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## SCIENTIFIC REPORT OF EFSA

# Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food[1]

**European Food Safety Authority**[2, 3]

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### ABSTRACT

Proposals to improve the harmonisation of monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter coli* and *jejuni*, indicator *Escherichia coli* and *Enterococcus* from food producing animals and derived meat by the European Union Member States are presented. In establishing a list of combinations of bacterial species, food-producing animal populations and food products and in setting up priorities for the monitoring of antimicrobial resistance from a public health perspective, the potential exposure of the consumers has been considered as the first variable to be taken into account. As the prevalence of *Salmonella* is decreasing, monitoring of antimicrobial resistance should be enforced in indicator bacteria. The concept of a threshold is introduced for some animal populations and their derived meat (whose consumption is limited to certain Member States) to determine whether monitoring of antimicrobial resistance should be mandatory. Currently used phenotypic monitoring of antimicrobial resistance in bacterial isolates is to be retained but recommendations are given for broadening the harmonised panel of antimicrobials used for *Salmonella*, *E. coli* and *Enterococcus* spp. with the inclusion of substances that are either important for human health or that can provide clearer insight into the resistance mechanisms involved. The use of microdilution methods for testing is confirmed as the preferred option and this should be accompanied by the application of epidemiological cut off values for the interpretation of microbiological resistance. A two-step testing strategy has been devised to further characterise those isolates of *E.coli* and *Salmonella* spp. showing resistance to extended spectrum cephalosporins and carbapenems. Several analytical methods are suggested for monitoring of ESBL/AmpC-producing *E.coli.* Finally, full support is given to the collection and reporting of data at isolate level, in order to enable more in-depth analyses to be conducted, in particular on the occurrence of multi-resistance.



### KEY WORDS

Harmonisation, monitoring, reporting, antimicrobial resistance, *Salmonella*, *Campylobacter*, indicator *Escherichia coli*, *Enterococcus* spp.

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**SUMMARY**

Provisions for monitoring of antimicrobial resistance in zoonotic and indicator bacteria in food producing animals and derived meat are laid down in Directive 2003/99/EC. In addition, Commission Decision 2007/407/EC, implementing Directive 2003/99/EC, lays down detailed and harmonised rules for the monitoring of antimicrobial resistance in *Salmonella* in poultry and pigs. The technical specifications of this Decision are applicable until the end of 2012. This legislative framework has been translated into practical recommendations in two technical specifications documents issued by the European Food Safety Authority in 2007 and 2008 and providing guidance on the harmonised monitoring of antimicrobial resistance in *Salmonella*, *Campylobacter*, indicator *E. coli* and enterococci in several food producing animal categories and derived meat. The implementation of these specifications by the European Union Member States has led to more harmonised and better comparable data on antimicrobial resistance; however, further enhancements are still required.

The European Food Safety Authority received a mandate from the European Commission to assess whether, in light of the experience accrued with the production of the European Union Summary Reports on Antimicrobial Resistance, the latest scientific opinions issued by European Food Safety Authority on the matter on antimicrobial resistance, and the efforts to increase the comparability between the findings from the food and animal sector with those gathered in the humans, there is need to revise existing specifications. This report includes a proposal for the revision of existing legislation and implementing guidance documents. The conclusions are partially built on the proposals and considerations from the recently issued scientific report on the analysis and reporting of data on antimicrobial resistance in the European Union Summary Report.

The evidence from the European Union Summary Reports on Antimicrobial Resistance has shown that guidance issued by the European Food Safety Authority has now mostly been followed by the Member States and has led to the production of more comparable data over the years. This is particularly true for the monitoring of *Salmonella* and *Campylobacter*, whereas for the indicator bacteria data are available for a limited number of Member States with no sign of increase over the past years. With the prevalence of *Salmonella* becoming increasingly low, thanks to the success of the control measures in place in the European Union Member States, it becomes particularly relevant to use indicator bacteria for the monitoring of trends and occurrence of resistance. As a first measure, it is therefore recommended that monitoring of antimicrobial resistance in *E. coli* and in the two enterococcal species, *Enterococcus faecium* and *E. faecalis* should also become mandatory as it is already for *Salmonella* and *Campylobacter* in all major food-producing animal species and their derived meat.

In defining combinations of bacteria/animal/food to become subject to mandatory monitoring, the approach followed was to prioritise potential consumers' exposure. Contrary to the previous recommendations and in order to obtain more informative and comparable results, sampling should no longer be stratified at the level of the different animal species (e.g. *Gallus gallus*, cattle, pigs) but should instead take into account the extremely diverse farming practices, including very different use of antimicrobials, that are in use in the different production types. Sampling should therefore be performed at the level of the different animal populations with the aim of collecting data that could be combined with those on exposure to antimicrobials. It is acknowledged that this approach would lead to an increase in the number of samples to be collected, and that this is a resource consuming activity for the Member States. In designing a sample scheme, therefore, special efforts have been made where possible to exploit samples that would be collected under other existing control programmes. Moreover, for those food-producing animal species and their derived fresh meat for which consumption is more specific to certain Member States (e.g. lamb, ducks, geese, goats) a threshold mechanism, calculated on the basis of the animals slaughtered, has been envisaged for the monitoring to become performed consistently in a given Member State. Similarly, for those animal production types that are not aimed at direct consumption and are characterised by lower antimicrobial resistance (e.g. dairy cows, laying hens) it is proposed to have less frequent samplings.

As regards the harmonised set of antimicrobial substances to be used for susceptibility testing, it is noted that the panels currently included in the technical specifications have been broadly used across the Member States and only minor revisions and additions are needed.

In particular, it is proposed to complement the existing panel of antimicrobials for *Salmonella* and *E. coli* with colistin and ceftazidime. Inclusion of the former is motivated by its recent and increased importance as a last resort treatment in human medicine; the latter is recommended, in addition to the already included cefotaxime, to improve the possibility of identifying isolates with resistance to extended-spectrum cephalosporins. In order to enhance the monitoring of extended-spectrum β-lactamase-producing bacteria it is also proposed to add a further step for those isolates that exhibit resistance to a third-generation cephalosporin. In these cases, isolates will be tested for resistance to cefoxitin, cefepime, meropenem and to synergy testing with ceftazidime + clavulanic acid and cefotaxime + clavulanic acid. In addition, a third panel of antimicrobial substances recommended (but not mandatory) for testing because of their relevance to public health is also proposed, comprising azithromycin, tigecycline and florfenicol.

As regards *Campylobacter*, no changes were deemed necessary to the currently recommended panel, whereas for *Enterococcus faecium* and *Enterococcus faecalis* it is proposed to complement the panel with the inclusion of the novel antimicrobial substances tigecycline and daptomycin given their importance for human health. In addition, it is proposed to include teicoplanin as a complement to vancomycin and thereby allow for deduction of the presumptive genotype of glycopeptide-resistant enterococci.

As regards the laboratory methodologies, it is confirmed that microdilution is the preferred method and that European Committee on Antimicrobial Susceptibility Testing epidemiological cut-off values should be used as interpretative criteria to define microbiological resistance. The concentration ranges to be used should ensure that both the epidemiological cut-off value and the clinical breakpoint are included so that comparability of results with human data is made possible. Also, in principle, the optimal concentration range should be tested for each substance although for some substances this could be reduced to a minimum range.

The monitoring of antimicrobial resistance in Shiga toxin/verotoxin-producing *E.coli* is not recommended as a priority. Several analytical methods are suggested for monitoring of extended-spectrum β-lactamase-producing *E. coli*, each of the methods having advantages and disadvantages. It is recommended that an experimental study the usefulness of these methods for monitoring purposes is carried out.

As regards the best format for the reporting of the data, the recommendations are the same as those from the technical specifications for the collection and reporting of data at isolate based level recently published.

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**BACKGROUND AS PROVIDED BY EC**

In accordance with Directive 2003/99/EC on monitoring of zoonoses and zoonotic agents, Member States must ensure that monitoring provides comparable data on the occurrence of AMR in zoonotic agents and, in so far as they present a threat to public health, other agents. In particular, Member States must ensure that the monitoring provides relevant information at least with regard to a representative number of isolates of *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli* from cattle, pigs and poultry and food of animal origin derived from these species.

Commission Decision 2007/407/EC, implementing Directive 2003/99/EC, lays down detailed and harmonised rules for the monitoring of AMR in *Salmonella* in poultry and pigs. The technical specifications of this Decision are applicable until the end of 2012.

Control of AMR is a high priority for the Commission, which is preparing a Communication to the European Parliament and the Council on a 5-year action plan to fight against AMR in the EU that will be adopted on 17 November 2011. In order to follow trends on AMR in zoonotic agents and to evaluate the results of the strategy, new implementing provisions on AMR monitorig in Directive 2003/99/EC must be considered.

In 2007 and 2008 the EFSA Task Force on Zoonoses Data Collection endorsed reports including guidance for harmonised monitoring and reporting of AMR in *Salmonella*, *Campylobacter* and commensal *Escherichia coli* and *Enterococcus* spp. from food animals. These reports provided the technical, science-based input for the detailed rules on AMR monitoring which are in force until the end of 2012.

In the meantime, EFSA's Panel on Biological Hazards has adopted several opinions on AMR in zoonotic agents such as

- The Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals, adopted on 7 July 2011;
- Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections, adopted on 28 October 2009;
- Assessment of the Public Health significance of meticillin-resistant *Staphylococcus aureus* (MRSA) in animals and foods, adopted on 5 March 2009;
- Foodborne antimicrobial resistance as a biological hazard, adopted on 9 July 2008.

In addition, EFSA has published several reports on AMR monitoring in zoonotic agents in the EU such as

- European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2009, approved on 29 April 2011;
- The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008, approved on 15 June 2010;
- The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2004-2007, approved on 28 February 2010.

The Commission would like to review the monitoring requirements for AMR in zoonotic agents. Before doing that, it would be useful to consider the need for updates to the 2007 and 2008 EFSA reports, taking into account the most recent scientific opinions on AMR, technological developments, recent trends in AMR occurrence and knowledge on consequences for human health.

## TERMS OF REFERENCE AS PROVIDED BY EC

In accordance with Article 31 of Regulation (EC) No 178/2002, EFSA is requested to provide scientific and technical assistance proposing updates, where relevant, to the 2007 and 2008 EFSA reports on harmonised monitoring and reporting of AMR in *Salmonella*, *Campylobacter* and commensal *Escherichia coli* and *Enterococcus* spp. from food animals. Comparability with results from human monitoring should also be ensured. In particular EFSA should:

1. Provide detailed guidance on the monitoring of bacterial species, food animal species and/or food products and methodologies which should be considered as most relevant for AMR monitoring from a public health perspective, taking into account AMR mechanisms;

2. Reconsider the antimicrobials, epidemiological cut-offs values and recommended optimum concentration range to be tested at least for the combination selected under Terms of Reference 1;

3. Assess the need and, if considered relevant, propose harmonised parameters for the specific monitoring of Shigatoxin-producing *Escherichia coli* and ESBLs;

4. Indicate the best format for the collection and reporting of data.

**CONSIDERATION/SCIENTIFIC REPORT**

## 1. Introduction

In the European Union (EU), antimicrobial resistance (AMR) in bacteria from food-producing animals and food thereof is monitored pursuant to obligations in the Directive 2003/99/EC[4] and secondary legislation. The Directive obliges Member States (MSs) to monitor and report on AMR in zoonotic agents, such as *Salmonella* and *Campylobacter*, in poultry, pigs and cattle, and food of animal origin derived from those species. The monitoring must provide comparable data on the occurrence of AMR derived from the investigation of a representative number of isolates as well as relevant complementary information on the monitoring system in place. MSs shall assess trends and sources of zoonotic agents and AMR in their territory. Also foreseen is the possibility of broadening the scope of AMR monitoring to other agents in so far as they present a threat to public health. The recital 16 of the Directive clarifies that the monitoring of indicator organisms (commensal *E. coli* and enterococci) for AMR might be appropriate, as such organisms may constitute a reservoir of resistance genes, which they can transfer to pathogenic bacteria. Such monitoring of resistance in indicator bacteria is not presently required on a mandatory basis. In addition, more detailed requirements on the monitoring and reporting of AMR in *Salmonella* isolates deriving from different poultry and pig populations subject to mandatory monitoring and control programmes for the reduction of the prevalence of *Salmonella* at national level, are laid down in Commission Decision 2007/407/EC.[5]

Under the framework of Directive 2003/99/EC, the European Food Safety Authority (EFSA) has collected and analysed data on AMR in *Salmonella*, *Campylobacter* and indicator organisms from food-producing animals and food thereof since 2004. To date, EFSA has issued four European Union Summary Reports (EUSRs), the latest two of which, in collaboration with the European Centre for Disease Prevention and Control (ECDC), are specifically dedicated to AMR covering the period 2004-2010 (EFSA, 2010a,b; EFSA and ECDC, 2011, 2012). In addition, the general provisions on AMR monitoring and reporting provided for in the Directive were subsequently translated into technical specifications to address the need for harmonised monitoring and improved comparability of the data. For these purposes, EFSA issued two scientific reports providing guidance for the harmonised monitoring scheme and the methodology for susceptibility testing and reporting of AMR in *Salmonella* and *Campylobacter* (EFSA, 2007), and in indicator (commensal) *E. coli* and *Enterococcus* spp., from food and animals (EFSA, 2008a). The key technical requisites foreseen by Commission Decision 2007/407/EC were derived from this scientific report on *Salmonella* issued by EFSA in 2007.

