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Harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from food animals in the European Union

*European Food Safety Authority—Working Group on Developing Harmonised Schemes for Monitoring Antimicrobial Resistance in Zoonotic Agents**

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ABSTRACT

Many Member States of the European Union (EU) currently monitor antimicrobial resistance in zoonotic agents, including *Salmonella* and *Campylobacter*. According to Directive 2003/99/EC, Member States shall ensure that the monitoring provides comparable data on the occurrence of antimicrobial resistance. The European Commission asked the European Food Safety Authority to prepare detailed specifications for harmonised schemes for monitoring antimicrobial resistance. The objective of these specifications is to lay down provisions for a monitoring and reporting scheme for *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs, and for *Campylobacter jejuni* and *Campylobacter coli* in broiler chickens. The current specifications are considered to be a first step towards a gradual implementation of comprehensive antimicrobial resistance monitoring at the EU level. These specifications propose to test a common set of antimicrobial agents against available cut-off values and a specified concentration range to determine the susceptibility of *Salmonella* and *Campylobacter*. Using isolates collected through programmes in which the sampling frame covers all epidemiological units of the national production, the target number of *Salmonella* isolates to be included in the antimicrobial resistance monitoring per Member State per year is 170 for each study population (i.e., laying hens, broilers, turkeys and slaughter pigs). The target number of *Campylobacter* isolates to be included in the antimicrobial resistance monitoring per Member State per year is 170 for each study population (i.e., broilers). The results of the antimicrobial resistance monitoring are assessed and reported in the yearly national report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance.

Keywords Antimicrobial resistance, *Campylobacter*, European Food Safety Authority, food animals, review, *Salmonella*

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INTRODUCTION

The introduction of antimicrobial agents for use in human clinical medicine and animal husbandry has been an important achievement. The first antimicrobial agents were introduced during the 1930s, but unfortunately, emergence of antimicrobial resistance has always followed the introduction of new antimicrobial compounds [1].

Modern food animal production uses large amounts of antibiotics for disease control. This provides favourable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans. During the last decade, there has been an increase in awareness of the potential problems that selection of antimicrobial resistance among food-producing animals could cause for human health. In addition, food animals and food of animal origin are traded worldwide. This has emphasised the need for global initiatives and the establishment of standardised monitoring

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systems for determining the occurrence of resistance among food animals in all countries [2–5].

Recommendations concerning antimicrobial agents to be included in susceptibility testing, the methodology to use and suggestions for breakpoints have been published previously. However, detailed specifications on the procedures to follow, the antimicrobial agents to include and the breakpoints/cut-off values to use have not been agreed. There is a considerable lack of standardisation among the monitoring programmes that have already been established, making comparison among countries difficult [5,6]. According to Article 7 of Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, Member States must establish a monitoring system that provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents originating from animals, food and feed and, insofar as they present a threat to public health, other agents [7].

To address this need, in 2006 the European Commission asked the European Food Safety Authority (EFSA) to prepare detailed specifications for harmonised schemes on monitoring antimicrobial resistance. The EFSA convened a working group under its Task Force on Zoonoses Data Collection, consisting of scientists with expertise in epidemiology and microbiology, and experience in monitoring antimicrobial resistance, as well as staff from relevant European institutions. The working group developed detailed specifications for monitoring antimicrobial resistance in, initially, *Salmonella* and *Campylobacter*, to be used in all 27 Member States of the European Union (EU) [8]. On the basis of these specifications, the Commission adopted Commission Decisions regarding the monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* [9,10].

This review considers the specifications for a harmonised monitoring scheme of antimicrobial resistance in *Salmonella* from fowl (*Gallus gallus*), turkeys and pigs, and *Campylobacter jejuni* and *Campylobacter coli* from broiler chickens. However, many aspects of the laboratory methodology (susceptibility test methods, choice of antimicrobial agents and interpretive criteria), as well as sample size, are applicable regardless of the source of the isolate of a particular bacterial species. The current specifications are considered to be a first step towards a gradual implementation of comprehensive antimicrobial resistance monitoring for different animal and bacterial species.

ELEMENTS OF A MONITORING SCHEME

Several issues need to be addressed when establishing a monitoring scheme, e.g., determining the study population, bacterial species to be included, sampling strategies, isolation procedures, number of samples to be tested, susceptibility testing methods, and data recording, computing and reporting [4,6,11–13]. The following sections consider detailed specifications for the following elements: animal species and bacterial species; study population; sampling plan; sample size; detection, identification and storage of isolates; methods for susceptibility testing; antimicrobial agents to include; cut-off values to use; and data collection and reporting.

