European Commission

Food and Veterinary Office

OVERVIEW REPORT

Residue monitoring for critically important antimicrobials

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OVERVIEW REPORT ON EU MEMBER STATES' 2013 RESIDUE MONITORING PLANS AS REGARDS CRITICALLY IMPORTANT ANTIMICROBIALS

Executive Summary

This report forms part of the road map of the European Commission's action plan against the rising threats from antimicrobial resistance, specifically in the course of evaluating EU Member States' residue monitoring plans, to focus on the ability of the national laboratories to effectively monitor for residues of critically important antimicrobials (CIAs), including fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

Council Directive 96/23/EC, on measures to monitor certain substances and residues thereof in live animals and animal products, provides that Member States shall submit an updated residue monitoring plan to the Commission by 31 March each year. Since 2011, the Food and Veterinary Office (FVO) has been responsible for the assessment of these plans.

The FVO assessed Member States' 2013 residue monitoring plans concerning monitoring for residues of CIAs, with additional input from the EU reference laboratory for antimicrobial residues (ANSES, Fougères). The report found that Member States monitor animal products for a range of residues of veterinary antimicrobials (including most of the CIAs evaluated in this report).

Since the authorisation and use of CIAs in food-producing animals is relevant to the overall discussion regarding antimicrobial resistance, the ability of the Member States to test for residues of these substances is useful. Any maximum residue limit (MRL) violations would demonstrate one type of misuse of these drugs (i.e. using excessive dosage rates and/or failure to observe a sufficiently long withdrawal period prior to slaughter of the animal).

However, residues testing is not suitable for detecting all types of inappropriate use of CIAs (e.g. long-term treatment of animals or sub-therapeutic treatment) as such treatment regimens would probably not result in MRL violations. Given the current knowledge on the role of residues in the development of antimicrobial resistance, it is not considered that there would be any added benefit in the Member States measuring and reporting sub-MRL concentrations in food.

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ABBREVIATIONS AND DEFINITIONS USED IN THIS REPORT

Abbreviation	Explanation
AGISAR	(WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMEG	(EMA) Antimicrobial Advice ad hoc Expert Group
CIA	Critically Important Antimicrobial
EEA	European Economic Area
ELISA	Enzyme Linked Immunosorbent Assay
EMA	European Medicines Agency
EPMAR	European Public Maximum Residue Limit Assessment Report
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
EURL	EU Reference Laboratory
FAO	Food and Agriculture Organization
FVO	Food and Veterinary Office
MIC	Minimum Inhibitory Concentration
MRL	Maximum Residue Limit
OIE	World Organisation for Animal Health
WHO	World Health Organization

1. PURPOSE

This report forms part of the road map of the European Commission's action plan against the rising threats from antimicrobial resistance[1], specifically under *Heading A: Appropriate use of antimicrobials, Action No. 3: Introduce recommendations for prudent use in veterinary medicine, including follow-up reports*. This set out the operational objective, in the course of evaluating Member States' residue monitoring plans, to focus on the ability of the national laboratories to effectively monitor for residues of critically important antimicrobials (CIAs), including fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

2. BACKGROUND

Council Directive 96/23/EC, on measures to monitor certain substances and residues thereof in live animals and animal products[2], provides that European Union Member States shall submit an updated residue monitoring plan to the Commission by 31 March each year. This residue monitoring plan shall set out the groups of residues or substances to be detected, in live animals and animal tissues and products such as meat, milk, eggs and honey. The plan shall take into account the specific situation of each Member State, including, inter alia, legislation on the use of substances such as anti-bacterial substances, provisions on their prohibition or authorisation, distribution, placing on the market and rules governing their administration. The plan shall also include a list of the approved laboratories for that Member State, details of their capacity for processing samples, a list of the substances to be detected and methods of analysis.

Directive 96/23/EC also provides that the Commission shall examine the residue monitoring plans submitted by Member States, and, once it has established their conformity, the plans shall be approved in accordance with a regulatory procedure of the Committee on Plants, Animals, Food and Feed. Since 2011, the Food and Veterinary Office (FVO: Directorate F of the Commission's Directorate-General for Health and Food Safety) has been responsible for the assessment of the updated residue monitoring plans submitted by Member States each year.

Under the road map of the Commission's action plan against the rising threats from antimicrobial resistance, it was agreed that, based on the evaluation of Member States' residue monitoring plans for 2013, the FVO would produce a report on the ability of national laboratories to effectively monitor for residues of CIAs, including fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

3. CRITICALLY IMPORTANT ANTIMICROBIALS

A joint Food and Agriculture Organization (FAO)/ World Organisation for Animal Health (OIE)/ World Health Organization (WHO) Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance, held in 2003 and 2004, recommended that the OIE should develop a list of critically important antimicrobial agents in veterinary medicine, and

[1] http://ec.europa.eu/dgs/health_consumer/docs/road-map-amr_en.pdf
[2] OJ L 125, 23.5.1996, p. 10.

that the WHO should also develop a list of critically important antimicrobial agents in human medicine.