Since the issuing of the technical specifications in 2007 and 2008, complementary EFSA opinions and joint opinions of ECDC, EFSA, the European Medicines Agency (EMA, formerly known as EMEA) and the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) on AMR hazards, and in particular on meticillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamases (ESBLs), have been published (EFSA, 2008b; ECDC, EFSA, EMEA and SCENHIR, 2009; EFSA Panel on Biological Hazards (BIOHAZ), 2011). The conclusions and recommendations of these opinions have to be accounted for when updating the technical specifications and overarching legislation.

In addition, EFSA has recently published a scientific report on "Technical specifications for the analysis and reporting of data on antimicrobial resistance in the European Union Summary Report" (EFSA, 2012a). Based on a critical review of the European Union Summary Reports (EUSRs) on resistance previously issued, the report makes proposals for improved reporting and analyses of data on AMR at EU level. The report also acknowledges that, although the gradual implementation of the

[4] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31–40.

[5] Commission Decision of 12 June 2007 on a harmonised monitoring of antimicrobial resistance in *Salmonella* in poultry and pigs. OJ L 153, 14.6.2007, p. 26–29.

EFSA technical specifications by MSs yielded positive results in terms of comparability of AMR data from the food chain, further enhancements of harmonisation of data collection and reporting are still needed. A review of the past EUSRs on AMR in zoonotic and indicator bacteria prepared by EFSA with data from 2004 to 2010 reveals some of the critical areas that would probably deserve consideration for the next legislative revision.

This scientific report covers monitoring, collecting and reporting comparable AMR data on *Salmonella*, *Campylobacter*, indicator *E. coli* and enterococci from animals and food under Directive 2003/99/EC. The report provides a rationale and presents the key elements for a harmonised monitoring of AMR yielding comparable data. The proposals are based on the thorough review of the current technical specifications (EFSA, 2007, 2008a), the EFSA opinions on AMR published since then and the AMR data reported by MSs in the EUSR covering the period 2008-2010. Some proposals made in this report reinforce a number of recommendations already made elsewhere. Finally, recommendations on the monitoring of MRSA will be addressed in a subsequent report to account for the peculiarities of MRSA monitoring and typing.

## 2. Recommendations on bacterial species, food animal species and/or food products to be considered for AMR monitoring from a public health perspective

On the basis of the considerations previously presented in the scientific report on the analysis and reporting of AMR data in the EUSR (EFSA, 2012a), a recommendation on the combinations of bacterial species and animal populations/food categories to be regarded as a priority in a routine AMR monitoring is presented in synoptic tables, Table 1 and 2, detailing the number of isolates to be tested, the corresponding biological samples to be collected and the frequency of the sampling. It is proposed that AMR monitoring should primarily be focused on domestic production, so that the putative relationships between antimicrobial resistance and antibiomicrobial usage can be analysed. In addition to the combinations focusing on domestic productions presented in Tables 1 and 2, it is also suggested to complementarily monitor AMR in meat imported from third countries, for example poultry meat, at the EU level. The major considerations on which these recommendations are based are presented in the sections 2 and 3 of this report.

### 2.1. Bacterial species to be considered for AMR monitoring

AMR monitoring in animals and food should cover both zoonotic agents, in the first instance *Salmonella* and *Campylobacter*, and indicator organisms of the commensal flora. Such monitoring should supplement AMR testing in isolates from humans. Although the monitoring of AMR is mandatory for *Salmonella* and *Campylobacter* in food-producing animals and meat thereof, full reporting of quantitative data from all MSs is yet to be achieved. The number of MSs reporting quantitative AMR data, either as minimum inhibitory concentration (MIC) or as inhibition zone diameter (IZD), in these two zoonotic agents is below the expected total of 27 MSs, although huge differences exist between the different animal species and food categories, as shown in Appendix A (Table 1). In addition, with the success of control programmes for *Salmonella* in poultry, the number of *Salmonella* isolates available has started to dramatically diminish.

By contrast, indicator organisms of commensal intestinal flora are commonly isolated from animal intestinal content and faeces. Commensal *E. coli*, *Enterococcus faecium* and *E. faecalis* can be used as indicators of the Gram-negative and Gram-positive commensal intestinal flora, respectively, as most resistance phenotypes present in animal populations are present in these species. Commensal bacteria that contaminate food may be also considered a potential AMR hazard, as they can harbour transferable resistance genes. During the passage through the intestine, these bacteria may transfer their resistance genes to host-adapted bacteria or to pathogens. Exchange of resistance genes between bacteria from different sources can also occur in the environment, including in the kitchen (EFSA, 2008c). In addition, the effects of use patterns of antimicrobials in a given country and animal populations, as well as trends in the occurrence of resistance, can be studied more accurately in indicator bacteria than in food-borne pathogens, because all animals generally carry such indicator

bacteria. Complementary testing of commensal *E. coli* and enterococci from meat is also highly relevant as such testing facilitates exposure assessment for consumers, as the prevalence of zoonotic bacteria, such as *Salmonella*, may be or become low or extremely low.

**Table 1:** Recommendations on the combinations of bacterial species/food animal populations and desirable numbers of isolates to be included in susceptibility testing

| Animal populations | *Salmonella* | | | *Campylobacter* | | | Indicator commensal *E. coli* | | | Indicator commensal enterococci | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Where to collect | Samples to collect | Target no. isolates | Where to collect | Samples to collect | Target no. isolates | Where to collect | Samples to collect | Target n0. isolates | Where to collect | Samples to collect | Target no. isolates |
| **Monitoring recommended to be performed consistently on a yearly basis** | | | | | | | | | | | | |
| Laying hens | Farm[(a)] | boot swabs | 170[(b)] | - | - | - | - | - | - | - | - | - |
| Broilers | Farm[(a)] | boot swabs | 170[(b)] | Slaughterhouse | caecal spl. | 170[(c)] | Slaugterhouse | caecal spl. | 170 | Slaughterhouse | caecal spl. | 170 |
| Fattening turkeys | Farm[(a)] | boot swabs | 170 | - | - | - | - | - | - | - | - | - |
| Fattening pigs | Slaugterhouse | caecal spl. | 170 | Slaugterhouse | caecal spl. | 170[(d)] | Slaugterhouse | caecal spl. | 170 | Slaugterhouse | caecal spl. | 170 |
| Calves under 1 year | Slaugterhouse | caecal spl. | 170 | - | - | - | Slaugterhouse | caecal spl. | 170 | Slaugterhouse | caecal spl. | 170 |
| **Monitoring recommended to be performed on a yearly basis, if production exceeds 10.000 tons/year slaughtered** | | | | | | | | | | | | |
| Fattening turkeys | - | - | - | Slaugtherhouse | caecal spl. | 170 | Slaughterhouse | caecal spl. | 170 | Slaughterhouse | caecal spl. | 170 |
| Sheep | Slaughterhouse | caecal spl. | 170 | - | - | - | Slaughterhouse | caecal spl. | 170 | - | - | - |
| Goats | Slaughterhouse | caecal spl. | 170 | - | - | - | Slaughterhouse | caecal spl. | 170 | - | - | - |
| **Monitoring recommended to be performed on a regular basis (every 3 years)** | | | | | | | | | | | | |
| Laying hens | - | - | - | - | - | - | Farm | boot swabs | 170 | Farm | boot swabs | 170 |
| Breeders of *Gallus gallus*, egg sector | Farm | boot swabs | 170 | - | - | - | Farm | boot swabs | 170 | Farm | boot swabs | 170 |
| Breeders of *Gallus gallus*, meat sector | Farm | boot swabs | 170 | - | - | - | Farm | boot swabs | 170 | Farm | boot swabs | 170 |
| Turkey breeders | Farm | boot swabs | 170 | - | - | - | Farm | boot swabs | 170 | Farm | boot swabs | 170 |
| Calves under 1 year | - | - | - | Slaughtherhouse | caecal spl. | 170 | - | - | - | - | - | - |
| Dairy cattle | Slaughterhouse | caecal spl. | 170 | - | - | - | Slaughterhouse | caecal spl. | 170 | Slauhgterhouse | caecal spl. | 170 |
| Young bovines (1 to 2 years) | Slaughterhouse | caecal spl. | 170 | - | - | - | Slaughterhouse | caecal spl. | 170 | Slaughterhouse | caecal spl. | 170 |

(a): In the framework of the national *Salmonella* control programme. If prevalence is low and fewer than 170 isolates are available, all isolates from national control programmes to be tested for AMR.

(b): Or one isolate per serovar per epidemiological unit per year.

(c): At least 170 *C. jejuni* strains in poultry. Available *C. coli* strains isolated in the framework of the monitoring should be also tested for antimicrobial susceptibility.

(d): Only *C. coli* from pigs.

**Table 2:** Recommendations on the combinations of bacterial species/food categories and desirable numbers of isolates to be included in susceptibility testing

| Type of Meat | *Salmonella* | | *Campylobacter* | | Indicator commensal *E. coli* | | Indicator commensal enterococci | |
|---|---|---|---|---|---|---|---|---|
| | Where to collect | Target no isolates | Where to collect | Target no isolates | Where to collect | Target no isolates | Where to collect | Target no isolates |
| **Monitoring recommended to be performed consistently on a yearly basis** | | | | | | | | |
| Broiler | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Turkey | Cutting plant or at retail | 170 | - | - | - | - | - | - |
| Pork | Cutting plant or at retail | 170 | - | 170 | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Beef | Cutting plant or at retail | 170 | - | 170 | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| **Monitoring recommended to be performed on a yearly basis, if consumption exceeds 10.000 tons/year** | | | | | | | | |
| Veal | Cutting plant or at retail | 170 | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Turkey | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Ducks | Cutting plant or at retail | 170 | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Geese | Cutting plant or at retail | 170 | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Sheep | - | - | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |
| Goats | - | - | - | - | Cutting plant or at retail | 170 | Cutting plant or at retail | 170 |

no: number

As regards the reporting of AMR in indicator *E. coli* and enterococci performed so far, this is currently done by MSs on a voluntary basis; for these bacterial species a decreasing number of data have been reported by a limited number of MSs, as shown in Appendix A (Table 1A). It is therefore proposed to strongly reinforce the previous recommendation for the monitoring of AMR in indicator bacteria (EFSA, 2008a, 2012a), as resulting AMR data are more representative and comparable.