Animal and bacterial species

Requirements exist to establish national control programmes for *Salmonella*. These requirements specify that EU targets for a reduction in *Salmonella* prevalence should be established for flocks of poultry and turkeys, and in herds of pigs [14]. Within these control programmes, food business operators must have samples taken and analysed, and official controls, including sampling schemes, are required. This entails regular testing of the total populations of these animal species, or of representative subsets, in all Member States. It is opportune to use these isolates for the purposes of antimicrobial resistance monitoring, as the underlying schemes through which they are collected are already harmonised across Member States.

Study population

While it may be of interest to monitor antimicrobial resistance in various production phases, the greatest benefit may result from focusing on those populations to which the consumer is most likely be exposed. This means that isolates from broilers, turkeys and pigs should preferably be collected close to or at slaughter, whereas isolates from laying hens should be collected periodically throughout the egg production cycle. For this reason, the current specifications are limited to

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the production phases specified for the different study populations in Table 1.

Sampling plan

Monitoring of antimicrobial resistance can be based on isolates from clinical samples submitted to a diagnostic laboratory, or on actively collected isolates from healthy or diseased animals. Animal pathogens are normally included because it is important to observe trends in pathogenic organisms. The selection of isolates from clinical infections will depend in most cases on the submission of isolates from local veterinarians. This will lower the value of these isolates in a surveillance programme because of bias in the selection criteria used over time, and because participation varies among veterinarians. Some infections are more likely to generate symptoms, and isolates from such infections are more likely to be sent for susceptibility testing. Furthermore, in many cases, isolates are sent to a laboratory only after the animals have received antimicrobial treatment, and some veterinarians will send samples only after they have observed treatment failure. Thus, the data obtained from these isolates might overestimate the occurrence of resistance.

To provide an unbiased estimate of the proportion of resistance, the sampling frame should cover all epidemiological units (flocks or holdings) of the national production. This is achieved most readily if isolates originate from national control programmes in which the prevalence of *Salmonella* is determined, and details on where the sampling is to take place and the type of material to collect are already fixed. The epidemiological unit for laying hens, broilers and turkeys is the flock, because most holdings practice all-in-all-out production. For pigs, the epidemiological unit is the holding, because many farms do not practice strict all-in-all-out production.

Table 1. Study population and sampling plan (adapted from Annex IIB of Regulation 2160/2003/EC)

Animal species	Study population	Production phase at primary production
Fowl (*Gallus gallus*)	Flocks of laying hens	Every 15 weeks during the laying phase
	Broiler flocks	Animals leaving for slaughter
Turkeys	All flocks	Animals leaving for slaughter
Pigs	Slaughter pig herds	Animals leaving for slaughter or carcasses at slaughterhouse

Sample size

The number of isolates to be tested should allow, with predetermined accuracy, the calculation of the proportion of resistance to a particular antimicrobial agent in the Member States, and the detection of changes in this proportion over time. The target sample size may vary, depending on whether the sample size is calculated for the purpose of estimating the proportion of resistance or for the purpose of determining a trend. In addition, the sample size differs greatly according to the magnitude of the change that it is desired to detect, or the accuracy of the estimate and the initial resistance situation. If resistance is already widespread, only a relatively large decrease or increase in the proportion of resistance is considered to be relevant. However, for the detection of the initial emergence of resistance, an increase of a few per cent should be detectable.

Based on the assumptions of (i) an infinite population size for the number of bacterial isolates in each study population and Member State, (ii) a 95% CI and a power of 80%, and (iii) 100% sensitivity and specificity of the diagnostic test (i.e., categorisation of isolates into susceptible or resistant categories by means of antimicrobial susceptibility testing), an adequate target number of isolates from a public health perspective for susceptibility testing/study population/Member State/year is 170. This sample size allows the detection of a change of 15% in a setting with widespread resistance (50% resistance) and an increase of 5% in a setting with few pre-existing resistant isolates (0.1% resistance), and provides an accuracy of ±8% in the worst-case scenario of 50% resistance. If a linear trend exists within a country, smaller changes in proportion can be detected over time. Over a 3-year period of continuous monitoring, an average 5% decrease in the proportion of resistant isolates/year can be detected, starting from an initial proportion of resistance of 50%, and an average increase of 2%/year can be detected starting from an initial proportion of resistance of 0.1% [8]. At the aggregate European level, the monitoring programme will be even more precise than this. Thus, the monitoring programme will also provide valuable information on sub-populations with a small sample size/Member State, e.g., rare *Salmonella* serovars.

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Detection, identification and storage of isolates

For isolation and confirmation of bacteria, validated methods need to be followed, as defined in the national control programmes, which foresee that Member States will store isolates for at least 2 years.