An antimicrobial agent is defined in the glossary of the OIE Terrestrial Animal Health Code as "a naturally occurring, semi-synthetic or synthetic substance that exhibits antimicrobial activity (kill or inhibit the growth of micro-organisms) at concentrations attainable *in vivo*. Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition".

3.1. WHO LIST OF CRITICALLY IMPORTANT ANTIMICROBIALS

Starting with the first WHO Expert Meeting on CIAs for Human Health in 2005, the WHO has classified antimicrobials used in human medicines into three groups: "critically important", "highly important" and "important".

At the second meeting in 2007, participants were asked to prioritise antimicrobials within the "critically important" group towards those substances for which management of the risks from antimicrobial resistance were considered as being needed most urgently. The antimicrobial classes identified were fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

The third meeting of the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) in 2011, including experts in both human health and veterinary medicine, also added to the list veterinary medicines falling in the same classes of antimicrobials as those entered in the human medicine list, considering their potential to impact resistance among the CIAs for human medicine.

Within all of these antimicrobials, the 2011 WHO list[3] identifies the highest priority CIAs as fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides and glycopeptides[4]. The criteria used to compile the WHO list were as follows:

- Criterion 1: An antimicrobial agent which is the sole or one of limited available therapy to treat serious human disease;

- Criterion 2: An antimicrobial agent is used to treat diseases caused by either: (1) organisms that may be transmitted to humans from non-human sources or, (2) human diseases caused by organisms that may acquire resistance genes from non-human sources.

[3] WHO list of Critically Important Antimicrobials (CIA), 3rd revision 2011: http://www.who.int/foodborne_disease/resistance/cia/en/

[4] Five antibiotic feed additives used as growth promoters (avoparcin, bacitracin zinc, spiramycin, virginiamycin and tylosin phosphate) belonging to classes of compounds also used in human medicine were already prohibited in the EU and their authorisations withdrawn in 1997 and 1998 to help decrease resistance to antibiotics used in medical therapy. Antibiotics, other than coccidiostats and histomonostats, could be marketed and used as feed additives in the EU only until 31 December 2005. http://ec.europa.eu/food/food/animalnutrition/feedadditives/authowithdrawal_en.htm

In 2011 the criteria were further refined and focused as follows:

- Criterion 1:
    - Application 1.1 – High absolute number of people affected by diseases for which the antimicrobial is the sole or one of few alternatives to treat serious human disease.
    - Application 1.2 – High frequency of use of the antimicrobial for any indication in human medicine, since usage may favour selection of resistance.
- Criterion 2:
    - Application 2.1 – Greater degree of confidence that there are non-human sources that result in transmission of resistant bacteria (*Campylobacter* spp.), or their resistance genes, to humans (high for *Salmonella* spp., *Escherichia coli* and *Enterococcus* spp.).

Within these categories, the following substances were identified as being for veterinary use only:

- 3rd and 4th generation cephalosporins: cefovecin, cefquinome, ceftiofur;
- Fluoroquinolones: danofloxacin, difloxacin, enrofloxacin, ibafloxacin, marbofloxacin, orbifloxacin;
- Macrolides: gamithromycin, kitasamycin, tildipirosin, tilmicosin, tulathromycin, tylosin, tylvalosin;
- Glycopeptides: avoparcin[5].

3.2. OIE LIST OF ANTIMICROBIAL AGENTS OF VETERINARY IMPORTANCE

The OIE's preliminary list of antimicrobial agents of veterinary importance was published in 2006, based on answers received to a questionnaire sent to OIE member countries. Based on an analysis of further data and comments received from member countries, this preliminary list was refined and further developed. In May 2007, the OIE's World Assembly of Delegates approved the revised list of antimicrobial agents of veterinary importance (OIE List), which was to be used within the framework of work activities with the WHO, FAO and the Codex Alimentarius Commission on antimicrobial resistance. The following criteria were used to determine the degree of importance for classes of veterinary antimicrobial agents:

- Criterion 1: Response rate to the questionnaire regarding Veterinary Important Antimicrobial Agents. This criterion was met when a majority of the respondents (more than 50%) identified the importance of the antimicrobial class in their response to the questionnaire;

[5] Avoparcin, which was formerly authorised as a feed additive in the EU, has not been commercially produced since the late 1990s

- Criterion 2: Treatment of serious animal disease and availability of alternative antimicrobial agents. This criterion was met when compounds within the class were identified as essential against specific infections, and there was a lack of sufficient therapeutic alternatives.

The following categories were then established on the basis of these criteria:

- Veterinary Critically Important Antimicrobial Agents: meet both criteria 1 and 2;
- Veterinary Highly Important Antimicrobial Agents: meet either criterion 1 or 2;
- Veterinary Important Antimicrobial Agents: meet neither criterion 1 nor 2.

A revised OIE list[6], including accompanying recommendations, adopted at the 2013 OIE Global Conference on the responsible and prudent use of antimicrobial agents for animals, was adopted and published by the OIE in 2013. This list identified the following categories of veterinary critically important antimicrobial agents: aminoglycosides, 3rd and 4th generation cephalosporins, macrolides, penicillins, phenicols, fluoroquinolones, sulphonamides and tetracyclines.