### 2.2. Combinations of bacterial species/food animal populations or food categories to prioritise for AMR monitoring

As foreseen by the EU legislation and recommended from a number of EFSA scientific reports, AMR monitoring in zoonotic agents should be performed in *Salmonella* and *Campylobacter* isolates in all major food-producing livestock species. Concerning meat from these animal species, testing of *Salmonella* and *Campylobacter* isolates is also mandatory. Decision 2007/407/EC foresees the monitoring of AMR in *Salmonella* isolates derived from the *Salmonella* monitoring and control programmes in different populations of *Gallus gallus* (domestic fowl) and, therefore, the ability to distinguish the AMR monitoring between the *Salmonella* strains isolated from laying hens and broilers. Indeed, levels of AMR can be quite distinct between animals of different production types or different production stages (within a pyramidal production sector), reflecting the widely differing treatment regimes, management practices and hygienic conditions encountered. Therefore, an important refinement would be to structure AMR monitoring and reporting in *Salmonella*, *Campylobacter* and indicator organisms systematically according to production types/animal populations instead of the generic categories of animal species generally used to date.

In establishing a list of mandatory requirements for monitoring, the greatest benefit may result from focusing on the animal populations, to which the consumer will most likely be exposed through food thereof, in particular meat products. These animal populations correspond to various production types of the main food-producing animal species, such as broilers, laying hens, fattening turkeys, fattening pigs, veal calves, and the subsequent meat and food products. As regards the possible inclusion of other foodstuffs, such as dairy products and vegetables, among the food categories to be monitored, it is acknowledged that dairy products mainly derive from pasteurised milk, in which the bacteria are destroyed, and that the available evidence has shown that AMR observed in vegetables was actually due to the manure being spread on the soil. In light of the above, it is therefore recommended to limit the scope of regular, annual monitoring to animal productions and the derived meat. The set of food targeted by national AMR monitoring programmes can be voluntarily expanded by MSs as necessary.

In countries where other meats are also commonly consumed (e.g. lamb, veal), the appropriate food animal populations should be also targeted. A possible way forward would be represented by the introduction of a threshold, calculated on the basis of the animal production (e.g. tons of slaughtered animals), to determine whether AMR monitoring should be performed in a specific animal population in a given MS. This mechanism should cater for the differences observed at national level in consumption of specific types of meat (e.g. lamb, ducks). Production statistics issued by Eurostat are shown in Appendix B (Tables 1B and 2B). These highlight great differences in animal production at national level. Those of animal populations whose national production are above the threshold should be targeted by the AMR monitoring.

It is proposed that the AMR monitoring in *Campylobacter* spp. should focus on animal populations and (fresh) meat thereof known for a high load of *Campylobacter*. Testing of *Campylobacter* spp. from meat is therefore proposed to be performed consistently on a yearly basis for broiler and turkey meat. Only *C. jejuni* and *C. coli* are tested for susceptibility, all other *Campylobacter* species being excluded from the programme. In addition, the monitoring of AMR in *Campylobacter* spp. might preferentially focus on *C. coli* in pigs and on *C. jejuni* in poultry, as they are respectively the more prevalent *Campylobacter* species in these food animals respectively. As *C. coli* occurs regularly in broilers and turkeys and may be more resistant than *C. jejuni*, it would be worthwhile to test for susceptibility the *C. coli* strains isolated in the framework of the AMR monitoring in *Campylobacter* spp. in poultry.

# 3. Recommendations on the methodologies considered as most relevant for AMR monitoring from a public health perspective

## 3.1. Analytical methods in routine monitoring and quality control

Concerning the laboratory methodologies to be used for AMR monitoring, it is acknowledged that molecular techniques are the gold standard for detecting AMR genes and that they will become more and more used in the coming years. On the other hand, at this stage it is deemed too premature to move away from the current phenotypic monitoring in favour of these molecular methods, since this would require a radical change of the current approach and substantial investments from the MSs.

Standardised dilution methods give a semi-quantitative measurement of the susceptibility as an antimicrobial concentration (expressed in mg/L) that is reproducible between different laboratories with a biological variation (± one dilution step). As the European Committee on Antimicrobial Susceptibility Testing (EUCAST) website (http://www.eucast.org/) gives access to aggregated distributions of Minimum Inhibitory Concentration (MIC) for these bacterial species, as well as defining epidemiological cut-off values (ECOFFs) (Kahlmeter et al., 2003) and clinical breakpoints in human medicine, data obtained by making use of dilution methods can be interpreted for both epidemiological and clinical purposes, provided that the dilution range used frames both thresholds. By contrast, the quantitative inhibition zone diameter (IZD) data (expressed in mm) derived from diffusion methods are often collected using various methods, whose methodological differences influence the results obtained (EFSA, 2012a). Considering the multiplicity of methods in use, derivation of ECOFFs appropriate for all methods is thus difficult and EUCAST has worked on defining a standard disc diffusion method and related ECOFFs. It is therefore proposed to strongly reinforce the previous recommendation for the use of standardised dilution methods for antimicrobial susceptibility testing of bacterial strains targeted by the harmonised monitoring (EFSA, 2007, 2008, 2012a). More generally, standardised dilution methods should therefore be used to test the susceptibility to, at least, a specified concise list of antimicrobials, given appropriate dilution ranges and ECOFFs (see section 4).

In addition, a quality assurance part should be undertaken in the national AMR monitoring programme to detect any potential differences between the laboratories performing susceptibility tests relating to methods and thresholds, particularly if laboratories other than the national reference laboratory on AMR (NRL-AMR) are involved. At EU level, proficiency tests of the EU reference laboratory (EURL) on AMR for susceptibility testing of *Campylobacter*, *Salmonella*, *E. coli*, enterococci and staphylococci, which are performed annually for the NRL-AMR, support the harmonisation process.

## 3.2. Sampling plans

### 3.2.1. General considerations on a representative and random sampling

Isolates which are tested for antimicrobial susceptibility should ideally be derived from active monitoring programmes. This would ensure the determination of bacterial prevalence in the studied animal populations, whether *Salmonella*, *Campylobacter* or indicator bacteria. Randomised sampling strategies should be preferentially emphasised, allowing for proper statistical data analysis and reducing the effect of sampling bias. It is particularly important that the bacterial isolates originate from healthy animals sampled from randomly selected holdings or flocks or randomly selected within the slaughterhouses.

A random sample in each animal population targeted ensures the representativeness of the entire population, and reflects variability in managerial and hygienic practices in holdings and different country regions. An approximately equal distribution of the collected samples over the year enables the different seasons to be covered. If diseased animals are sampled, these susceptibility results should be reported separately.

### 3.2.2. Sampling plan of bacterial species in certain poultry populations

It is proposed that the AMR monitoring in *Salmonella* spp. from various poultry populations (i.e. breeders of *Gallus gallus* of the meat sector, breeders of *Gallus gallus* of the egg sector, laying hens, broilers, breeders of turkeys, fattening turkeys) targeted by national control and monitoring programmes of *Salmonella*, are based on *Salmonella* isolates collected in the framework of these control programmes. For the national control and monitoring programmes of *Salmonella* in such poultry populations, minimum requirements on the collection of type of material and where the sampling is to take place are already fixed by EU legislation. An unbiased estimate of the proportion of resistance may be obtained through a sampling frame covering all epidemiological units (flocks) of the national production. This is most readily achieved if *Salmonella* isolates originate from the national control programmes. The epidemiological unit for the various poultry populations concerned is the flock, because most holdings practise all-in–all-out production. It is assumed that *Salmonella* isolates of the same serovar from the same epidemiological unit (flock) show a similar pattern of resistance. To ensure representativeness, susceptibility testing should be done for no more than one isolate per *Salmonella* serovar from the same epidemiological unit per year. The number of isolates to be tested per animal population is 170. In the case of higher number of *Salmonella* isolates available, a random selection of 170 isolates should be performed from the collection of yearly available isolates in the MS. In the case of low prevalence, all the *Salmonella* isolates should be tested for susceptibility (see section 3.2.7).

### 3.2.3. Sampling plan of other bacterial species in food-producing animal populations

For all the other combinations of bacterial species/animal populations, it is proposed that AMR monitoring is based on the collection of caecal samples at the slaughterhouse. Sampling performed at the slaughterhouse is emphasised, as in many of the MSs it will be most cost-effective way to collect the samples. In addition, regarding monitoring of AMR in *Campylobacter* spp., the use of on-farm samples collected in the framework of the national *Salmonella* monitoring and control programmes may be problematic, as environmental samples (boot swabs and dust samples) are not optimal for the isolation of *Campylobacter*. Indicator *E. coli* and enterococci may be isolated from the same biological samples.

It is recommended that at least 60 % to 80 % of the domestic animal population in a MS are included in the sampling frame, meaning that slaughterhouses processing at least 60 % to 80 % of the domestic animals (starting with the slaughterhouses of largest throughput) are eligible for sampling. In the case of sampling performed at the slaughterhouse, an active monitoring programme should be based on random sampling of healthy animal carcasses (e.g. broilers, pigs). The sampling plan should be typically stratified per slaughterhouse by allocating the number of samples collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse. An approximately equal distribution of the collected samples over the year enables the different seasons to be covered. Only one representative sample of caecal content per epidemiological unit (e.g. flock, batch or farm), derived either from a unique carcass or from a number of carcasses, is gathered to account for clustering. The number of biological samples to be collected is determined in order to achieve 170 isolates by accounting for the prevalence of the bacteria species monitored.

### 3.2.4. Sampling meat at cutting plant or at retail

Samples of (fresh) meat can be either collected at the cutting plant or at retail level. As cutting plants are frequently co-located with slaughterhouses, sampling at the cutting plant most likely facilitates distinguishing between the domestic and imported productions. Data obtained on AMR resistance in domestic production could then be linked to antimicrobial use in the MS. Sampling at retail, on the other hand, will provide a better estimate of consumers' exposure to resistant bacteria, although a differentiation of domestic and imported products may be problematic in some MSs. In the case of sampling at retail, products from domestic and imported raw material should be differentiated. A stratified sampling plan is proposed.

### 3.2.5. Sampling frequency and targeted monitoring

For the most sensitive detection of emerging AMR and trends in a number of combinations of interest, the sampling should be performed consistently on an annual basis. Similarly, for those MSs where additional food-producing animal populations and meat thereof are importantly produced or consumed, the priority combinations may be complemented accordingly. A lower-priority monitoring may be granted to some other combinations for which low resistance and/or little change in the situation may be expected. A monitoring interval of 2 or 3 years may therefore be applied for these combinations. Having an annual baseline sampling, with changing specific animal/food categories being subject to more intensive sampling every second or third year, should be considered an option. Ideally, the timing of these more intensive programmes should be harmonised between MSs to optimise comparability of results.

With regard to the frequency for testing, there was general agreement that this should be done on a yearly basis. Ideally, such testing should also supplement the monitoring of antimicrobial consumption which should be performed optimally at the same level of the animal populations of interest. With regard to the latter point, it is acknowledged that this is currently not achievable with the data collected through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project.

### 3.2.6. Specific AMR monitoring in imported meat from third countries

As the AMR monitoring previously described is intended to primarily target meat domestically produced, it is proposed that this monitoring is complemented with a specific monitoring of AMR in imported (fresh or frozen) meat from third countries at the level of the EU. To this end, a sampling design stratified per MS with proportional allocations of the number of isolates to the quantities imported in the MS of origin, appears to be the best and the most cost-effective option. The monitoring of AMR in poultry meat imported from third-countries may be the first target of this kind of monitoring regarding imported meat. The statistics on broiler meat importations issued by Eurostat should be used to construct the sampling plan.

### 3.2.7. Sample size calculation

The sample size (i.e. number of isolates to be tested for susceptibility) should allow, within a predetermined accuracy, the calculation of the proportion of resistance to a particular antimicrobial for a given combination of bacterial species/animal populations or food categories and to detect changes in this proportion over time.