For *Salmonella*, all isolates selected for antimicrobial susceptibility testing should be identified to the serovar level. For *Salmonella* Enteritidis and *Salmonella* Typhimurium, it is recommended that all isolates selected for antimicrobial susceptibility testing are phage-typed to allow proper interpretation of the observed frequencies of resistance.

For *Campylobacter*, all the isolates selected for antimicrobial susceptibility testing should be identified to the species level. Monitoring is restricted to *C. jejuni* and *C. coli*, which are the most important species causing infections in humans.

Methods for susceptibility testing

To achieve optimum sensitivity for detection of acquired resistance, it is proposed that epidemiological cut-off values, rather than 'clinical' breakpoints, are used as interpretive criteria. Disk-diffusion is not advocated for European monitoring, because different methodologies are used with different interpretive criteria, and epidemiological cut-off values have not been defined for disk-diffusion. In addition, the disk-diffusion method does not guarantee reproducibility of results for *Campylobacter* spp. Therefore, to improve the comparability of the data provided by Member States, only quantitative data providing MIC values will be accepted.

For non-fastidious microorganisms, e.g., *Salmonella* spp., the EUCAST and CLSI methods for determining MICs have been accepted as international reference methods through CEN and ISO. Dilution methods should be performed according to these methods, as described in ISO Standard 20776-1:2006 [15].

For *Campylobacter* spp., dilution methods should be performed according to the methods described by CLSI [16,17]. At present, the CLSI recommendations are the only international standards giving guidance on broth microdilution testing and quality assurance for *Campylobacter* spp.

To control the quality and comparability of MIC results, laboratories performing susceptibility testing should participate successfully in proficiency testing conducted regularly by the Community Reference Laboratory for Antimicrobial Resistance.

Antimicrobial agents to include in resistance monitoring programmes

Many different antimicrobial agents are currently used in national monitoring programmes. Some examples for *Salmonella* and *Campylobacter* spp. are given in Tables 2 and 3, respectively. The five national monitoring programmes listed for *Salmonella* test a total of 36 different antimicrobial agents or combinations. While each programme includes between 12 and 19 antimicrobial agents, only four agents are tested in all five programmes. For *Campylobacter* spp., between six and 11 antimicrobial agents are included, covering 17 different antimicrobial agents or combinations, but only two agents are tested in all five programmes. This shows a clear need for harmonisation of monitoring programmes.

Antimicrobial agents to be included in a monitoring programme should provide valuable information and should be selected to ensure the highest possible sensitivity in detecting the presence of different resistance mechanisms. In many cases, antimicrobial resistance mechanisms can be inferred by determining the MIC of a given antimicrobial agent or groups of agents. It is then possible, without further testing, to infer which other antimicrobial agents will probably be inactivated by the same resistance mechanism [18,19]. In addition, some types of resistance genes, e.g., those encoding resistance to cephalosporins, might give a complex pattern of resistance. In such cases, it is advisable to choose the antimicrobial agent that is most likely to detect resistance to the entire group, and then to select resistant isolates for further testing or research studies.

Thus, monitoring based on a relatively limited number of antimicrobial agents can give information about the likely resistance to a much broader group of agents and/or information concerning which isolates should be tested with additional antimicrobial agents. The list of recommended antimicrobial agents to be tested is given in Table 4, together with concentration ranges to be tested and interpretative criteria. Only the most relevant antimicrobial agents are included, based

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Table 2. Antimicrobial agents included in different monitoring programmes for *Salmonella*

Antimicrobial group	Antimicrobial agent	National monitoring programme					Common to all five systems
		DANMAP	NARMS	Japan	MARAN	CIPARS	
Aminoglycosides	Amikacin		X			X	
	Apramycin	X		X			
	Gentamicin	X	X	X	X	X	X
	Kanamycin		X	X		X	
	Neomycin	X			X		
	Spectinomycin	X					
	Streptomycin	X	X	X		X	
Aminopenicillins	Ampicillin	X	X	X		X	
	Amoxycillin				X		
β-Lactamase inhibitor combinations	Amoxycillin + clavulanic acid	X	X			X	
Cephalosporin (first generation)	Cephalothin	X	X				
Cephalosporins (third generation)	Cefazolin			X			
	Ceftiofur	X	X	X		X	
	Cefotaxime				X		
	Ceftriaxone		X			X	
	Cefuroxime			X			
Cephamycins	Cefoxitin		X			X	
Carbapanems	Imipenem				X		
Folate pathway inhibitors	Sulphamethoxazole	X	X		X		
	Sulphisoxazole		X			X	
	Trimethoprim	X			X		
	Trimethoprim + sulphonamides		X	X		X	
Macrolides	Azithromycin		X				
	Erythromycin		X				
Phenicols	Chloramphenicol	X	X	X	X	X	X
	Florfenicol	X			X		
Polypeptides	Colistin	X		X			
Quinolones	Ciprofloxacin	X	X		X	X	
	Enrofloxacin			X			
	Ofloxacin			X			
	Oxolinic acid			X			
	Nalidixic acid	X	X	X	X	X	X
Tetracyclines	Oxytetracycline	X	X	X	X	X	X
Other	Destromycin			X			
	Bicozamycin			X			
	Olaquindox			X			
Total number of antimicrobial agents included		17	19	19	12	15	4