3.3. EMA CATEGORISATION OF ANTIMICROBIAL AGENTS OF VETERINARY IMPORTANCE

In April 2013, the European Commission requested advice (via a number of specific questions) from the European Medicines Agency (EMA) on the impact of the use of antibiotics in animals on public and animal health and measures to manage the possible risk to humans. EMA's Antimicrobial Advice ad hoc Expert Group (AMEG) dealt with this request, the responses to which were adopted by the Committees for Medicinal Products for Veterinary use (CVMP) and human use (CHMP) in December 2014[7]. Question 2 from the Commission dealt with the categorisation of antimicrobials. AMEG considered three levels of classification:

- Category 1: Antimicrobials used in veterinary medicine where the risk for public health is estimated as low or limited;
- Category 2: Antimicrobials used in veterinary medicine where the risk for public health is estimated higher and;
- Category 3: Antimicrobials not approved for use in veterinary medicine.

Category 2 includes those antimicrobial classes listed as CIAs by WHO for which the risk to public health from veterinary use is only considered acceptable provided that specific restrictions are placed on their use (i.e. fluoroquinolones and systemically administered (parenteral and oral) 3rd and 4th generation cephalosporins). AMEG considers that these reserved antimicrobials should be used only when there are no alternative antimicrobials authorised for the respective target species and indication.

AMEG also proposed that, pending risk assessment, two other classes of antimicrobials should be included in Category 2 (penicillins and aminoglycosides). Penicillins form a

6 http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/OIE_List_antimicrobials.pdf
7 http://www.ema.europa.eu/docs/en_GB/document_library/Other/2014/07/WC500170253.pdf

diverse class that can be divided into subclasses, some of which have efficacy against *Enterobacteriaceae* and have a high risk for transfer of resistance. AMEG considers that further risk profiling is needed to decide if these particular penicillins may be regarded in the same way as 3rd and 4th generation cephalosporins. For the aminoglycosides, AMEG concluded that there may be a resistance risk associated with the use of this class which has as yet not been addressed.

4. MONITORING FOR RESIDUES OF CRITICALLY IMPORTANT ANTIMICROBIALS IN MEMBER STATES

In the context of the Commission's action plan against the rising threats from antimicrobial resistance, it was decided to evaluate the Member States' 2013 residue monitoring plans, as regards the ability of the national laboratories to effectively monitor for residues of antimicrobials of concern/ CIAs. There are differences between the lists of CIAs elaborated by the WHO and OIE – the latter including aminoglycosides, penicillins, phenicols, sulphonamides and tetracyclines in addition to the WHO list. In general, these 'extra' families of antimicrobials are long established and commonly used compounds in veterinary medicine, and are well catered for in terms of residue monitoring. Consequently, for the purpose of this evaluation, it was decided to focus solely on the WHO list, namely, fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides.

For each relevant commodity covered by the residue monitoring plan (cattle, sheep, goats, pigs, horses, poultry, farmed fish, milk, eggs, rabbits, farmed game and honey), Member States' plans were assessed as regards the screening and confirmatory methods in place for the following compounds within each of the selected therapeutic groups:

- Fluoroquinolones: enrofloxacin (ciprofloxacin), difloxacin, marbofloxacin, danofloxacin and sarafloxacin[8];

- Macrolides (and related substances): erythromycin, josamycin, tilmicosin, kitasamycin (not approved for use in food-producing animals in the EU but used in food animal production in some third countries), spiramycin and tylosin (not used in human medicine);

- Cephalosporins: ceftiofur, cefoperazone and cefquinome.

These individual compounds were selected as they are present in authorised veterinary medicinal products in various EU Member States. EMA has published 'European Public Maximum Residue Limit (MRL) assessment reports' (EPMARs) on each of these active substances and these are publicly available (see Table 1 below). The routes of administration (by injection – intramuscular (i/m), subcutaneous (s/c), intra-mammary application (in dairy cows) or by mouth (in feed or water)) indicated by the sponsors in their MRL applications to EMA are also listed in Table 1.

[8] Orbifloxacin was also considered for inclusion, but the standard database used for submission of Member States' residue monitoring plans did not provide for its specification.

Table 1: Formulation and MRL information on the CIAs evaluated

Compound	Formulations	EPMAR
Ceftiofur	i/m and s/c injection: cattle	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011909.pdf
Cefoperazone	intra-mammary: cattle	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011869.pdf
Cefquinome	i/m injection and intra-mammary: cattle	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011892.pdf
Erythromycin	i/m injection: cattle; oral: (water) poultry	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014185.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014184.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014182.pdf
Josamycin	oral (poultry and pigs) (water); not currently authorised for food producing animals in the EU	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014509.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014512.pdf
Tilmicosin	s/c injection: cattle, sheep, rabbits, intra-mammary: dairy cows oral: pigs, poultry (water)	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015577.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015584.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015597.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015593.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015591.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015587.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015586.pdf