The approach and the results of the sample size analyses and calculation in the EFSA technical specifications (EFSA, 2007, 2008a) were reviewed through an alternative approach. First, the baseline requirements used in the framework of the previous EFSA technical specifications (EFSA, 2007) for the calculation and the choice of the minimum sample size recommended were carefully reviewed and their relevance were confirmed. These requirements should foresee that the sample size should allow:

- Detection of a change of 15 % in the situation of widespread resistance (50 % proportion of resistance) and to detect an increase of 5 % in the situation of few pre-existing resistant isolates (0.1 % proportion of resistance); and
- Provision of an accuracy of ± 8 % for the purpose of determining the proportion of resistance in the worst case scenario of 50 % resistant isolates.

In case a linear trend exists within a country, smaller changes in proportion can be detected over time. In the case of 3 years' continuous monitoring:

- starting from an initial proportion of resistance of 50 %: a 5 % decrease in proportion of resistance per year can be detected; and
- starting from an initial proportion of resistance of 0.1 %: an increase by 2 % per year can be detected.

The choice of a sample size of 170 per year was re-evaluated, by re-calculating the effective power by a logistic regression model with bias reduction and profile likelihood confidence intervals as an improved method allowing examination of different time trends and designs (technical note in Appendix C). The added value of using a logistic regression model for computing the sample size is that a logistic regression model also allows determination and testing of non-linear trends or particular known or unknown change points. In the calculations, it is assumed that the change in proportion of resistance over time within one MS is linear, and that it is sufficient to demonstrate statistical significance of the change in proportion of resistance after 2, 3, 4 or 5 years. This could be achieved by applying an interval of 2 or more years for testing antimicrobial resistance in a given study population. Based on this latter consideration, alternative designs exhibiting similar power are additionally proposed and discussed. Nevertheless, testing samples every year is recommended, because trends in proportion of resistance might be detected earlier, even if the statistical power is not sufficient to reliably detect a trend after that time.

It is concluded and acknowledged as desirable from a public health perspective, that an adequate target number of bacterial isolates to be susceptibility tested per study animal population, per country and per year is 170 (EFSA, 2007, 2008a).The number of biological samples to be collected from each animal population in order to achieve 170 isolates depends on the prevalence of the bacterial species monitored. In the particular case of very low bacterial prevalence, whenever a large number of samples has to be collected to achieve a sufficient number of isolates, a passive surveillance scheme can be implemented using isolates deriving from oriented or systematic sampling. Nevertheless, such samples must not be used for isolation of the indicator organisms *E. coli* or enterococci because the samples will not be representative of the total population, but would represent the targeted population which was sampled. *E. coli* and enterococci are to be representative of the total population because a future objective will be to link the amount of antimicrobial usage in the animal population to the occurrence of resistance in that population and, therefore, it is not appropriate to study only a targeted subset of the population when considering this requirement.

### 3.2.8. Complementary molecular analyses

In certain situations additional genetic and phenotypic analyses of certain isolates may be desirable. This may be the case when the strains isolated are of particular concern to human health care, such as ESBL- and pAmpC β-lactamase-producing and carbapenemase-producing strains. The potential impact of such isolates on public health necessitates the availability of additional information on the isolates, so that the need for counter-measures can be assessed. The required information may consist of a combination of genetic and phenotypic features, for example the presence of resistance and genes and the actual MICs towards the relevant antimicrobials. Should whole genome sequencing (WGS) become a standard analytical tool, only phenotypic characterisation may be necessary.

The need for additional analyses in some cases may be clear. It will be difficult to define the situations that require further investigations unambiguously. Criteria based on the now known properties of bacteria are likely to be insufficient in the future, as new strains and new resistance mechanisms evolve over time. When the potential impact on human health care of the resistance patterns discovered in the monitoring programmes is greater than usual, the competent authority should have additional investigations carried out to assess the need for counter-measures. These investigations should supply further information on the genetics of the strains concerned, and on certain the physiological properties, such as the actual resistance for a number of antimicrobials. In the long term, it is envisaged that the use of genome sequencing for monitoring of antimicrobial resistance will become more and more used for bacteria from food production animals and foods thereof, at least as a means to obtain data that are immediately comparable with data from humans.

## 4. Recommendations on antimicrobials, epidemiological cut-off values and recommended optimum concentration ranges to be used for susceptibility testing of isolates

The EFSA 2007 and 2008 technical specification documents were the first steps towards a progressive improvement of AMR monitoring at the EU level. The experience from the previously published 2008, 2009 and 2010 EU SRs on AMR (EFSA, 2010a; EFSA and ECDC, 2011, 2012) showed that the recommendations regarding the common test panel of antimicrobials (EFSA, 2007, 2008a) have been mostly implemented by the MSs. Regular review, future developments and refinement of technical specifications were expected, particularly regarding the harmonised antimicrobial panels, ranges of concentration and ECOFFs. In the light of the recommendations of the EFSA opinion on extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals (EFSA Scientific Panel on Biological Hazard (BIOHAZ), 2011) and the conclusions of recent scientific publications, it has been indeed recently proposed (EFSA, 2012a) to further review the concise common set of antimicrobials so that the phenotypic monitoring of ESBL-producing and pAmpC β-lactamase- producing bacteria in animals and food can be addressed. In addition, certain antimicrobials such as the carbapenems and colistin, are assuming importance as 'last-resort' antimicrobials in the treatment of certain highly-resistant Gram-negative infections in humans, and their inclusion also needs to be considered when panels of antimicrobials are reviewed. These preliminary considerations were used in the present report as a basis for the re-evaluation of the current recommendations on antimicrobials to include in the mandatory monitoring.

### 4.1. Harmonised panel of antimicrobials for susceptibility testing of *Enterobacteriacae*: *Salmonella* and indicator *E. coli*

#### 4.1.1. Complementary antimicrobials to be inserted in the harmonised panel

To provide continuity of monitoring data and allow epidemiological tracing of isolates with particular patterns of resistance (particularly in relation to certain *Salmonella* serovars), it is recommended that those antimicrobials listed in previous recommendations should remain in future testing requirements. The rationale for inclusion of the antimicrobials recommended for use in current monitoring programmes has been previously described (EFSA, 2007, 2008a). Those recommendations should be complemented with the addition of the following antimicrobials:

- **Colistin**. This antimicrobial has been used for many years in livestock and is increasingly also used in human medicine, where it is one of the antimicrobials of last resort in extremely resistant Gram-negative bacterial infections. Mechanisms of acquired resistance have been described in both *E. coli* and *S.* Typhimurium (Landman et al., 2008). It is therefore considered important to monitor resistance to colistin in food animals and food thereof.[6]

- **Ceftazidime**. In the current recommendations, cefotaxime is included as a marker for resistance to extended-spectrum cephalosporins (ESCs). Testing for ceftazidime would complement the testing of cefotaxime and would improve the ability to identify isolates with transferable ESC resistance. In particular, this would enhance the sensitivity to identify isolates producing certain beta-lactamases belonging to the SHV and TEM families of enzymes, which are ceftazidimases and have much lower activity than cefotaximases. Currently such enzymes appear to be less common in animals and food in the EU, but omission of ceftazidime from test procedures would mean that their presence would go undetected. Because the situation is dynamic and the *status quo* may not necessarily be maintained, it is considered essential to ensure that monitoring for ESBL and similar enzymes is comprehensive.[7] In order to promote harmonisation between

[6] If colistin resistance is detected, subculture and purity checks before retesting are recommended, because a degree of resistance can be shown by some bacterial contaminants, such as *Proteus* spp.

[7] The alternative to inclusion of cefotaxime and ceftazidime would be the inclusion of cefpodoxime, but this is not the preferred option in view of the greater number of false positive results which would be likely to arise using cefpodoxime as the sole indicator of ESC resistance (EFSA, 2008a). The replacement of cefotaxime with ceftriaxone was also considered; however, these compounds were considered broadly equivalent in their ability to detect cefotaximases.

medical and veterinary laboratories, the combination of cefotaxime and ceftazidime is the preferred option. Resistance to ceftazidime and/or cefotaxime is further discussed below.

- A **carbapenem** is also considered very important for complementary inclusion. The occurrence of *Enterobacteriacae* resistant to carbapenems is a growing threat in human medicine. The presence of such resistance in bacteria from animals is largely unknown, although such resistance in pigs in one European country has been recently recorded (Fischer et al., 2012). Detection of the existence and spread of carbapenem-resistant bacteria in animal populations is thus considered extremely important for the assessment of potential zoonotic risks. It is therefore recommended that phenotypic testing for carbapenem resistance in *Salmonella* and *E. coli* should be performed consistently. The detection of carbapenem resistance is not straightforward, since carbapenemases belong to several different classes of beta-lactamases and no single test is likely to give high sensitivity as well as high specificity for all types of enzymes.

  It was firstly addressed whether resistance to ceftazidime and/or cefotaxime could be used as a reliable first screening indicator for detection of all carbapenemases. A pragmatic approach would indeed be to focus on the subset of *Salmonella* and *E. coli* isolates resistant to ESCs, as it is likely that isolates producing carbapenemases also show MICs above the EUCAST ECOFFs for cefotaxime and/or ceftazidime. In addition, many isolates carrying carbapenemases also concurrently possess ESBL or AmpC enzymes. Following this approach, this subset of isolates should be tested for susceptibility to carbapenems by determination of MIC using microdilution or gradient test or by disc diffusion (see below). The exceptions are the enzyme OXA-48, and certain other OXA enzymes, which would not be detected as they do not confer resistance to either cefotaxime or ceftazidime (Walther-Rasmussen and Høiby, 2006). Some OXA carbapenemases also have low hydrolytic activity against imipenem and meropenem, and many of these enzymes tend to occur in bacterial species which are not subject to current surveillance procedures, for example *Klebsiella* and *Acinetobacter*. Carbapenemase-producing *Acinetobacter* spp. bacteria were very recently reported from cattle in France and had MICs of imipenem and meropenem above those for the reference strains (Poirel et al., 2012). It is likely that the proposed revision will not detect all OXA enzymes and this position will need to be reviewed in future.

  Therefore, as an additional check for the presence of carbapenemases, and in an attempt to circumvent some of these methodological difficulties, the inclusion of **meropenem at two concentrations**[8] in the harmonised panel is suggested. The concentrations chosen will be the ECOFF and one dilution above the ECOFF. The relative merits of including meropenem, imipenem or ertapenem have therefore been addressed and meropenem is considered to be the optimal single compound for detection of the majority of carbapenemases. The major problem with the inclusion of ertapenem alone in the panel of antimicrobials used for susceptibility testing is that bacterial isolates with permeability changes and with either ESBL or AmpC enzymes may show resistance to to this antimicrobial. Thus putative resistance to ertapenem might be a reflection of AmpC, ESBL or carbapenem resistance, and not of 'true' resistance to the compound. Therefore, should ertapenem be included as a single representative of the carbapenem compounds, isolates resistant to this antimicrobial will need to be characterised further to determine the mechanism of resistance. To minimise the burden on MSs, while at the

8 The most relevant antimicrobial for phenotypic detection of carbapenemase resistance in Enterobacteriacae is under debate, but the three substances, imipenem, ertapenem and meropenem, are mostly discussed. In a recent publication, presenting susceptibility data on E. coli producing carbapenemases of Ambler class A (KPC), class B (NDM, VIM, IMP) and class D (OXA), all isolates had MICs above the ECOFFs for both ertapenem and meropenem (Nordmann et al., 2012). However, a number of isolates, mainly OXA-48 producers, had MICs to imipenem below the ECOFF. This implies that the sensitivity for detecting carbapenem resistance would be similar whether ertapenem or meropenem was used, whereas imipenem would give a lower sensitivity. In the same publication, three and two of 20 non-carbapenemase-producing E. coli strains had MICs above the ECOFF for ertapenem and meropenem, respectively, and would be falsely identified as carbapenemase producers. Accordingly, meropenem or ertapenem interpreted by EUCAST ECOFFs would perform equally well for detection of carbapenemase resistance in E. coli.

same time maximising the useful information available, testing meropenem in the harmonised and secondary panels and adding ertapenem to the optional third panel is considered the optimal way forward. It is considered that many MSs will not have the resources to investigate whether reduced permeability and the possession of either a ESBL or AmpC enzymes are responsible for observed ertapenem resistance. Therefore, meropenem is considered optimal for inclusion as the single compound for detection of carbapenem resistance, with ertapenem being included in the optional third panel which MSs may voluntarily choose to include in their monitoring programmes.