DANMAP, Danish Integrated Antimicrobial Resistance Monitoring and Research Programme; NARMS, National Antimicrobial Resistance Monitoring System (USA); MARAN, Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands; CIPARS, Canadian Integrated Program for Antimicrobial Resistance Surveillance.

on their relevance for human therapeutic use and/or epidemiological relevance for monitoring and/or detecting new resistance mechanisms of public health importance.

Salmonella spp.

Aminoglycosides. It recommended that streptomycin and gentamicin should be included, but not neomycin, kanamycin or apramycin. Streptomycin is considered to be an important antibiotic to include in monitoring programmes for detecting resistance in *Salmonella* spp. because it is used as an indicator for the presence of the penta-resistance phenotype of *S.* Typhimurium DT104 or DT104-like phenotypes. Because of the variations in phenotype, the wild-type susceptibility distribution has not been defined by EUCAST; it is therefore recommended that the resistance (R) breakpoint of >32 mg/L proposed by the Concerted Action on Antibacterial Resistance in Bacteria of Animal Origin (ARBAO) [20] should be used to identify all highly-resistant isolates. The resistance gene that encodes streptomycin resistance in DT104 is *aadA2*. Variants of this gene are known to occur in other serovars or phage types of *S.* Typhimurium.

Gentamicin, which is related to kanamycin and amikacin, is considered to be an important agent for routine monitoring, as it is used in both animals and humans for the treatment of serious infections. Resistance is encoded by a variety of genes encoding aminoglycoside acetylase (AAC), aminoglycoside nucleotidyl transferase (ANT) and aminoglycoside phosphorylase (APH) enzymes. Cross-resistance to other aminoglycosides can occur, and this depends on the precise gene present.

Neomycin is an aminoglycoside for which a variety of resistance mechanisms exist, including APH(2′), APH(3″), AAC(2′) and AAC(6′)-III, that result in cross-resistance to other aminoglycosides.

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Table 3. Antimicrobial agents included in various monitoring programmes for *Campylobacter* spp.

		National monitoring programme					
Antimicrobial group	**Antimicrobial agent**	**DANMAP**	**NARMS**	**SVARM**	**MARAN**	**CIPARS**	**Common to all five systems**
Aminoglycosides	Gentamicin	X	X	X		X	
	Neomycin				X		
	Streptomycin	X			X		
Aminopenicillins	Ampicillin			X			
	Amoxycillin				X		
Folate pathway inhibitors	Trimethoprim + sulphonamides				X		
	Sulphamethoxazole				X		
Lincosamides	Clindamycin		X			X	
Macrolides	Azitromycin		X			X	
	Erythromycin	X	X	X	X	X	X
Phenicols	Chloramphenicol	X	X		X		
Quinolones	Ciprofloxacin	X	X		X	X	
	Enrofloxacin			X			
	Nalidixic acid	X	X	X	X	X	X
Tetracyclines	Oxytetracycline	X	X	X		X	
	Doxycycline				X		
	Metronidazole				X		
Total number of antimicrobial agents included		7	8	6	11	8	2

DANMAP, Danish Integrated Antimicrobial Resistance Monitoring and Research Programme; NARMS, National Antimicrobial Resistance Monitoring System (USA); MARAN, Monitoring of Antimicrobial Resistance and Antibiotic usage in Animals in The Netherlands; CIPARS, Canadian Integrated Program for Antimicrobial Resistance Surveillance; SVARM, Swedish Veterinary Antimicrobial Resistance Monitoring.

Table 4. Antimicrobial agents suggested for antimicrobial resistance monitoring programmes

	Antimicrobial agent	**Epidemiological cut-off value (mg/L)**	**Advised optimum concentration range to be tested (mg/L)**
Salmonella	Cefotaxime	0.5	0.06–8
	Nalidixic acid	16	2–256
	Ciprofloxacin	0.06	0.008–8
	Ampicillin	4	0.5–64
	Tetracycline	8	0.5–64
	Chloramphenicol	16	2–256
	Gentamicin	2	0.25–32
	Streptomycin	32[b]	2–256
	Trimethoprim[a]	2	0.25–32
	Sulphonamides	256[c]	8–1024
Campylobacter jejuni	Erythromycin	4	0.5–64
	Ciprofloxacin	1	0.06–8
	Tetracycline	2	0.125–16
	Streptomycin	2	0.5–32
	Gentamicin	1	0.125–16
Campylobacter coli	Erythromycin	16	0.5–64
	Ciprofloxacin	1	0.06–8
	Tetracycline	2	0.125–16
	Streptomycin	4	0.5–32
	Gentamicin	2	0.125–16