Compound	Formulations	EPMAR
Spiramycin	intra-mammary: cattle oral: (feed): pigs	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015985.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015984.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015983.pdf
Tylosin	i/m injection: cattle, pigs, sheep. oral (feed/water): pigs, poultry (incl laying hens)	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015758.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015760.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015764.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015766.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015766.pdf
Enrofloxacin	s/c injection: cattle, sheep i/m injection: pigs oral: cattle, pigs, sheep, poultry (water)	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014139.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014139.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014144.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014147.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014151.pdf
Difloxacin	oral: poultry (water) s/c injection: cattle i/m injection: cattle, pigs	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013811.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013813.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013820.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013849.pdf
Marbofloxacin	i/m injection: cattle, pigs oral: cattle	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014864.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014865.pdf

Compound	Formulations	EPMAR
Danofloxacin	i/m injection: cattle, pigs oral: poultry (water)	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013465.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013474.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013494.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013494.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013518.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013523.pdf
Sarafloxacin	oral: poultry (water) and fish (feed)	http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015846.pdf http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015848.pdf

With regard to analytical methods used within the scope of residue monitoring plans, screening methods are designed to identify putative non-compliant samples, have a high sample throughput, are cheap and simple to operate and should have a false negative rate of 5% or less i.e. 95% of truly non-compliant samples (> MRL) will be identified. They tend to be non-selective (i.e. several related compounds in the sample may give a 'positive result') and non- or semi-quantitative.

Screening methods for antimicrobials include biological methods (inhibition of bacterial growth) and biochemical methods (molecular interactions between analytes and antibodies or receptor proteins are the basis of Enzyme Linked Immunosorbent Assays – ELISA). Chemical labelling of either the analyte or antibody/receptor allows the interaction to be monitored and measured. These methods are either selective for a family of analytes having related molecular structures, or are sometimes analyte-specific.

Confirmatory methods are applied to 'screening positive' samples. Such methods must unequivocally identify and quantify the analytes in question and should have a false positive rate of 5% or less. As such, they are based on physico-chemical principles and distinguish the chemical structure and molecular characteristics of analytes by separation of molecules (e.g. by chromatography) and the detection of signals related to molecular characteristics (e.g. ultraviolet absorption, diode-array detection, fluorescence, (tandem- mass spectrometry etc.). They are able to distinguish between similar molecular structures (particularly tandem- mass spectrometry), and allow for the simultaneous analysis of several analytes.

Notwithstanding the limited scientific evidence that residues (of these or other antimicrobials) in food contribute significantly to the development of antimicrobial resistance (see section 5), it was still considered useful to examine whether Member States were testing for these substances, if only to see whether there were tools in place to verify compliance with corresponding MRLs in edible tissues.

This exercise covered 27 Member States: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom. Croatia was not included since the Member States' plans were to be submitted by 31 March 2013, before Croatia's accession as the 28th EU Member State on 1 July 2013.

In order to aggregate and summarise the submitted data, the following assumptions were made:

- The sensitivity of microbial growth inhibition plate test methods, used by some Member States for screening, is considered similar to the EU STAR[9] method developed by the EU Reference Laboratory (EURL);

- All physico-chemical methods used by the Member States to test samples are fully validated, in accordance with Commission Decision 2002/657/EC[10], and capable of detecting the analytes in question at the level of interest (MRL).

The EURL for antimicrobial residues (ANSES, Fougères) carried out a detailed analysis of the particulars of the analytical methods used by the Member States and these data were used by the FVO in compiling this report. An example of the analysis carried out for a typical Member State is given in Annex 1. The FVO performed a general assessment of the Member States' plans and provided each Member State with a summary extraction of submitted testing details concerning CIAs. Based on these assessments, several Member States provided clarifications and additional information concerning their residue monitoring plans and these data have been taken into account in the aggregated EU-27 data presented in the following tables.

Such comments included several Member States' commitments to give greater consideration to the inclusion of CIAs when developing and implementing their future residue monitoring plans, taking into account the range of authorised veterinary medicinal products for each species and patterns of use in considering the residues to be analysed for in each species/ commodity and an intention to expand the number of substances tested for and validate additional analytical methods as appropriate/ necessary.

[9] A five plate test developed several years ago at the EURL for the screening of antimicrobial residues in milk and muscle.

[10] OJ, L 221, 17/08/2002 pp. 8-36.

Table 2

Fluoroquinolones: Total number of EU-27 Member States having CIA screening/confirmatory methods in place in the 2013 national residue monitoring plans for specific antimicrobials

Fluoroquinolones

Commodity	Method	Enrofloxacin/ciprofloxacin	Difloxacin	Marbofloxacin	Danofloxacin	Sarafloxacin
Bovine	Screening	25	21	20	23	15
	Confirmatory	26	23	21	23	17
Ovine/ Caprine	Screening	21	18	16	19	12
	Confirmatory	21	19	16	19	13
Swine	Screening	23	17	17	20	13
	Confirmatory	24	18	18	21	15
Equine	Screening	12	11	9	11	6
	Confirmatory	13	12	10	12	5
Poultry	Screening	23	19	16	20	15
	Confirmatory	25	22	17	21	17
Aquaculture	Screening	21	18	14	16	16
	Confirmatory	23	9	15	17	17
Milk	Screening	21	15	18	19	12
	Confirmatory	23	18	19	20	14
Eggs	Screening	17	14	15	18	15
	Confirmatory	17	13	15	18	15
Rabbit	Screening	14	10	10	11	8
	Confirmatory	14	10	10	11	8
Farmed game	Screening	15	11	10	14	8
	Confirmatory	16	12	11	15	9
Honey	Screening	7	6	6	6	5
	Confirmatory	9	8	7	8	7
Overall % of commodities tested for each drug	Screening	**67%**	**54%**	**51%**	**60%**	**42%**
	Confirmatory	**71%**	**55%**	**54%**	**62%**	**46%**