### 4.1.2. Antimicrobials to be inserted in the harmonised panel on a voluntary basis

The testing of susceptibility to additional antimicrobials is also considered useful in some circumstances, but a cost-effective approach does not currently allow their inclusion within the harmonised set of antimicrobials for which testing is recommended. Indeed, inclusion of these antimicrobials on primary plates is likely to be at the expense of other antimicrobials. Inclusion is recommended for consideration in the examination of isolates on secondary or tertiary plates, in particular for isolates demonstrating resistance to third-generation cephalosporins.

- **Florfenicol** is authorised in the EU for treatment of animals but is not used in humans. The *floR* resistance gene confers resistance to florfenicol and chloramphenicol, whereas a number of chloramphenicol resistance genes do not confer resistance to florfenicol. The *floR* gene is found on *Salmonella* Genomic Island-1 (SGI-1), which is present in some multi-drug resistant *Salmonella* isolates – e.g., *S.* Typhimurium DT 104 (Boyd et al., 2001). Although there are therefore some advantages in the additional information which may be gleaned by inclusion of florfenicol, there is insufficient capacity on the testing plate to allow inclusion of this substance.

- **Tigecycline** is considered useful for inclusion as it is one of the antimicrobials which may be used in the treatment of highly resistant Gram-negative bacterial infections in humans. Tigecycline is not used in animals in the EU, and at this stage it is therefore not considered necessary to make testing consistent/mandatory. Monitoring of resistance to tigecycline in addition to the harmonised set is encouraged.

- **Azithromycin** is considered useful for inclusion as it is one of the antimicrobials which may be used in the treatment of highly resistant Gram-negative bacterial infections in humans, in particular invasive salmonelloses. In addition, it is not known to what extent the newer, long-acting 'modern macrolides' authorised in veterinary medicine for food-producing animals (i.e. tulathromycin, gamithromycin and tildipirosin) select for resistance to macrolides in *E. coli* and *Salmonella*. Monitoring of resistance to azithromycin in addition to the harmonised set is therefore encouraged on a voluntary basis so that relevant data can be gathered together to construct MIC distributions and determine ECOFFs.

Inclusion of the combination amoxicillin/clavulanate was discussed in the previous recommendations (EFSA, 2007), and was not considered necessary. These considerations[9] still apply and that this compound should not therefore be included in the current recommended panel of antimicrobials.

[9] The issues regarding the inclusion of amoxicillin/clavulanate relate to the optimal concentrations of amoxicillin and clavulanate (and the ratio of concentrations between the two compounds) that should be used for susceptibility testing, the stability of clavulanate in liquid media and the ECOFFs which should be selected to discriminate between wild-type and non-wild-type organisms. In the case of beta-lactam resistance, the main resistance phenotypes will be detected using the core and supplementary panels of antimicrobials described in this document and although it could be anticipated that some additional resistance phenotypes might be detected by inclusion of amoxicillin/clavulanate (for example, hyper-production of TEM-1 enzymes), the inclusion of amoxicillin/clavulanate in the monitoring programme is not considered justified at this stage.

### 4.1.3. Further testing of *Enterobacteriacae* isolates resistant to ceftazidime and/or cefotaxime and/or meropenem

#### 4.1.3.1. Objectives and step-wise strategy

In order to provide better insight into the epidemiology of AMR, to investigate possible links between different environmental niches (e.g. various animal populations and humans) and to assess zoonotic risks, it is highly recommended to determine whether ESC resistant *Salmonella* and *E. coli* isolates are of either ESBL, AmpC or ESBL+AmpC phenotypes. Carbapenemase phenotypes should also be detected.

For the purpose of harmonisation, the following definitions of phenotypes are proposed for use in the monitoring programme, as it is important that the monitoring outputs are comparable between MSs. In the definitions below, the term "resistant isolates" refers to microbiologically-resistant isolates, also called non-wild-type isolates[10] (which exhibit MIC above the ECOFF). To facilitate EFSA's requirement to collate and report the final results, not all MSs may proceed to genotype isolates. A standardised nomenclature is therefore required to describe equivalent outputs which are comparable between MSs. The following descriptive terms are proposed:

- ESBL phenotype: resistant to ceftazidime and/or cefotaxime; resistant to cefepime; susceptible to cefoxitin.
- Presumptive ESBL: resistant to ceftazidime and/or cefotaxime; resistant to cefepime; susceptible to cefoxitin; synergy shown in clavulanate synergy tests.
- AmpC phenotype: resistant to ceftazidime, cefotaxime and cefoxitin.
- ESBL and AmpC phenotype: resistant to ceftazidime, cefotaxime, cefoxitin and cefepime.
- Carbapenemase phenotype: resistant to meropenem.

These definitions are based on the relationships between phenotypic and genotypic patterns of resistance to different cephalosporins, as illustrated in Table 3.

[10] A microorganism is categorised as non-wild type for a given bacterial species by applying the appropriate ECOFF value in a defined phenotypic test system; non-wild-type organisms are considered to show 'microbiological resistance' (as opposed to 'clinical resistance'). ECOFF values separate the naive, susceptible wild-type bacterial population from non-wild-type bacterial isolates that have developed reduced susceptibility to a given antimicrobial agent (Kahlmeter et al., 2003)

**Table 3:** Relationship between phenotypic and genotypic patterns of microbiological resistance to cephalosporins and meropenem according to ECOFFs

| Genotype | Microbiological resistance phenotype (i.e. non-wild type) | | | | |
|---|---|---|---|---|---|
| | 3rd-generation cephalosporin: Cefotaxime | 3rd-generation cephalosporin: Ceftazidime | 4th-generation cephalosporin: Cefepime | Cephamycin: Cefoxitin | Carbapenem: Meropenem |
| ***ESBL*** | | | | | |
| . TEM-ESBL | R | R | R | S | S |
| . SHV-ESBL | R | R | R | S | S |
| . CTX-M | R | S/R | R | S [(a)] | S |
| ***pAmpC*** | | | | | |
| . CMY-2, CMY-1, ACC | R | R | S/R(low level) [(b)] | R | S |
| ***ESBL+pAmpC*** [(c)] | R | R | R | R | S |
| ***Carbapenemases*** | | | | | |
| . Class A carbapenemases: KPC | R | R | R | S | R |
| . Class B metallo beta-lactamases: IMP, NDM-1, VIM | R | R | R | R | R |
| . Class D carbapenemases: OXA-48 and variants | S | S | S/R | S/R | R |

ESBL, extended-spectrum beta-lactamase; pAmpC, plasmidic AmpC beta-lactamase; R, resistant; S, susceptible.

(a): most of the CTX-M types are below the ECOFF (8mg/L); MICs just above the ECOFF have rarely been described (CTX-M-5).

(b): Usually MICs are below 4 mg/L.

(c): ESBL and porin loss can be confused with pAmpC.

A stepwise strategy for determining phenotypes is proposed, as illustrated on flowcharts shown in Figure 1. The strategy includes testing the subset of ESC-resistant isolates for susceptibility to cefoxitin, cefepime and meropenem. The synergy test to assess the presence of an ESBL is also necessary for any strain resistant to either cefotaxime or ceftazidime. A subset of isolates phenotypically confirmed as resistant to ESC and/or carbapenems should subsequently be tested for genotype by molecular methods. More details are presented below on the proposals made for the different steps, in particular the secondary panel, the synergy testing and the molecular typing.

#### 4.1.3.2. Secondary panel of antimicrobial substances

The susceptibility testing of the subset of cefotaxime and/or ceftazidime-resistant *Salmonella* and *E. coli* isolates to cefoxitin and cefepime, included in a secondary panel of antimicrobials, is recommended.

Testing for susceptibility to meropenem should be also consistently performed on the same isolates in preference to ertapenem. This is because ertapenem is particularly affected by the combination of either ESBL or AmpC enzyme production and porin loss/reduced permeability of the bacterial cell, which results in the consequent acquisition of a degree of resistance to ertapenem. ESBL and AmpC resistance will be detected by the other procedures described in this report. Most MSs do not have the resources available to characterise such isolates. Full characterisation would ideally include some form of typing of the host *E. coli* strain, as ESBL and AmpC resistance tend to be associated with spread of particular clones of *E. coli* which have acquired the necessary permeability changes. It is considered that monitoring of ertapenem resistance and characterisation of ertapenem-resistant isolates should not be included in the current recommendations. Recent medical guidelines have also concluded that ertapenem is not advised as an indicator of carbapenem resistance (Cohen Stuart et al., 2010). These recommendations for animal and food isolates should be harmonised with those for isolates from humans, which are currently undergoing discussion at ECDC.

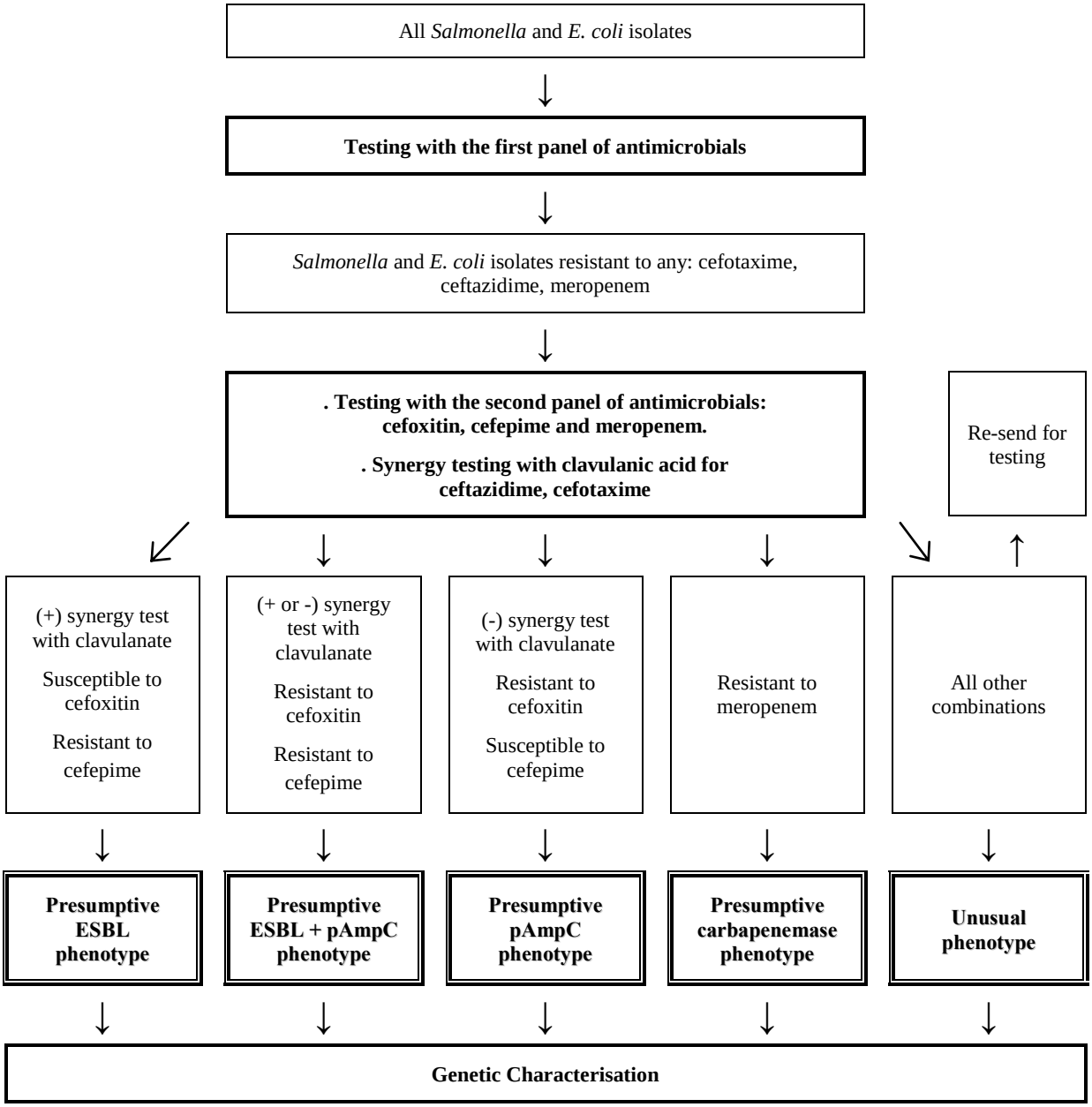


**Figure 1:** Stepwise strategy for testing isolates microbiologically resistant to ESCs or meropenem (i.e. non-wild type isolates).