[a]Trimethoprim is often used in combination with sulphonamides because of synergy in clinical treatment. However, for susceptibility testing it is important to test and report both substances separately.
[b]Breakpoint recommended by the Concerted Action on Antibiotic Resistance in Bacteria of Animal Origin (ARBAO) project.
[c]CLSI breakpoint.

Cross-resistance to kanamycin depends on the precise gene present. Similarly, kanamycin is related to gentamicin, tobramycin, amikacin and netilmicin. Resistance is mediated by genes encoding AAC(3), ANT(2″)-I and APH. Cross-resistance to other aminoglycosides can occur, but also depends on the precise gene present. Neomycin and kanamycin are not used currently for the treatment of infections in humans, and the mechanisms of resistance to these agents have no application in the identification of specific clones of *Salmonella*.

Apramycin is used for oral treatment of infections in pigs and calves. This agent is not used in humans, and is not recommended for inclusion in surveillance programmes, although cross-resistance to other aminoglycosides, including gentamicin, can occur [21].

Amphenicols. It is considered to be more important to include chloramphenicol than florfenicol. Chloramphenicol has been prohibited for use in food-producing animals in the EU since 1994 [22], but substantial resistance levels are

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still reported in Enterobacteriaceae. Linkage of resistance genes and co-selection may explain this phenomenon. Cross-resistance to florfenicol depends on the precise resistance gene present. Genes encoding a chloramphenicol acetyltransferase enzyme confer resistance only to chloramphenicol, while the *floR* gene encodes resistance to both substances [23]. It is recommended that chloramphenicol should be included in surveillance programmes.

Florfenicol is also considered to be an important agent for monitoring purposes, as the *floR* gene is part of the multidrug resistance gene cluster in *S*. Typhimurium DT104. However, all isolates resistant to florfenicol are also resistant to chloramphenicol.

Extended-spectrum penicillins and β-lactamase inhibitors. It is recommended to include ampicillin rather than amoxycillin or β-lactams combined with β-lactamase inhibitors. Ampicillin represents the extended-spectrum aminopenicillins with intrinsic activity against Enterobacteriaceae. Cross-resistance to amoxycillin is complete, although slight differences in potency exist. Ampicillin and amoxycillin are inactivated by all β-lactamases produced by Enterobacteriaceae, including TEM-1 and SHV-1. Furthermore, ampicillin is traditionally used as an indicator agent for the presence of β-lactamases in Enterobacteriaceae.

Amoxycillin–clavulanic acid is a combination of a broad-spectrum β-lactam and a β-lactamase inhibitor that inhibits the hydrolytic activity of β-lactamases and classical extended-spectrum β-lactamases (ESBLs). Resistance detected in routine monitoring gives limited additional information and can be methodologically difficult to detect because the resistant and susceptible populations are close together in terms of MICs.

Cephalosporins. Of the large variety of generations and compounds available, it is suggested that cefotaxime should be used for routine monitoring of resistance in *Salmonella*.

Cephalothin is a first-generation cephalosporin with an identical spectrum of antimicrobial activity to the broad-spectrum aminopenicillins. Cephalothin is inactivated by all types of β-lactamases produced by Enterobacteriaceae, including TEM-1 and SHV-1. Therefore, the inclusion of cephalothin provides no added value over the inclusion of ampicillin.

Third-generation cephalosporins are important drugs for the treatment of systemic infections, including those caused by *Salmonella* in humans, especially children. Thus, bacteria that produce ESBLs are considered to be a major public health threat. The establishment of optimum phenotypic testing systems for sensitive, specific and rapid detection of ESBLs is therefore a very important component of routine monitoring programmes [24].

Escherichia coli and *Salmonella* isolates resistant to oxyiminocephalosporins because of the production of ESBLs have emerged worldwide, and a number of different ESBL genes, e.g., the bla_{SHV}, bla_{TEM}, bla_{CTX} and bla_{CMY} gene families, have been identified [25–28]. Resistant isolates are found in bacteria from infections in humans, as well as in production and companion animals.