The overall % of commodities tested for each drug is calculated by dividing the actual number of tests by the theoretical maximum (i.e. all 27 Member States testing all 11 commodities). However, in certain Member States there may be no sampling of a particular species under the residue monitoring plan, for example if there is no production of farmed rabbits (e.g. United Kingdom and Ireland) or slaughtering of horses for food production (e.g. Cyprus).

Table 3

Macrolides: Total number of EU-27 Member States having CIA screening/confirmatory methods in place in the 2013 national residue monitoring plans for specific antimicrobials

Commodity	Method	Erythromycin	Josamycin	Tilmicosin	Kitasamycin	Spiramycin	Tylosin
Bovine	Screening	19	12	18	0	16	21
	Confirmatory	19	14	20	0	19	23
Ovine/Caprine	Screening	18	10	16	0	15	20
	Confirmatory	18	11	17	0	16	20
Swine	Screening	20	10	16	0	17	21
	Confirmatory	20	13	19	0	20	24
Equine	Screening	12	6	12	1	11	14
	Confirmatory	13	8	14	1	12	16
Poultry	Screening	19	11	13	0	16	20
	Confirmatory	21	14	16	0	19	23
Aquaculture	Screening	13	5	9	0	8	11
	Confirmatory	12	6	10	0	8	13
Milk	Screening	16	6	14	0	17	19
	Confirmatory	16	8	16	0	18	20
Eggs	Screening	16	8	11	0	12	15
	Confirmatory	16	9	12	0	13	16
Rabbit	Screening	14	6	9	0	9	11
	Confirmatory	14	7	10	0	9	13
Farmed game	Screening	14	7	10	0	10	14
	Confirmatory	15	8	11	0	12	16
Honey	Screening	14	6	8	0	9	17
	Confirmatory	14	6	8	0	9	19
Overall % of commodities tested for each drug	Screening	**59%**	**29%**	**46%**	**0%**	**47%**	**62%**
	Confirmatory	**60%**	**35%**	**52%**	**0%**	**52%**	**68%**

Table 4

3rd and 4th generation cephalosporins: Total number of EU-27 Member States having CIA screening/confirmatory methods in place in the 2013 national residue monitoring plans for specific antimicrobials

Commodity	Method	Ceftiofur	Cefoperazone	Cefquinome
Bovine	Screening	16	8	13
	Confirmatory	14	9	13
Ovine/Caprine	Screening	14	7	11
	Confirmatory	13	8	11
Swine	Screening	16	8	13
	Confirmatory	14	9	13
Equine	Screening	7	4	6
	Confirmatory	8	5	7
Poultry	Screening	11	7	10
	Confirmatory	10	8	10
Aquaculture	Screening	5	4	4
	Confirmatory	4	4	4
Milk	Screening	14	13	13
	Confirmatory	13	14	13
Eggs	Screening	6	5	6
	Confirmatory	5	5	6
Rabbit	Screening	6	4	5
	Confirmatory	5	4	5
Farmed game	Screening	8	5	7
	Confirmatory	8	6	8
Honey	Screening	2	4	3
	Confirmatory	2	4	3
Overall % of commodities tested for each drug	Screening	**35%**	**23%**	**31%**
	Confirmatory	**32%**	**26%**	**31%**

5. RESIDUES AND ANTIMICROBIAL RESISTANCE

Directive 86/469/EEC[11] originally introduced rules on the monitoring of certain residues of pharmacological substances and environmental contaminants in farm animals and fresh meat derived thereof. Directive 96/23/EC extended this monitoring to cover other animal species and all animal products for human consumption. Regulation (EC) No 470/2009 of the European Parliament and of the Council[12] lays down the Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin and MRLs in foodstuffs of animal origin are listed in Table 1 of the Annex to Commission Regulation (EU) No 37/2010[13].

[11] OJ, L 275, 26.9.1986 p. 36.
[12] OJ, L 152, 16/6/2009, pp. 11–22.
[13] OJ L 15, 20.1.2010 p. 1

MRLs are considered safe limits and, compliance with these limits indicates that good veterinary and agricultural practice has been adhered to (i.e. the correct dose rate and meat/milk withholding period has been followed) and that the foodstuff is safe.

Given the fact that antimicrobials are the most commonly used therapeutic class of veterinary medicines, monitoring of their residues is a major component in the Member States' (and trading partners') residue monitoring plans covering, *inter alia*, the edible tissues of cattle, sheep, goats, pigs, horses, poultry, farmed finfish and crustaceans[14], milk, eggs, farmed rabbits, farmed game and honey. Commission Decision 97/747/EC[15] lays down the levels and frequencies of sampling, provided for by Directive 96/23/EC, for the monitoring of certain substances and residues thereof in various animal products.

