella* spp. follows the algorithm depicted in Figure 1.

Figure 1. Schematic view of the proposed phenotypic testing for detection and confirmation of ESBL-, acquired AmpC, and carbapenemase-producing *Salmonella* spp.*



Modified from EFSA [8]

6. Genotyping for further identification of resistance mechanisms

The main aim of collecting information on precise resistance mechanisms at the EU level would be to aid recognition of epidemic cross-border spread of multi-drug resistant *Salmonella* strains (e.g. floR and catA genes in *Salmonella* Typhimurium DT104 in the 1990s). This could be done by genotyping a subset of strains. The genes and identification methods should be determined by the individual NPHRL at present (e.g. PCR for family detection/ DNA sequence-based identification) because a diverse range of methods are available, technologies are changing rapidly and there is no consensus that one approach is superior to others. The possibility of having a centre(s) of excellence for AMR mechanisms characterisation can also be looked into since not all NPHRL may wish to, or have capacity to develop all the testing capabilities. An expert FWD network working group could be formed to define testing selection criteria and there should be a correlation done with serotype and clonal type to monitor plasmid/clonal spread. Until further agreements are made therefore, genotyping for identification of resistance mechanisms beyond the phenotypic testing described in section 5 will not be included in the EU level reporting.

7. Interpretive criteria to use

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is determining, reviewing and revising European clinical breakpoints and epidemiological cut-off values for antimicrobial susceptibility testing for those antimicrobials which are used for treatment of human infections. EUCAST is supported by all the national breakpoint committees, ECDC and the European Society of Clinical Microbiology and Infectious Diseases. Also the European Medicines Agency acknowledges the role of EUCAST as the European breakpoint committee for new drugs put on the market.

Clinical breakpoints are used to determine the likelihood of therapeutic success or failure, and may alter with legitimate changes in circumstances (e.g. alterations in dosing regimen, drug formulation, patient factors) [12]. Epidemiological cut-off values (ECOFFs) on the other hand, define whether a microorganism is wild-type or has any acquired mechanisms of resistance to the antimicrobial in question. As such, the ECOFF value should not alter due to changing circumstances.

Reporting of interpreted results by Member States

The EU case definitions (Commission Implementing Decision 2012/506/EU) state that EUCAST clinical breakpoints should be the interpretive criteria used when defining a microorganism as clinically susceptible, intermediate or resistant [4]. This should therefore be followed when reporting susceptible, intermediate, resistant (SIR) values to ECDC (see section 8). EUCAST interpretive criteria do not yet exist for all antimicrobials listed as first priority. Until they are developed, quantitative reporting is recommended for those antimicrobials.

Interpretation by ECDC of quantitative data reported by Member States

It was agreed that ECDC should interpret the data reported quantitatively to the Centre with either clinical breakpoints or ECOFFs, depending on the purpose. When the purpose is to present the resistance situation in terms of clinical treatment possibilities, clinical breakpoints will be applied. When comparing resistance levels in humans with those in animals and food, ECOFFs will be applied as the AST for animal and food isolates is interpreted with ECOFFs to facilitate early detection of acquired resistance. For the antimicrobials where EUCAST interpretive criteria do not yet exist, ECDC may use interpretive criteria from other guidelines, such as the Clinical and Laboratory Standards Institute (CLSI), where appropriate. Annex 1 and 2 provides the current EUCAST clinical breakpoints and ECOFFs for the antimicrobials listed in Tables 1 and 2.

8. Reporting format

At present, Member States are reporting case-based, interpreted information on isolates to ECDC by category as susceptible, intermediate or resistant, as captured in their national surveillance systems.

Quantitative reporting (i.e. real numerical values expressed as MIC in mg/L or IZD in mm) would however allow ECDC and Member States to choose the specific interpretive criteria at the analysis phase depending on the surveillance objectives and indicators (see previous section). It would also allow for comparisons of AMR over time.

Reporting of quantitative MIC or IZD data

Countries are encouraged to report AST data to ECDC in a quantitative format. Quantitative data should only be submitted by NPHRLs and/or other laboratories which use the agreed standardised EUCAST AST methods, and have received permission to upload data to TESSy. Laboratories reporting quantitative data are encouraged to participate in available EQA schemes.