Different ESBLs show varying degrees of activity against the different cephalosporins. Thus, the MICs for ESBL-producing isolates might vary considerably, depending on the β-lactamase and the cephalosporin tested. For routine surveillance purposes, it is essential to use a cephalosporin that will detect ESBL-producing isolates with high sensitivity and specificity, using the most optimal breakpoints. The CLSI has recommended a number of different cephalosporins (cefpodoxime, ceftazidime, aztreonam, cefotaxime, ceftriaxone) for initial screening, and also states that the use of more than one cephalosporin will increase the sensitivity of screening. However, the breakpoints recommended by the CLSI have been criticised. The UK Health Protection Agency recommends the use of cefotaxime–ceftazidime or cefpodoxime in its published guidance for diagnostic laboratories [29]. Several cephalosporins have been approved for use in food animals and pets. The value of these veterinary cephalosporins, e.g., cefoperazone, cefquinome and ceftiofur, for detection of ESBLs is not yet well-defined.

Testing for cefpodoxime resistance will result in a large number of false-positive isolates [30]. Thus, although this cephalosporin is very sensitive for detecting almost all ESBL-producing isolates, it might give a false high prevalence for cephalosporin resistance, and is thus of limited value in routine monitoring. Ceftazidime, together with cefpodoxime, is considered to be the best substrate for most TEM- and SHV-derived ESBLs [19,24]. Cefotaxime is a good substrate for

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the CTX-M enzymes, which are the ESBLs that are currently most common, whereas ceftazidime is a poor substrate and gives a large number of false-positive results. Other cephalosporins, including the veterinary cephalosporins, might be as efficient as cefotaxime in detecting ESBLs, but such data are not currently available.

Quinolones. It is recommended that both nalidixic acid and ciprofloxacin should be included in susceptibility testing programmes. Compared with ciprofloxacin, enrofloxacin is slightly less potent, and a CLSI resistance breakpoint for canine and feline enteric bacilli has been set at ≥4 mg/L. Neither the epidemiological cut-off value for enrofloxacin nor clinical breakpoints for Enterobacteriaceae have been defined by EUCAST. Furthermore, ciprofloxacin is used in human medicine as the first-choice drug for antimicrobial therapy of *Salmonella* infections in patients at risk. Therefore, it is advised to use ciprofloxacin rather then enrofloxacin for monitoring purposes, since the latter is used mostly in veterinary medicine.

Until recently, chromosomal mutations in different genes involved in DNA transcription and replication were considered to be the main mechanisms of quinolone resistance in Enterobacteriaceae. A single point mutation can mediate full resistance to the first-generation agent nalidixic acid, and reduced susceptibility to ciprofloxacin and other fluoroquinolones. However, a new transferable resistance mechanism was described in *Klebsiella pneumoniae* in 1998 [31]. The responsible gene, *qnrA*, encodes a protein that blocks the action of fluoroquinolones. Two other *qnr* genes (*qnrB* and *qnrS*) and a number of different variants have subsequently been identified. These resistance genes have been detected in several species, including *Salmonella enterica* [32–34]. The presence of the *qnr* genes alone does not necessarily mediate full resistance to nalidixic acid, and thus the use of only nalidixic acid to screen for fluoroquinolone resistance is unreliable. Low-level fluoroquinolone resistance is difficult to detect in routine diagnostic laboratories, and such isolates might easily be considered susceptible, especially when using disk-diffusion methods.

Another mechanism of transferable quinolone resistance was reported in 2006 [35]. The *cr* variant of the *aac(6′)Ib* gene encodes an AAC that confers resistance to ciprofloxacin by N-acetylation of its piperazinyl amine. This variant has two amino-acid changes, W102R and D179Y, which together enable this aminoglycoside resistance mechanism to also modify ciprofloxacin. This new resistance mechanism may be very common, and the limited number of reports to date probably reflects the fact that this gene has only recently been discovered. The *aac(6′)Ib-cr* gene appears not to encode resistance to enrofloxacin, but the activity of this resistance mechanism against other veterinary fluoroquinolones and nalidixic acid is currently unknown.

Folate synthesis pathway inhibitors. Sulphamethoxazole and trimethoprim are recommended for separate use in routine monitoring. Resistance to sulphonamides is important for epidemiological purposes, e.g., the detection of particular types of *Salmonella*, including *S.* Typhimurium DT104. The sulphonamide resistance genes that have been identified in *Salmonella* encode resistance to all known sulphonamides. Thus, sulphamethoxazole is commonly used as a single representative of the sulphonamide class in a monitoring programme. Trimethoprim is often used in therapy and is frequently tested in combination with sulphonamides because of apparent synergy between these agents. These agents are often prescribed as a combination (co-trimoxazole); therefore, when monitoring resistance, these agents should be tested individually because isolates are only resistant to the combination if they are resistant to both trimethoprim and sulphonamides.