Whether within the animal itself, or via the environment, it is possible that exposure of bacteria to a certain concentration of an antimicrobial (including its residues) may exert selective pressure on clinically relevant bacteria, and lead to the development of antimicrobial resistance. However, the contribution of residues in food to the development of antimicrobial resistance is not thought to be significant in comparison to the impact of resistant bacteria in or on foodstuffs[16] and there is no documented evidence that antimicrobials (when presented as residue concentrations approved as safe by regulatory agencies) cause adverse human health effects (e.g. no prolonged antimicrobial therapy, prolonged hospital stay, predisposition to infection, treatment failure)[17].

Following the use of antimicrobials in farmed animals, whether for the treatment or prevention of a certain infection / disease, residues of the antimicrobials used may end up in the environment following their excretion from the animal via faeces or urine, or by possible direct environmental contamination if the antimicrobial has been administered via feed or drinking water to the animal with associated spillage, feed wastage etc. (See Table 1 for the list of administration routes of the CIAs evaluated).

Measured environmental concentrations of antibiotics such as ciprofloxacin (not used in veterinary medicine but is the main metabolite of enrofloxacin), erythromycin and tetracyclines have been found to be high enough to exert selective pressure on clinically relevant bacteria, with a build-up level of up to several hundred mg/kg of persistent antibiotics, such as tetracyclines and sulphonamides, being detected in liquid manure[18] (note that both tetracyclines and sulphonamides are extensively used as feed-based medications; most of the CIAs evaluated in the report are not administered in feed or water but by injection).

'Hot spots' for resistance selection may include river sediments, liquid manure, pig faeces lagoon sediments and farmed soil, where residues of antimicrobials such as tetracyclines may accumulate. Tetracycline aggregates in dried liquid manure soil have been found at

[14] No EU MRLs have been established for crustaceans. MRLs established for finfish are used for regulatory purposes.

[15] OJ L 303, 6.11.1997 p. 12.

[16] Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment; Geneva, December 1 – 5, 2003.

[17] Giguère, S., J.F. Prescott, and P.M. Dowling.: "Antimicrobial Therapy in Veterinary Medicine", Wiley Blackwell – 5th Edition, 2013 – Text is included in Chapter 25 (introductory text): Antimicrobial Drug Residues in Food of Animal origin.

[18] "Antimicrobial resistance in animals: both use and residues count?" Marc Heyndrickx and Jeroen Dewulf, VDRA Congress, Ghent, Belgium, 3-5 June 2014.

concentrations of up to 1.5 mg/kg, well within the Minimum Inhibitory Concentration (MIC) values of many bacteria. Sub-inhibitory antimicrobial concentrations may also modulate the transcription of genes and antimicrobials targeting DNA replication may activate competence in certain bacteria (e.g. *Streptococcus pneumonia*), leading to an increased uptake of foreign DNA. Environmental antibiotic concentrations may also induce stress responses in the indigenous microbial communities, promoting horizontal gene transfer. In these ways residues of antimicrobials, whether in animals, animal products or the environment may play some role in the development of acquired antimicrobial resistance, and potential spread of such resistant microorganisms to animals and humans alike.

The use of the CIAs fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides in farm animals will be influenced by the availability of authorised veterinary medicinal products containing these antimicrobials, their indications for use (see Table 1) as well as any possible extra-label or off-label use. Under the auspices of EMA, four European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) reports[19] have already been produced covering, in 2009, the sales of veterinary antimicrobial agents (including CIAs) in nine EU countries for the period 2005-2009, 19 EU and European Economic Area (EEA) countries in 2010, 25 EU/EEA countries in 2011 and 26 EU/EEA countries in 2012. Such sales data, based on a harmonised approach for the collection and reporting of data on the sales and use of veterinary antimicrobial agents, should prove a useful tool to inform EU policy concerning veterinary antimicrobials and their responsible use in food-producing animals.

Notwithstanding the absence of evidence that residues of CIAs in food play a role in the transfer of antimicrobial resistance, having in place sensitive testing methods capable of detecting residues of these substances, at their respective EU MRLs (and below) is an important means to be able to verify that MRLs have not been exceeded and that the antimicrobials have been used in accordance with label instructions.

6. DISCUSSION OF RESULTS

In the context of national residue monitoring plans, EU Member States monitor animal products for a range of residues of veterinary antimicrobials (including most of the CIAs evaluated in this report).

The data demonstrate that, with regard to fluoroquinolones, enrofloxacin (and its main metabolite ciprofloxacin) is the predominant compound tested for in the Member States' plans, with overall 71% estimated coverage by a confirmatory method. The lowest percentage was for sarafloxacin (46%), although it should be noted that this drug only has EU MRLs assigned for chicken and fish (*Salmonidae*).