Quantitative AMR data for *Salmonella* can be reported to ECDC through the SALMISO record type which allows laboratories to report their isolate-based *Salmonella* AMR data directly to TESSy after the approval of the national surveillance centre. The isolate-based record type allows reporting of both quantitative and qualitative (SIR interpretation) data. A new record type, CAMPISO, will be created to allow quantitative and qualitative reporting of isolate-based data for the antimicrobials listed for *Campylobacter* in this protocol. Considering the difference in antimicrobial resistance between *Campylobacter* species, AST results should only be reported for isolates where the *Campylobacter* species is known. Please note that quantitative reporting is possible only through the CAMPISO and SALMISO record types.

Reporting of qualitative SIR data

Reporting of interpreted results as SIR-values is possible either through the case-based reporting or through the isolate-based reporting in TESSy. The qualitative reporting will be kept in the case-based reporting in TESSy for a transition period of some years before quantitative data can be reported by a representative proportion of participating NPHRLs allowing reliable comparisons and further analyses.

9. Comparison of data from human isolates and animal and food isolates

For monitoring of AMR in isolates from food-producing animals and food, EFSA has agreed with its counterparts in the Member States on the following methodology:

- To use standardised dilution method for antimicrobial susceptibility testing
- To use EUCAST ECOFF values for the interpretation of microbiological resistance (non-wild type resistance)
- To report quantitative data (mg/l) instead of qualitative results (SIR)
- To collect antimicrobial resistance data at the isolate level
- To use the harmonised set of antimicrobials
- To use phenotypic monitoring to detect the new emerging resistance types, like ESBL.

The technical specifications from EFSA [8] served as basis for the Commission Implementing Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria [9] which entered into force 1 January 2014. The panel of antimicrobials, the interpretive criteria and the concentration ranges to test are presented in Annex 3.

The 2013/652/EU corresponds to the specifications of this protocol to a large extent. Some differences exist in the panel of antimicrobials to be tested. Where the first panel for *Salmonella* isolates from animal and food contain four antimicrobials which are listed as optional for human isolates (nalidixic acid, colistin, azithromycin and tigecycline), the second panel, for confirmation of ESBL-, acquired AmpC, and carbapenemase-producing *Salmonella* spp. contains one antimicrobial listed as optional for human isolates (ertapenem) and two which are not included in the protocol for human isolates (temocillin and imipenem). The panel for *Campylobacter* isolates from animal and food include three antimicrobials which are either optional (gentamicin) or not included (nalidixic acid and streptomycin) in the protocol for human isolates. The difference in the antimicrobials which are not on both panels is not considered a critical issue as the most important agents are included in both panels.

Another difference between the protocols is that clinical breakpoints would primarily be used as the interpretive criteria for human isolates if reported by SIR-values while ECOFFs are used for animal and food isolates. This

reflects the difference in the reason for performing AST, with treatment of clinical illness being the primary focus for testing in human isolates and early detection of acquired resistance and increased resistance in zoonotic bacteria being the goal for AST in animal and food isolates. Quantitative data can however be reliably compared as the data can then be interpreted with either clinical breakpoints or ECOFFs, depending on the purpose of the analysis.

An important consideration in relation to comparison of data is that only dilution susceptibility test data (MICs expresses as mg/L) are accepted in the monitoring in animals and food. Consideration has been given to adopting an MIC only policy also for human isolates, however the costs of testing all isolates by MIC methods are likely to be prohibitive for many NPHRL or to be so high that the laboratories are further restricted in the proportion of submitted isolates on which they can perform susceptibility testing. These effects would be entirely counter-productive in terms of European surveillance of antimicrobial resistance. ECDC will therefore accept both dilution and disk diffusion data and consider the challenge of effective surveillance based on a combination of disk diffusion zone diameter and MIC determination to be manageable. This is provided that the disc diffusion data are generated by a well-controlled and standardised method and are collected in a quantitative manner. It is also considered that although agreement between the two methods is not perfect it is generally high and sufficient to ensure that trends of public health importance of human and veterinary medicine can be identified by appropriate analysis of data from either set of data or from a combination of data from both sources.