Tetracyclines. Tetracycline should be used for monitoring purposes as a representative of this class of agents, which includes the related substances oxytetracycline, chlortetracycline, doxycycline and minocycline. Resistance is encoded by *tet* genes that result in active efflux of tetracycline from the bacterial cell. Although differences in antimicrobial potency exist among the agents in this class, cross-resistance is complete, with the exception of minocycline.

Campylobacter spp.

Macrolides. It is recommended to include erythromycin as the representative agent for the macrolides. Macrolides (erythromycin, clarithromycin, azithromycin) are considered to be the first-choice drugs for therapy of human infections

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caused by *Campylobacter* spp. Acquired resistance is caused by single point mutations in the rDNA, resulting in cross-resistance among the different macrolides [36]. Macrolides are also commonly used to treat food animals, and resistance to macrolides has been reported, predominantly in *C. coli* [37–39]. Erythromycin is commonly used in test panels.

Quinolones. It is recommended that only ciprofloxacin should be included in monitoring programmes for *Campylobacter* spp. Resistance to quinolones and fluoroquinolones is caused by a single point mutation in the *gyrA* gene, resulting in resistance to both substances [36]. However, isolates showing resistance to fluoroquinolones, but not to nalidixic acid, have been reported. Because intermediately-resistant sub-populations of *Campylobacter* spp. do not exist, nalidixic acid and ciprofloxacin are equally sensitive for the detection of acquired resistance if epidemiological cut-off values are used. Ciprofloxacin is particularly relevant because of its high level of use in the treatment of human infections.

Tetracyclines. The inclusion of tetracycline in monitoring programmes for *Campylobacter* spp. is relevant. Tetracyclines are considered to be alternative agents for treatment of campylobacteriosis, and acquired resistance has been observed. Resistance in *Campylobacter* spp. is encoded by *tet*(*O*), which is a gene that encodes a ribosomal protection protein [36].

Aminoglycosides. Streptomycin and gentamicin should be tested as representatives of the aminoglycoside class. Resistance to aminoglycosides in *Campylobacter* spp. is mediated by genes encoding APH and ANT enzymes [36]. Streptomycin resistance can be used as an indicator for acquired resistance genes and can give useful epidemiological information. Aminoglycosides administered by the intravenous route, e.g., gentamicin, are the only alternative to macrolides and fluoroquinolones for systemic infections caused by *Campylobacter* spp. [40].

β-*Lactams.* Extended-spectrum penicillins and cephalosporins are not used for therapy of *Campylobacter* infections in humans, and are therefore considered to be of low priority for surveillance purposes.

Cut-off values

EUCAST has developed interpretive criteria for classification of resistance to a variety of compounds, based on wild-type MIC distributions (http://www.eucast.org). The procedure for developing epidemiological cut-off values has been described previously [13,41,42]. The epidemiological cut-off value is the highest MIC value of the wild-type population (WT) that is appropriate to detect biological resistance, expressed as WT ≤X mg/L. EUCAST collects MIC data from as many different sources as possible. These data are collated into a single large distribution and a cut-off value is proposed. An example is given in Fig. 1, which shows MIC data for 1424 *C. jejuni* and 2087 *C. coli* isolates from 17 different data sources. Fig. 1 also shows why different cut-off values are proposed for different species in some cases. Qualitative results should be reported as susceptible (S) or resistant (R), expressed as S ≤X mg/L or R >X mg/L.

EUCAST has not proposed cut-off values for streptomycin and sulphamethoxazole for use with *Salmonella*, as tests of isolates with known streptomycin resistance genes in different centres have revealed a large number of deviations [43]. A cut-off value of R >32 mg/L will probably identify all highly-resistant isolates, but does not exclude the possibility that isolates with acquired resistance genes could be missed when using these criteria.

It has been suggested that the concentration range for a particular antimicrobial agent should include the susceptible wild-type population and at least four concentrations above the cut-off values (Table 4). Most MIC determinations are performed in microtitre plates, in which the format means that the range would consist of a minimum of eight two-fold dilutions. On occasions, wider ranges are advised in order to detect high-level resistance (e.g., to ciprofloxacin). The objective of the relatively wide range is to detect isolates with acquired resistance with optimum sensitivity by including the wild-type susceptible population within this range.