With regard to macrolides, tylosin unsurprisingly is the predominant compound tested for in the Member States' plans, with overall 68% estimated coverage by a confirmatory method. This drug, which has been authorised for many years in the EU and elsewhere, has EU MRLs assigned for all food-producing species. The lowest coverage was for kitsamycin (only one Member State was testing for this in equidae), although this particular macrolide is not authorised for use in food-producing animals in the EU (it is authorised in other jurisdictions as a pre-mix for medicated feedingstuffs). With regard to josamycin, this had 35% estimated coverage by a confirmatory method, but it is not currently authorised for use in food-

[19] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp

producing animals in the EU (formerly it had provisional MRLs for poultry but these MRLs expired in the early 2000s).

With regard to cephalosporins, the overall coverage by a confirmatory method was low for all three compounds selected (estimated 26% to 32%). However, this percentage improves when one only selects those species/commodities where each of the three compounds have EU MRLs assigned, with coverage ranging from 37% for ceftiofur (all commodities excluding eggs and honey) to 52% for cefoperazone (milk only).

7. CONCLUSIONS

The authorisation and use of CIAs in food-producing animals is relevant to the overall discussion regarding antimicrobial resistance. To this end, the ability of the Member States to test for residues of these substances is useful, as MRL violations would demonstrate one type of misuse of these drugs (i.e. using excessive dosage rates and/or failure to observe a sufficiently long withdrawal period prior to slaughter of the animal).

It is recognised that residues testing is not suitable for detecting all types of inappropriate use of CIAs (e.g. long-term treatment of animals or sub-therapeutic treatment) as such treatment regimens would probably not result in MRL violations. Whilst many Member States have sufficiently sensitive instrumental methods in place which would allow the quantification of sub-MRL concentrations of CIAs and other antimicrobials, such concentrations have no regulatory impact (sanctions cannot be applied as the regulatory limit has not been breached). Given the current knowledge on the role of residues in the development of antimicrobial resistance, it is not considered that there would be any added benefit in the Member States measuring and reporting sub-MRL concentrations in food.

ANNEX 1: EXAMPLE OF ANALYSES CARRIED OUT BY THE EURL FOR A TYPICAL MEMBER STATE

GENERAL INFORMATION

The data reported hereafter are extracted from those reported by the MS in the data file "Plan Data" of NRMP 2013.

In order to summarize as far as possible the obtained data it has been necessary to assume some prerequisites.

Firstly, only the screening methods are evaluated hereafter: any false negative result will never be monitored with a confirmatory method applied only when screening is effective.

Secondly, it has been assumed that all physico-chemical methods proposed hereafter are fully validated; and thus are fit for the purpose.

The cell colored in Green means that to the EU-RL knowledge of the "RESIDUE file" for the substance / matrix / method combination is in accordance with regulated MRLs

The cell colored in Pink means that the EU-RL knowledge of the "RESIDUE file" is not sufficient to give an advice on relevance of the substance / matrix / method's accordance with the regulated MRLs

The cell colored in Red means that to the EU-RL knowledge of the "RESIDUE file" for the substance / matrix / method combination is NOT in accordance with the regulated MRLs

Thirdly, as an approximation it is assumed the sensitivity of the "Plates Tests" methods here-after considered was evaluated as similar to the one sensitivity as for the EU "STAR" also called "5-PT" method, this for any combinations of substance / matrix.

Quinolones:

For Eggs analyzed by HPLC Fluo it is assumed that all substances of interest are properly determined. Reversely for the other matrices only Danofloxacin and Marbofloxacin are properly analyzed. Honey, Milk and Rabbit are not of concern.

Ciprofloxacin	X
Danofloxacin	X
Difloxacin	X
Enrofloxacin	X
Marbofloxacin	X
Orbifloxacin	
Sarafloxacin	X

Macrolides: All analyzed matrices are covered by LC-MS/MS but Aquaculture, Eggs, Honey, Milk and Rabbit are not of concern.

Erythromycin	X
Josamycin	X
Kitasamycin	
Spiramycin	X
Tilmicosin	X
Tylosin	X

Cefalosporins: All analyzed matrices are covered by STAR: the sensitivity for Cefoperazone is not known; therefore for Cefoperazone the sensitivity is not sufficient while for Ceftiofur the sensitivity is sufficient. Eggs, Honey, and Rabbit are not of concern.

Cefoperazone	X
Cefquinome	X
Ceftiofur	X

EU-RL extractions:

	Aquaculture	Bovine	Eggs	Farmed game	Honey	Horses	Milk	Pigs	Poultry	Rabbit	Sheep/Goats
Screening	Five plate Test (STAR)	Five plate Test (STAR)	HPLC-fluo	Five plate Test (STAR)		Five plate Test (STAR)		Five plate Test (STAR)	Five plate Test (STAR)		Five plate Test (STAR)
Confirmation	HPLC-fluo	HPLC-fluo	HPLC-fluo	HPLC-fluo		HPLC-fluo		HPLC-fluo	HPLC-fluo		HPLC-fluo
Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin		Ciprofloxacin		Ciprofloxacin	Ciprofloxacin		Ciprofloxacin
Danofloxacin	Danofloxacin	Danofloxacin	Danofloxacin	Danofloxacin		Danofloxacin		Danofloxacin	Danofloxacin		Danofloxacin
Difloxacin	Difloxacin	Difloxacin	Difloxacin	Difloxacin		Difloxacin		Difloxacin	Difloxacin		Difloxacin
Enrofloxacin	Enrofloxacin	Enrofloxacin	Enrofloxacin	Enrofloxacin		Enrofloxacin		Enrofloxacin	Enrofloxacin		Enrofloxacin
	Flumequine	Flumequine	Flumequine			Flumequine		Flumequine	Flumequine		Flumequine
Marbofloxacin	Marbofloxacin	Marbofloxacin		Marbofloxacin		Marbofloxacin		Marbofloxacin	Marbofloxacin		Marbofloxacin
	Nalidixic acid	Nalidixic acid	Nalidixic acid	Nalidixic acid		Nalidixic acid		Nalidixic acid	Nalidixic acid		Nalidixic acid
Orbifloxacin											
	Oxolinic acid	Oxolinic acid	Oxolinic acid	Oxolinic acid		Oxolinic acid		Oxolinic acid	Oxolinic acid		Oxolinic acid
Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin	Sarafloxacin		Sarafloxacin		Sarafloxacin	Sarafloxacin		Sarafloxacin

EU-RL extractions:

	Aquaculture	Bovine	Eggs	Farmed game	Honey	Horses	Milk	Pigs	Poultry	Rabbit	Sheep/Goats
		KIDNEY		KIDNEY		KIDNEY		KIDNEY	MUSCLE		KIDNEY
Screening		LC-MSMS		LC-MSMS		LC-MSMS		LC-MSMS	LC-MSMS		LC-MSMS
Confirmation		Same as screening		Same as screening		Same as screening		Same as screening	Same as screening		Same as screening
Erythromycin		Erythromycin (Erythromycin A)		Erythromycin		Erythromycin		Erythromycin (Erythromycin A)	Erythromycin (Erythromycin A)		Erythromycin (Erythromycin A)
Josamycin		Josamycin		Josamycin		Josamycin		Josamycin	Josamycin		Josamycin
Kitasamycin											
		Neospiramycin		Neospiramycin		Neospiramycin		Neospiramycin	Neospiramycin		Neospiramycin
		Oleandomycin		Oleandomycin		Oleandomycin		Oleandomycin	Oleandomycin		Oleandomycin
		Roxithromycin		Roxithromycin		Roxithromycin		Roxithromycin	Roxithromycin		Roxithromycin
Spiramycin		Spiramycin		Spiramycin		Spiramycin		Spiramycin	Spiramycin		Spiramycin
		Tiamulin		Tiamulin		Tiamulin		Tiamulin	Tiamulin		Tiamulin
Tilmicosin		Tilmicosin		Tilmicosin		Tilmicosin		Tilmicosin	Tilmicosin		Tilmicosin
		Troleandomycin		Troleandomycin		Troleandomycin		Troleandomycin	Troleandomycin		Troleandomycin
		Tulathromycin		Tulathromycin		Tulathromycin		Tulathromycin	Tulathromycin		Tulathromycin
Tylosin		Tylosin, Tylosin A		Tylosin, Tylosin A		Tylosin, Tylosin A		Tylosin, Tylosin A	Tylosin, Tylosin A		Tylosin, Tylosin A

EU-RL extractions

	Aquaculture	Bovine	Eggs	Farmed game	Honey	Horses	Milk	Pigs	Poultry	Rabbit	Sheep/Goats
Screening	Five plate Test (STAR)	Five plate Test (STAR)		Five plate Test (STAR)		Five plate Test (STAR)	Five plate Test (STAR)	Five plate Test (STAR)	Five plate Test (STAR)		Five plate Test (STAR)
Confirmation	LC-MSMS	LC-MSMS		LC-MSMS		LC-MSMS	LC-MSMS	LC-MSMS	LC-MSMS		LC-MSMS
	Cefalexin (Cefalexin Anhydrate)	Cefalexin (Cefalexin Anhydrate)		Cefalexin (Cefalexin Anhydrate)		Cefalexin (Cefalexin Anhydrate)	Cefalexin (Cefalexin Anhydrate)	Cefalexin (Cefalexin Anhydrate)	Cefalexin (Cefalexin Anhydrate)		Cefalexin (Cefalexin Anhydrate)
	Cefalonium	Cefalonium		Cefalonium		Cefalonium	Cefalonium	Cefalonium	Cefalonium		Cefalonium
	Cefapirin	Cefapirin		Cefapirin		Cefapirin	Cefapirin	Cefapirin	Cefapirin		Cefapirin
	Cefazolin	Cefazolin		Cefazolin		Cefazolin	Cefazolin	Cefazolin	Cefazolin		Cefazolin
Cefoperazone	Cefoperazon	Cefoperazon		Cefoperazon		Cefoperazon	Cefoperazon	Cefoperazon	Cefoperazon		Cefoperazon
Cefquinome	Cefquinom	Cefquinom		Cefquinom		Cefquinom	Cefquinom	Cefquinom	Cefquinom		Cefquinom
Ceftiofur	Ceftiofur	Ceftiofur		Ceftiofur		Ceftiofur	Ceftiofur	Ceftiofur	Ceftiofur		Ceftiofur

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