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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* spp. as of 11 Oct 2013

Antimicrobial	Criteria based on MIC dilution (mg/L)			Criteria based on disk diffusion (mm)		
	S≤	R>	ECOFF≤	S≥	R<	ECOFF≥
First priority						
Ampicillin (AMP)	8.0	8.0	8.0	14	14	18
Cefotaxime (CTX)	1.0[2]	2.0	0.5	20[2]	17	20
Ceftazidime (CAZ)	1.0[2]	4.0	2.0	22[2]	19	ND
Chlorampenicol (CHL)	8.0	8.0	16.0	17[1]	17[1]	ND
Ciprofloxacin (CIP)	0.5	1.0	0.064	22	19	ND
Colistin (COL)	2.0	2.0	2.0[1]	NA	NA	NA
Gentamicin (GEN)	2.0	4.0	2.0	17[1]	14[1]	ND
Meropenem (MEM)	2.0	8.0	0.125[2]	22[1]	16[1]	25[2]
Sulfamethoxazole (SMX)	ND	ND	ND	ND	ND	ND
Tetracycline (TCY)	ND	ND	8.0	ND	ND	ND
Trimethoprim (TMP)	2.0	4.0	2.0	18[1]	15[1]	ND
Second level testing ESBL-producers						
Cefepime (FEP)	1.0[1]	4.0[1]	ND	24[1]	21[1]	ND
Cefoxitin (FOX)	ND	ND	8.0[2]	19[1,2]	19[1]	ND
Optional						
Amoxicillin (AMX)	8.0	8.0	4.0	ND	ND	ND
Azithromycin (AZM)	ND	ND	ND	ND	ND	ND
Ceftriaxone (CRO)	1.0[1]	2.0[1]	ND	23[1,2]	20[1]	ND
Ertapenem (ETP)	0.5	1.0	0.064 (0.125)[2]	25[1,2]	22[1]	ND
Florfenicol (FLR)	ND	ND	16.0	ND	ND	ND
Nalidixic acid (NAL)	ND	ND	16.0	ND	ND	16
Tigecycline (TGC)	1.0	2.0	1.0	ND	ND	ND
Trimethoprim-sulfamethoxazole (SXT)	2.0	4.0	1.0	16	13	ND

1. Interpretive criteria for Enterobacteriaceae

2. Please note that these interpretive criteria should be used when screening for ESBL-production or carbapenemase-production, according to EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

ND – not determined
NA – not applicable as test method unsuitable for this antimicrobial
Interpretive criteria for Enterobacteriaceae
Please note that these interpretive criteria should be used when screening for ESBL-production or carbapenemase-production, according to EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

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 11 Oct 2013

Antimicrobial	Criteria based on MIC dilution (mg/L)			Criteria based on disk diffusion (mm)		
	S≤	R>	ECOFF ≤	S≥	R<	ECOFF≥
First priority						
Ciprofloxacin (CIP)	0.5	0.5	0.5	26	26	26
Erythromycin (ERY)*C. jejuni*	4.0	4.0	4.0	20	20	22
Erythromycin (ERY)*C. coli*	8.0	8.0	8.0	24	24	24
Tetracycline (TCY)	2.0	2.0	1.0	30	30	30
Optional						
Amoxicillin + clavulanic acid (AMC)	ND	ND	ND	ND	ND	ND
Azithromycin (AZM)	ND	ND	0.25	ND	ND	ND
Ertapenem (ETP)	ND	ND	ND	ND	ND	ND
Gentamicin (GEN)	ND	ND	2.0	ND	ND	ND
Imipenem (IMP)	ND	ND	ND	ND	ND	ND
Meropenem (MEM)	ND	ND	ND	ND	ND	ND

ND – not determined

Annex 3. Antimicrobial panels and concentration ranges to be tested in food and animal isolates (2013/652/EU)

The tables in this annex are taken from the Commission Implementing Decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria 2013/652/EU [9].