Data collection and reporting

Data should be collected and evaluated at the national level, and should also be evaluated centrally in order to make comparisons among

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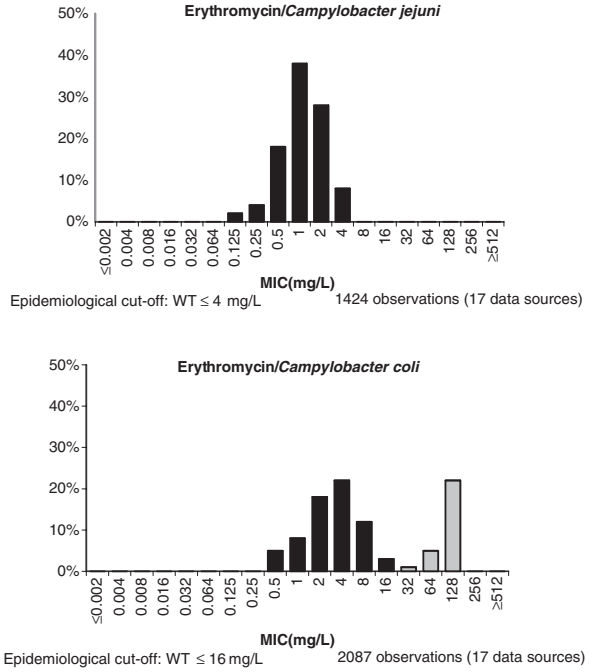

Fig. 1. MIC distributions of erythromycin for *Campylobacter coli* and *Campylobacter jejuni* (data obtained from EUCAST; available at http://www.eucast.org).

countries. In many cases, data are reported only as susceptible or resistant. However, for the purpose of a continuous monitoring programme, it would be optimal if data were reported as MICs to allow comparisons over time, even if breakpoints are changed.

Resistance to some antimicrobial agents can be associated with particular *Salmonella* serovars or phage types. MIC distributions for *S.* Typhimurium and *S.* Enteritidis should be reported separately because of their public health significance, and because these serovars are likely to be detected relatively frequently in many countries. Clonal spread of resistant *Salmonella* serovars or phage types is an important phenomenon in some countries. *S.* Typhimurium and *S.* Derby should be reported separately for pigs, as they occur at a reasonably high prevalence in pigs in many countries, and these data may also provide some additional information concerning the relative antimicrobial selective pressures being exerted on the study populations. The other serovars may be grouped together and reported for each study population separately.

There are marked differences in the prevalence of resistance shown by *C. jejuni* and *C. coli* to different antimicrobial agents, and it is therefore inappropriate to report both species together.

Reporting multiple resistance is of importance, and it is therefore recommended that reports should include information concerning the number of fully susceptible isolates and the number of isolates resistant to one, two, three, four and more than four agents. Results for additional antimicrobial agents should not be reported or included in resistance profiles in order to ensure a fair comparison of resistance data and the number of multidrug-resistant isolates among countries. Only resistance to different antimicrobial agents with unrelated resistance mechanisms should be reported. Thus, it is recommended to include data concerning resistance to ciprofloxacin, but not nalidixic acid, in the resistance profiles. For *Salmonella*, it is also suggested that the number of isolates with the penta-resistance (ACSSuT) phenotype should be reported separately. It is also recommended that the serovar and phage type of these isolates should be reported.

DISCUSSION

National antimicrobial resistance monitoring programmes have now been implemented in a number of countries worldwide. Most of these programmes focus on pathogenic bacteria, e.g., *Salmonella*, and some also report data concerning resistance in indicator bacteria isolated from healthy animals. Since these programmes are not coordinated centrally, they differ in the methodology used and the antimicrobial agents tested. In the EU, based on the specifications developed by the EFSA and subsequently adopted by the Commission and Member States, a harmonised monitoring programme will come into place during 2008. In conjunction with the help of a continuous external quality control scheme put in force following the appointment of a Community Reference Laboratory for Antimicrobial Resistance, this should enable the centralised collection of comparable antimicrobial resistance data of good quality. The use of the current standards will be evaluated during the years to come, and it is likely that changes may be proposed as the standards are used and new data become available.

The standards proposed in the Commission Decisions are binding for all EU Member States, but obviously not for countries in the rest of the

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world. Different antimicrobial agents are tested and different breakpoints are used in, e.g., the National Antimicrobial Resistance Monitoring System (NARMS) programme in the USA (http://www.cdc.gov/narms/), which hinders intercontinental comparisons. There is a need for continuous international discussion on standards for monitoring antimicrobial resistance.

At present, knowledge of antimicrobial resistance in food animals is incomplete in many Member States. Harmonised monitoring of antimicrobial resistance will help to identify and report emerging resistance problems at the earliest possible stage. In addition, knowledge of the use of antimicrobial agents in different food animal species is currently often unavailable or incomplete. Such monitoring is needed to assess the impact of antimicrobial use on the occurrence of resistance, and to determine where, and for which infections, most antimicrobial agents are used. By implementing harmonised monitoring schemes, Member States will make an important step towards monitoring antimicrobial resistance in bacteria of animal origin. It is hoped that, together with improved harmonised collection of data on the use of antimicrobial agents, it will be possible to address important questions concerning risks for humans and the most efficient interventions.

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