#### 4.1.3.3. Synergy testing

A number of methods are available for testing for the synergistic effect of clavulanate in potentiating the action of cephalosporins against ESBL-producing organisms as described in detail in the recent EFSA opinion on extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals (EFSA Scientific Panel on Biological Hazard (BIOHAZ), 2011). Such effects are commonly detected using one of the two following methods assessing the inhibitory effect of clavulanic acid on beta-lactamase activity.

Disc diffusion methods available include pairs of discs containing cephalosporin and cephalosporin plus clavulanate. An increase in the size of the zone of inhibition is observed for the disc containing cephalosporin and clavulanate in comparison with the disc containing the same amount of cephalosporin alone.

- **Combination disc synergy method** is performed by comparing zone diameters, on the same Mueller-Hinton agar plate, around discs containing cefotaxime and cefotaxime + clavulanate as well as ceftazidime and ceftazidime + clavulanate. If the presence of clavulanate increases zone diameters by at least 5mm for either ceftazidime or cefotaxime, then the test is considered positive for the production of an ESBL.

Gradient antimicrobial strips are also commercially available containing a cephalosporin at one end and the same cephalosporin plus clavulanate at the other; a reduction in the cephalosporin MIC by the clavulanate can be used to indicate ESBL production.

- **Gradient test** containing the combination of cefotaxime + clavulanic acid and ceftazidime + clavulanic acid must be performed as recommended by manufacturer. If any of the tests show at least an 8-fold lower MIC in the presence of clavulanate, or if any phantom zone is identified around either of the strips, the test is considered to be positive for production of an ESBL.

Isolates with a suspected ESBL phenotype, or an ESBL plus an AmpC phenotype should be examined for clavulanate synergy. In the case of suspected ESBL plus AmpC phenotype, detection of the synergy with clavulanate might be more difficult to assess, but several commercial kits can provide assistance. One method to investigate ESBLs in the presence of AmpC enzymes is to look for the synergy between clavulanate and a 4$^{th}$-generation cephalosporin (cefepime) by either gradient test or combination disc synergy test. Usually, cefepime susceptibility is less affected by the production of a plasmidic AmpC than are the 3$^{rd}$-generation cephalosporins and the synergy due to the presence of an ESBL is easier to visualise. Another method can be to use AmpC inhibitors, for example agar plates containing cloxacillin (usually 250 to 300 mg/L) to perform the combination disc synergy test. The presence of cloxacillin in the culture media inhibits the activity of any produced cephalosporinase and allows visualisation of the ESBL phenotype if present. Isolates which demonstrate clavulanate synergy will be designated "presumptive ESBL" and the occurrence of clavulanate synergy (and the cephalosporin against which synergy was shown) should be recorded as part of the data collected in relation to susceptibility monitoring.

Acceptable protocols for the application of synergy tests need to be developed and agreed for use within the monitoring programme depending on the laboratory experience. Synergy testing for presumptive identification of ESBL-producing *E. coli* will be particularly important in cases where MSs are unable to perform any further genetic characterisation.

#### 4.1.3.4. Genetic characterisation

Hitherto, genetic characterisation has largely been performed by specialist NRLs for antimicrobial resistance and may not be performed in all MSs. There is a need for capacity building and training to ensure that all MSs have the expertise required to perform at least some basic genetic characterisation of isolates. Extensive examinations are possible, but the genetic examination proposed seeks to

identify, at a useful though not fully comprehensive level, the common enzymes of greatest public health importance which might be encountered.

- **ESBL phenotype**

Minimum protocols for identification need to be agreed for the genetic characterisation of isolates with an ESBL phenotype. It is suggested that CTX-M, SHV and TEM enzymes should be the main focus of investigations. For CTX-M, SHV and TEM enzymes, ideally generic polymerase chain reactions (PCRs) will be used initially to detect the presence of the particular ESBL families of importance, for example for CTX-M types there is a generic PCR to detect their presence (Saladin et al., 2002), followed by specific PCRs and gene sequencing to identify specific enzyme types.

- **AmpC phenotype**

There are a number of AmpC enzymes which may occur in *E. coli*; wild-type *E. coli* also naturally posseses a chromosomal AmpC enzyme, which is normally not expressed, but which may be expressed as a result of certain promoter mutations. Phenotypic synergy tests are available to infer the likely presence of AmpC enzymes, though it was not considered that these should be routinely used in the monitoring programme at this stage.

Considering the relative importance of AmpC and ESBL enzymes in human medicine, from a clinical perspective in a number of MSs. ESBLs appear to be the most important of the two enzymes. In view of this and also to problems related to the presence of chromosomal AmpC enzymes in *E. coli*, it is recommended that the genetic characterisation of isolates with an AmpC phenotype is limited to *Salmonella* isolates (wild-type *Salmonella* do not possess a chromosomal AmpC enzyme). AmpC enzymes in *E. coli* are also considered important in a number of MSs. Therefore it is recommended that AmpC-producing *E. coli* should also be monitored in accordance with available national resources and priorities. Examination of a subset of isolates is recommended when large numbers of isolates are obtained.

The most common AmpC gene previously detected in *Salmonella* is $bla_{CMY-2}$, and genetic investigations will be limited to *Salmonella* strains and to examination for this enzyme. AmpC *E. coli* should be monitored in accordance with national priorities and resources.

*Salmonella* isolates with an AmpC phenotype which are negative for $bla_{CMY-2}$ gene should be further examined, where possible at the NRL, the EURL, or at another suitable laboratory. Technical development is required to prioritise other families of AmpC enzymes and individual AmpC enzymes which should be included in any further expansion of the monitoring.

- **Carbapenemase phenotype**

Isolates with a carbapenemase[11] phenotype (not showing wild-type susceptibility to meropenem) should be re-tested against carbapenems to confirm such resistance, which is likely to be rare in veterinary and food isolates.

Isolates resistant to carbapenems should be further tested phenotypically using discs or gradient strips for synergy between carbapenems and EDTA (indicating a probable metallo-beta-lactamase) and for inhibition by boronic acid discs (indicating the possible presence of KPC enzymes). A detailed protocol for such investigations should be developed as part of the monitoring recommendations; metallo-beta-lactamases and KPC enzymes are considered of particular importance and the use of commercially available kits to detect these phenotypes may assist MSs in rapidly evaluating the significance of susceptibility test results as well as indicating which further genetic tests may be most appropriate.

[11] There are three broad families of carbapenemases: the KPC enzymes, the metallo-beta-lactamases, containing the enzymes IMP, NDM and VIM, and certain OXA enzymes.

Genetic characterisation of isolates should be undertaken at the NRL or the EURL.

### 4.2. Harmonised panel of antimicrobials for susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*

The antimicrobials which should be included in the panel against which *Campylobacter* spp. are tested remain unchanged and are as listed in the current recommendations, which also include the rationale for their inclusion (EFSA, 2007).

The inclusion of additional macrolides, such as azithromycin, telithromycin or clarithromycin, was considered, but since point mutations in ribosomal DNA result in cross-resistance between the different macrolides (Aarestrup and Engberg, 2001) their inclusion does not appear to provide any additional information. More recent research has also indicated that mutation at the target site on the 50S subunit ribosome confers resistance to all macrolides (Belanger et al., 2007) and that there is a high degree of cross-resistance, for example between erythromycin and telithromycin (Schonberg-Norio et al., 2006) and among erythromycin, azithromycin and telithromycin (Zhao et al., 2010), in *Campylobacter* spp. Erythromycin is included in the current panel and it is therefore considered that this substance is an adequate marker to indicate resistance to all macrolides. It is therefore considered neither relevant nor necessary to complement the current panel with additional macrolide compounds.

Amoxicillin or ampicillin is often included in national monitoring programmes for *Campylobacter* spp. (EFSA, 2007) and acquired resistance to these antimicrobials does occur (MARAN-2009, 2011; EFSA and ECDC, 2012). The EFSA's recommendations of 2007 noted that these compounds are not used for therapy of human infections with *Campylobacter* spp.and considered them as optional for monitoring at the EU level. Thus, it is considered that this position remains unchanged.

Invasive *Campylobacter* infections are uncommon in humans but when they occur, they may be treated with carbapenems, such as imipenem. If evidence emerges of carbapenem resistance in *Campylobacter* spp. then imipenem should be included in surveillance recommendations for food and animals. Currently there is no recommendation to include imipenem in routine surveillance procedures.

### 4.3. Harmonised panel of antimicrobials for susceptibility testing of *Enterococcus faecalis* and *Enterococcus faecium*

The rationale for inclusion of the current panel of antimicrobials for testing *Enterococcus faecalis* and *Enterococcus faecium* was previously described (EFSA, 2008a). No changes to those antimicrobials included in the earlier recommendations are advised, butt is recommended that the harmonised panel of substances tested is complemented with the following antimicrobials:

- **Tigecycline**. This antimicrobial is not used in animals but is considered of critical importance in human medicine. Surveillance of the occurrence of resistance to tigecycline in bacteria from animals is therefore important for the assessment of possible zoonotic risks. Because of the close relationship of tigecycline with tetracyclines, should tigecycline resistance be detected there may also be issues in relation to the degree of co-selection arising from the use of tetracyclines in food production animals.

- **Daptomycin**. This antimicrobial is not used in animals but is considered of critical importance in human medicine. Knowledge of the occurrence of resistance in bacteria from animals is therefore important for assessment of zoonotic risks.

- **Teicoplanin**. Vancomycin-resistant *E. faecium* (VRE) were previously common among farm animals, possibly resulting from the widespread use of the vancomycin analogue avoparcin as a growth promoter. When the use of avoparcin was discontinued, the occurrence of VRE in farm animals decreased, but such bacteria are still often found at a low prevalence (EFSA and ECDC,

2012). Vancomycin resistance in *E. faecium* from farm animals is almost exclusively of the *vanA* genotype. In VRE causing nosocomial infections in humans, the *vanA* gene is also common, but other variants also occur, mainly *vanB*. Knowledge of genotype is important for assessment of zoonotic risks and is obtained by testing by molecular methods. Knowledge of genotype can also be derived by determining MICs of vancomycin and teicoplanin for isolates, as shown in Table 5. Inclusion of teicoplanin in the panel for mandatory testing therefore allows the presumptive genotype of glycopeptide resistant enterococci to be deduced (EFSA, 2008a).

**Table 4:** Relationship between genotype (*van* genes) and susceptibility to vancomycin and teicoplanin in *E. faecalis* and *E. faecium*.(a)

| **Genotype**.(b) | **Range of MIC (mg/L)** | |
|---|---|---|
| | Vancomycin | Teicoplanin |
| *vanA* | 64-1000 | 16-512 |
| *vanB* | 4-1000.(c) | 0.5-1 |
| *vanD* | 64-128 | 4-64 |

(a): Adapted from Courvalin (2006).

(b): Note that many other van genotypes have been reported, but are all extremely rare, and can be identified only by genotypic methods.

(c): Note that the MIC range given includes vancomycin susceptible isolates (MIC 4 mg/L).