Table 1. Panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds for resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli* (First panel)

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF [a]	Clinical breakpoint [b]	
Ampicillin	*Salmonella*	>8	>8	1-64 (7)
	E .coli	>8	>8	
Cefotaxime	*Salmonella*	>0.5	>2	0.25-4 (5)
	E .coli	>0.25	>2	
Ceftazidime	*Salmonella*	>2	>4	0.5-8 (5)
	E .coli	>0.5	>4	
Meropenem	*Salmonella*	>0.125	>8	0.03-16 (10)
	E. coli	>0.125	>8	
Nalidixic acid	*Salmonella*	>16	NA	4-128 (6)
	E .coli	>16	NA	
Ciprofloxacin	*Salmonella*	>0.064	>1	0.015-8 (10)
	E .coli	>0.064	>1	
Tetracycline	*Salmonella*	>8	NA	2-64 (6)
	E .coli	>8	NA	
Colistin	*Salmonella*	>2	>2	1-16 (5)
	E .coli	>2	>2	
Gentamicin	*Salmonella*	>2	>4	0.5-32 (7)
	E .coli	>2	>4	
Trimethoprim	*Salmonella*	>2	>4	0.25-32 (8)
	E .coli	>2	>4	
Sulfamethoxazole	*Salmonella*	NA	NA	8-1024 (8)
	E .coli	>64	NA	
Chloramphenicol	*Salmonella*	>16	>8	8-128 (5)
	E .coli	>16	>8	
Azithromycin	*Salmonella*	NA	NA	2-64 (6)
	E .coli	NA	NA	
Tigecycline	*Salmonella*	>1 (*)	>2 (*)	0.25-8 (6)
	E .coli	>1	>2	

(a) EUCAST epidemiological cut-off values
(b) EUCAST clinical resistance breakpoints
** Data from EUCAST available for Salmonella Enteriditis, Typhimurium, Typhi and Paratyphi*
NA: not available

Table 4. Panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical resistance breakpoints and concentrations ranges to be used for testing only *Salmonella* spp. and indicator commensal *E. coli* isolates resistant to cefotaxime or ceftazidime or meropenem – (Second panel)

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF [a]	Clinical breakpoint [b]	
Cefoxitin	*Salmonella*	>8	NA	0.5-64 (8)
	E .coli	>8	NA	
Cefepime	*Salmonella*	NA	NA	0.06-32 (10)
	E .coli	>0.125	>4	
Cefotaxime + clavulanic acid*	*Salmonella*	NA (**)	NA (**)	0.06-64 (11)
	E .coli	NA (**)	NA (**)	
Ceftazidime + clavulanic acid*	*Salmonella*	NA (**)	NA (**)	0.125-128 (11)
	E .coli	NA (**)	NA (**)	
Meropenem	*Salmonella*	>0.125	>8	0.03-16 (10)
	E .coli	>0.125	>8	
Temocillin	*Salmonella*	NA	NA	0.5-64 (8)
	E .coli	NA	NA	
Imipenem	*Salmonella*	>1	>8	0.12-16 (8)
	E .coli	>0.5	>8	
Ertapenem	*Salmonella*	>0.06	>1	0.015-2 (8)
	E .coli	>0.06	>1	
Cefotaxime	*Salmonella*	>0.5	>2	0.25-64 (9)
	E .coli	>0.25	>2	
Ceftazidime	*Salmonella*	>2	>4	0.25-128 (10)
	E .coli	>0.5	>4	

(a) EUCAST epidemiological cut-off values
(b) EUCAST clinical resistance breakpoints
NA: not available
(*) 4 mg/L clavulanic acid
(**) The values shall be compared to the values of Cefotaxime and Ceftazidime and interpreted according to CLSI or EUCAST guidelines regarding synergy testing.

Table 2. Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni* and *C. coli*

Antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
		ECOFF [a]	Clinical breakpoint [b]	
Erythromycin	*C. jejuni*	>4	>4	1-128 (8)
	C. coli	>8	>8	
Ciprofloxacin	*C. jejuni*	>0.5	>0.5	0.12-16 (8)
	C. coli	>0.5	>0.5	
Tetracycline	*C. jejuni*	>1	>2	0.5-64 (8)
	C. coli	>2	>2	
Gentamicin	*C. jejuni*	>2	NA	0.12-16 (8)
	C. coli	>2	NA	
Nalidixic acid	*C. jejuni*	>16	NA	1-64 (7)
	C. coli	>16	NA	
Streptomycin[c]	*C. jejuni*	>4	NA	0.25-16 (7)
	C. coli	>4	NA	

(a) EUCAST epidemiological cut-off values
(b) EUCAST clinical resistance breakpoints
(c) At a voluntary basis
NA: not available