### 4.4. Epidemiological cut-off values

As recently pointed out (EFSA, 2012), and as discussed above, EUCAST ECOFFs values are to be used, when available, as the interpretative criteria to define microbiological resistance, thus separating the wild-type population from a population with acquired or mutational resistance towards a given antimicrobial substance. When no EUCAST data are available for a given antimicrobial substance, then criteria reviewed by the EURL-AMR might be used. If EUCAST ECOFFs are to be maintained in the legislation for the purpose of harmonisation, it is envisaged a potential issue in case their periodic update cannot be synchronised with the legislation.

### 4.5. Recommended concentration ranges to be tested

Recommendations on the optimum concentration range to be tested for each antimicrobial should take into account the feasibility and cost-effectiveness of such testing. More specifically, the space available on a 96-well plate has been considered in outlining proposals intended to favour the testing of more substances rather than extended ranges of concentrations. The proposals for the ranges to be tested should ensure that both EUCAST ECOFFs and clinical breakpoints are covered so to ensure comparability with human isolates.

In elaborating recommendations on antimicrobials and ranges to be tested in *Salmonella* and *E. coli*, it was taken into account that the same test panel could be used for both these bacterial genera. It was also considered that testing concentrations corresponding to the 'left' side of wild-type distributions (those lower than the modal concentrations) would be desirable but does not provide any additional information, since these values are meant to be constant over time. Rather it was considered more relevant to thoroughly encompass the MICs distributions of isolates with acquired reduced susceptibility.

An exception would be, for example, the susceptibility testing of ciprofloxacin. In view of the importance of fluoroquinolones in both human and veterinary medicine, both nalidixic acid and ciprofloxacin were retained in the harmonised panel of antimicrobials for mandatory testing. Additionally, information from such testing may be indicative of the presence of plasmidic resistance to quinolone antibiotics, as shown in Table 5.

**Table 5:** Relationship between various phenotypic and genotypic patterns of resistance to nalidixic acid and ciprofloxacin *Salmonella* and *E. coli*

| Genotype | | Phenotype | |
|---|---|---|---|
| **Mechanism** | **Location** | **Nalidixic acid** | **Ciprofloxacin** |
| No resistance mechanism | - | Susceptible | Susceptible |
| *gyrA* single mutation | chromosome | Resistant | Decreased susceptibility [(a)] |
| *gyrA, gyrB* multiple mutations | chromosome | Resistant | Resistant |
| *qnr* and/or *aac(6')-Ib-cr* | plasmid | Often susceptible | Decreased susceptibility [(a)] |

(a): Or "microbiologically resistant" according to the ECOFFs.

As bacteria may demonstrate a stepwise acquisition of resistance to fluoroquinolones, the determination of the ciprofloxacin MIC over a suitably wide concentration range, which should not be abbreviated, has been considered important. The recent occurrence of *Salmonella* Kentucky with high-level ciprofloxacin resistance in turkeys in some EU MSs (Wasyl and Hoszowski, 2012) underlines the potential usefulness of this measure.

### 4.6. Synoptic tables on antimicrobials, ECOFFs and concentration ranges recommended

In light of the above, proposals for the revisions of the antimicrobial substances to be used for testing of susceptibility in *Salmonella*, *E. coli*, *Campylobacter* and enterococci are presented in Tables 6-10. The proposals are mainly based on distributions of MICs available at the EUCAST website. In the tables, three different ranges are proposed for each combination of antimicrobial and bacterial species. The "*Optimum"* range is set to encompass the complete MIC distribution, including the wild-type and the subpopulation with acquired decreased susceptibility/resistance. Also the "*Advised"* range is set to give as good coverage as possible of the complete MIC distribution. This range, however, takes into account the room available on a 96-well plate by omitting the lower MICs of the wild-type but covering the MICs of the subpopulation with acquired reduced susceptibility/resistance. In the "*Minimum*" range the intention is to encompass the distribution from modal MIC of the wild-type and most of the subpopulation with acquired reduced susceptibility/resistance.

**Table 6:** Proposed set of antimicrobial substances to be included in AMR monitoring, EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints and concentration ranges to be tested in all *Salmonella* and *Escherichia coli* isolates

| **Antimicrobial** | **Species** | **EUCAST values**[a] (in mg/L) | | **Range of concentrations** (mg/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Current recommendation** | **New recommendation** (no of wells in brackets) | | |
| | | **ECOFF** | **Clinical resistance breakpoint** | | **Optimal** | **Advised** | **Minimum** |
| Ampicillin | *Salmonella* | >8[b] | >8 | 0.5–64 | 0.5–128 (9) | 1–128 (8) | 2–128 (7) |
| | *E. coli* | >8 | >8 | 1–128 | | | |
| Cefotaxime | *Salmonella* | >0.5 | >2 | 0.06–8 | 0.015–4 (9) | 0.03–4 (8) | 0.12–4 (6) |
| | *E. coli* | >0.25 | >2 | 0.015–2 | | | |
| Ceftazidime | *Salmonella* | >2 | >4 | Not included | 0.06–8 (8) | 0.06–8 (8) | 0.25–8 (6) |
| | *E. coli* | >0.5 | >4 | Not included | | | |
| Meropenem | *Salmonella* | >0.125 | >8 | Not included | 0.008–16 (12) | 0.12–0.25 (2) | 0.12–0.25 (2) |
| | *E. coli* | >0.125 | >8 | | | | |
| Nalidixic acid | *Salmonella* | >16 | – | 2–256 | 1–128 (8) | 4–128 (6) | 4–128 (6) |
| | *E. coli* | >16 | – | 1–128 | | | |
| Ciprofloxacin | *Salmonella* | >0.064 | >1 | 0.008–8 | 0.008–16 (12) | 0.008–8 (11) | 0.03–8 (8) |
| | *E. coli* | >0.064[b] | >1 | 0.004–4 | | | |
| Tetracycline | *Salmonella* | >8 | – | 0.5–64 | 0.5–128 (9) | 1–128 (8) | 2–128 (7) |
| | *E. coli* | >8 | – | 1–128 | | | |
| Colistin | *Salmonella* | >2[c] | >2 | Not included | 0.12–16 (8) | 0.5–16 (6) | 0.5–16 (6) |
| | *E. coli* | >2 | >2 | Not included | | | |
| Gentamicin | *Salmonella* | >2 | >4 | 0.25–32 | 0.25–32 (8) | 0.5–32 (7) | 0.5–32 (7) |
| | *E. coli* | >2 | >4 | 0.12–16 | | | |
| Streptomycin | *Salmonella* | >16[b] | – | 2–256 | 2–256 (8) | 2–256 (8) | 8–256 (6) |
| | *E. coli* | >16[d] | – | 2–256 | | | |
| Trimethoprim | *Salmonella* | >2 | >4 | 0.25–32 | 0.25–32 (8) | 0.25–32 (8) | 1–32 (6) |
| | *E. coli* | >2 | >4 | 0.12–16 | | | |
| Sulfamethoxazole | *Salmonella* | >256[e] | – | 8–1024 | 4–1024 (9) | 8–1024 (8) | 32–1024 (6) |
| | *E. coli* | >64[b] | – | 8–1024 | | | |
| Chloramphenicol | *Salmonella* | >16 | >8 | 2–256 | 2–256 (8) | 8–256 (6) | 8–256 (6) |
| | *E. coli* | >16 | >8 | 2–256 | | | |

(a): May 2012

(b): ECOFF changed from previous recommendation

(c): To be further evaluated due to differences between serovars of *Salmonella*. It is recommended that isolates with MIC>2mg/L for colistin in *Salmonella* spp. are evaluated at serotype level.

(d): To be further evaluated; cut-of >8 suggested by EURL

(e): EUCAST ECCOFF not defined; CLSI breakpoint (>256) advised by work group 2008

**Table 7:** Proposed panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints and concentration ranges to be used for testing only in *Salmonella* and indicator *E. coli* isolates resistant to cefotaxime or ceftazidime and/or meropenem—second panel

| Antimicrobial | Species | EUCAST values[a] (mg/L) | | Range of concentrations (mg/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Current recommendation | New recommendation (no of wells in brackets) | | |
| | | ECOFF | Clinical resistance breakpoint | | Optimal | Advised | Minimum |
| Cefoxitin | *Salmonella* | >8 | NA[b] | Not included | 0.5–64 (8) | 0.5–64 (8) | 0.5–64 (8) |
| | *E. coli* | >8 | NA | | | | |
| Cefepime | *Salmonella* | NA | NA | Not included | 0.008–8 (11) | 0.06–8 (8) | 0.06–8 (8) |
| | *E. coli* | >0.125 | >4 | | | | |
| Meropenem | *Salmonella* | >0.125 | >8 | Not included | 0.008–16 (12) | 0.003–16 (10) | 0.003–16 (10) |
| | *E. coli* | >0.125 | >8 | | | | |

(a): May 2012.

(b): Not available.

**Table 8:** Proposed panel of optional antimicrobial substances EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints and concentration ranges recommended for testing in *Salmonella* and indicator *E. coli*—third panel

| Antimicrobial | Species | EUCAST values[a] (mg/L) | | Range of concentrations (mg/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Current recommendation | New recommendation (no of wells in brackets) | | |
| | | ECOFF | Clinical resistance breakpoint | | Optimal | Advised | Minimum |
| Tigecycline | *Salmonella* | >1 | >2 | Not included | 0.06–8 (8) | 0.06–8 (8) | 0.25–8 (6) |
| | *E. coli* | >1 | >2 | | | | |
| Florfenicol | *Salmonella* | >16 | NA[b] | Not included | 2–256 (8) | 2–256 (8) | 1–128 (6) |
| | *E. coli* | >16 | NA | | | | |
| Imipenem | *Salmonella* | >1 | >8 | Not included | 0.06–16 (9) | 0.12–16 (8) | 0.12–16 (8) |
| | *E. coli* | >0.5 | >8 | | | | |
| Ertapenem | *Salmonella* | >0.06 | >1 | Not included | 0.004–2 (10) | 0.015–2 (8) | 0.015–2 (8) |
| | *E. coli* | >0.06 | >1 | | | | |
| Azithromycin | *Salmonella* | NA | NA | Not included | 1-64 (7)[c] | 1-64 (7) | 2-32 (5) |
| | *E. coli* | NA | NA | | | | |

(a): May 2012.

(b): Not available.

(c): Recommendations on ranges of concentrations are based on publication by Sjölund-Karlsson et al. (2011).

**Table 9:** Proposed panel of antimicrobial substances to be included in AMR monitoring, EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints and concentration ranges to be tested in *C. jejuni* and *C. coli*

| **Antimicrobial** | **Species** | **EUCAST values**[a] (mg/L) | | **Range of concentrations** (mg/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Current recommendation** | **New recommendation** (no of wells in brackets) | | |
| | | **ECOFF** | **Clinical resistance breakpoint** | | **Optimal** | **Advised** | **Minimum** |
| Erythromycin | *C. jejuni* | >4 | >4 | 0.5–64 | 0.25–128 (10) | 1–128 (8) | 1–128 (8) |
| | *C. coli* | >8 | NA[b] | | | | |
| Ciprofloxacin | *C. jejuni* | >0.5 | >1 | 0.06–8 | 0.06–32 (10) | 0.12–16 (8) | 0.12–16 (8) |
| | *C. coli* | >1 | >1 | | | | |
| Tetracycline | *C. jejuni* | >1 | NA | 0.12–16 | 0.25–128 (10) | 0.5–64 (8) | 0.5–64 (8) |
| | *C. coli* | >2 | NA | | | | |
| Streptomycin | *C. jejuni* | >4 | NA | 0.5–32 | 0.5–256 (10) | 1–128 (8) | 1–128 (8) |
| | *C. coli* | >4 | NA | | | | |
| Gentamicin | *C. jejuni* | >2 | NA | 0.12–16 | 0.12–16 (8) | 0.12–16 (8) | 0.12–16 (8) |
| | *C. coli* | >2 | NA | | | | |

(a): May2012.

(b): Not available.

**Table 10:** Proposed panel of antimicrobial substances to be included in AMR monitoring, EUCAST epidemiological cut-off values (ECOFFs) and clinical breakpoints and concentration ranges to be tested in *E. faecalis* and *E. faecium*

| **Antimicrobial** | **Species** | **EUCAST values**[a] (mg/L) | | **Range of concentrations** (mg/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Current recommendation** | **New recommendation** (no of wells in brackets) | | |
| | | **ECOFF** | **Clinical breakpoint** | | **Optimal** | **Advised** | **Minimum** |
| Streptomycin | *E. faecalis* | >512 | NA[b] | 8–1024 | 16–2048 (8) | 16–2048 (8) | 64–2048 (6) |
| | *E. faecium* | >128 | NA | | | | |
| Gentamicin | *E. faecalis* | >32 | NA | 4–512 | 4–1024 (9) | 8–1024 (8) | 16–1024 (7) |
| | *E. faecium* | >32 | NA | | | | |
| Chloramphenicol | *E. faecalis* | >32 | NA | 4–512 | 4–128 (6) | 4–128 (6) | 8–128 (5) |
| | *E. faecium* | >32 | NA | | | | |
| Ampicillin | *E. faecalis* | >4 | >8 | 0.25–32 | 0.25–64 (9) | 0.5–64 (8) | 2–64 (6) |
| | *E. faecium* | >4 | >8 | | | | |
| Vancomycin | *E. faecalis* | >4 | >4 | 1–128 | 0.5–128 (9) | 1–128 (8) | 1–128 (8) |
| | *E. faecium* | >4 | >4 | | | | |
| Teicoplanin | *E. faecalis* | >2 | >2 | Not included | 0.12–64 (10) | 0.5–64 (8) | 0.5–8 (5) |
| | *E. faecium* | >2 | >2 | | | | |
| Erythromycin | *E. faecalis* | >4 | NA | 0.5–64 | 0.25–128 (10) | 1–128 (8) | 2–128 (7) |
| | *E. faecium* | >4 | NA | | | | |
| Quinupristin/dalfopristin | *E. faecalis* | >16 | NA | 0.25–32 | 0.25–64 (9) | 0.5–64 (8) | 1–64 (7) |
| | *E. faecium* | >1 | >4 | | | | |
| Tetracycline | *E. faecalis* | >4[c] | NA | 0.5–64 | 0.25–128 (10) | 1–128 (8) | 1–128 (8) |
| | *E. faecium* | >4[c] | NA | | | | |
| Tigecycline | *E. faecalis* | >0.25 | >0.5 | Not included | 0.03–4 (8) | 0.03–4 (8) | 0.03–4 (8) |
| | *E. faecium* | >0.25 | >0.5 | | | | |
| Linezolid | *E. faecalis* | >4 | >4 | 0.5–64 | 0.5–64 (8) | 0.5–64 (8) | 0.5–64 (8) |
| | *E. faecium* | >4 | >4 | | | | |
| Daptomycin | *E. faecalis* | >4 | NA | Not included | 0.25–32 (8) | 0.25–32 (8) | 0.25–32 (8) |
| | *E. faecium* | >4 | NA | | | | |

(a): May 2012.

(b): Not available.

(c): ECOFF changed from previous recommendation.

## 5. Harmonised parameters for the specific monitoring of Shiga toxin-producing *Escherichia coli* and ESBLs

The aims of the monitoring of AMR in Shiga toxin-producing/verotoxigenic *E. coli* (STEC/VTEC) and the monitoring of extended spectrum beta-lactamases (ESBLs) differ strongly with respect to the objectives and needs of routine AMR monitoring.

### 5.1. Specific monitoring of AMR in STEC/VTEC

The rationale for monitoring AMR in indicator bacteria isolated from randomly selected healthy animals and food is to provide data on the pool of resistance determinants present in the commensal flora and thereby to give, in particular, an indication of the selective pressure exerted by the use of antimicrobials in food-producing animals. Monitoring AMR in STEC/VTEC is, from this perspective, a study of a particular subpopulation of intestinal bacteria. It is noteworthy that monitoring of AMR in STEC/VTEC is not included in the monitoring programmes of the USA[12] (NARMS) or Canada[13] (CIPARS).

The major threat of the STEC/VTEC species is not connected to the absence or presence of particular AMR patterns, as the pathogenicity of these strains is determined by genes coding for toxins and for the ability to adhere to the intestinal epithelium. Antimicrobials are not considered important in the treatment of STEC/VTEC infections in humans and can be contra-indicated. (EFSA, 2008c; ECDC, EFSA, EMEA and SCENHIR, 2009). A similar view is presented in a recent position paper from Die Deutsche Gesellschaft für Infektiologie although it is recognised that under certain circumstances treatment with antimicrobials can be warranted in some STEC/VTEC infections (DGI, 2011). From a clinical perspective, it is unlikely that data on AMR in STEC/VTEC strains isolated from animals and food would be of great epidemiological value.

One reason for monitoring AMR in STEC/VTEC could be to investigate linkage of virulence (i.e. shiga-toxin production) to certain resistance phenotypes. Such information could give insight in possible co-selection of virulence and resistance whereby use of a specific antimicrobial in a certain category of animals could select for STEC/VTEC. Also, wider knowledge of resistance phenotypes could be used as epidemiological typing for inference of relationships between outbreak strains. It is likely that other methods of epidemiological typing (e.g. multiple-locus variable number of tandem repeats analysis (MLVA), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST)) are better suited for this purpose.

The two outbreaks associated with STEC O104:H4 in Europe in 2011 (EFSA, 2011b) demonstrate that it is possible for epidemic STEC/VTEC strains possessing ESBL enzymes to emerge and therefore, exhibit resistance to cephalosporin and other beta-lactam antimicrobials. In such circumstances, resistance to cephalosporins may provide a useful selective criterion by which STEC/VTEC strains with ESBL resistance might be selectively recovered from the general population of *E. coli* occurring at a particular site.

### 5.2. Specific monitoring of ESBL-/AmpC-producing bacteria

#### 5.2.1. Rationale

Pathogens possessing ESBL enzymes both can and have caused significant problems for human health (EFSA, 2011a). Commensal bacteria carrying ESBL resistance also provide a reservoir of resistance genes which may enter the food chain. Transfer of ESBL genes from bacteria in broilers and broiler meat has been demonstrated (Leverstein-van Hall et al., 2011; Overdevest et al., 2011; Smet et al., 2010). Monitoring the development and spread of ESBL-carrying bacteria in the agricultural sector

[12] Available at: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm
[13] Available at: http://www.phac-aspc.gc.ca/cipars-picra/index-eng.php

will provide crucial information in designing cost-effective measures to reduce the burden of disease related to infection with ESBL-producing bacteria. Quantitatively, ESBL-producing bacteria in poultry farms seem the most prominent threat, followed by pig farms and cattle (Horton et al., 2011). In the case of cattle veal production should be preferentially emphasised in the monitoring. The situation in both animals and humans is dynamic. Monitoring of all animal production sectors is therefore considered important, especially given the considerable impact that infections caused by ESBL-producing bacteria have on human health. Because the continually evolving nature of the situation, the scope of the monitoring should be enlarged so as to also include AmpC-producing bacteria.

There are three possible broad levels of comparison which might be made between bacterial isolates of the same species detected in animals, food and humans and which show ESBL resistance: (1) the bacterium (e.g. *E. coli* sequence type); (2) the plasmid (e.g. plasmid replicon type); (3) the resistance enzyme (e.g. the ESBL CTX-M-15). When considering the significance for humans of ESBL resistance in the food chain, there can be different implications relating to the presence of either the same enzyme, the same enzyme on the same plasmid, or the same enzyme on the same plasmid in the same bacterium, being found in humans, food and animals. Therefore, it is important that the isolation methods applied to samples of food, or samples from animals or humans accurately reflect the bacterial flora in that substrate and have not (for example) artificially promoted the dissemination of resistance plasmids more widely than occurs in the natural or field situation. There is also the need to ensure that methods are sufficiently sensitive to allow detection of low numbers of ESBL *E. coli* in the sample under test.

It is important that all MSs use a single harmonised procedure to ensure that results are comparable at the EU level. The sensitivity of methods involving non-selective pre-enrichment then selective solid media can be as low as 100 cfu per gram of faeces (Randall et al., 2009). Non selective pre-enrichment is therefore considered adequate for routine surveillance purposes, although it should be realised that very low numbers of ESBL-/AmpC- producing organisms may not be detected without selective pre-enrichment (EFSA 2011a).

### 5.2.2. Discussion on different possible approaches

As explained in section 4, the phenotypic pattern of resistance in *Salmonella* and *E. coli* isolates will indicate whether they may possess an ESBL or an AmpC phenotype or whether they may be carbapenemase producers. The phenotypic tests are also indicative as to whether such isolates should be subject to further molecular characterisation. Genetic techniques are to be used to demonstrate the presence of genes coding for ESBLs and other resistances of importance. The use of WGS as a monitoring tool would automatically establish the presence or absence of ESBL genes. It is not possible to provide comprehensive and exhaustive recommendations to cover all scenarios and the scheme outlined is intended to cover what are considered to be the current issues of importance. This recommendation will need periodic revision and updating and the suggested interval for revision is at least every 3 years.

The overall aim and objective in such monitoring is to refine the information collected during current monitoring procedures in relation to ESBL and third-generation cephalosporin resistance. At the EU level, the recommended requirement (EFSA, 2007, 2008) is to determine cefotaxime resistance in the various categories of bacteria from animals and food. This allows comparison of the levels of cefotaxime resistance to be made in animal, food and human bacterial isolates, but this is rather a blunt tool, because cefotaxime resistance may be conferred by a number of different resistance mechanisms (for example ESBL or AmpC enzymes). It is considered that to maximise the useful information obtained through monitoring it is important to further classify and characterise the isolates showing resistance to third-generation cephalosporins. In any case, it is considered that the over-riding objectives are that the surveillance data produced should be comparable between MSs and that methods may need to be applicable to sectors which have a high prevalence of ESBL-producing *E. coli*, as well as those which may have a low prevalence of ESBL-producing *E. coli.* There are

various levels at which this may be done and it is possible that not all MSs will have the resources or expertise to immediately achieve this.

Monitoring for ESBL genes regardless of the bacterial species is technically feasible, but would not be very informative, particularly as many *Enterobacteriacae* species have chromosomal AmpC enzymes. Therefore, the present standard practice is to measure the abundance of ESBL-carriers in *E. coli* as an indicator organism (Woodford et al., 2011). For this reason, it is also important that isolates are presumptively identified as "*E. coli"* to a satisfactory standard. It will not be adequate to identify isolates to the level of "coliforms".

The methodological variables which may be altered when monitoring for ESBL *E. coli* include the following:

- Non-selective enrichment (for example in buffered peptone water) prior to plating on selective media. This increases recovery rates by allowing improved growth of minor components of the *E. coli* flora.
- Selective enrichment in broth containing a cephalosporin prior to plating on selective media. This will increase the sensitivity of the method.
- Use of chromogenic or other indicator media to presumptively identify *E. coli*.
- Incorporation of a selective antimicrobial agent into the agar to select for resistant isolates (for example cefotaxime).
- Incorporation of inhibitory agents into the agar to reduce the presence of undesired isolates (for example AmpC inhibitor compounds).
- Determination of presence or absence of resistant colonies.
- Enumeration of the resistant colonies and of the total population of *E. coli* so that the proportion of resistant *E. coli* can be estimated.

There are therefore a number of different approaches which can be used for processing samples taken to investigate ESBL occurrence, and there are certain advantages and disadvantages associated with these methods (Table 11). The first complementary method presented in the table is useful in situations where ESBL enzyme-carrying microorganisms are very rare. If, for example, fewer than half of the samples taken are ESBL-positive, then trends can be discerned over time. If, however, more than a few percent of the *E. coli* carry ESBL genes, then every sample is likely to test ESBL positive and the results become meaningless as regards trend analysis. In that case, the second complementary method is preferable, because it allows estimation of the percentage of *E. coli* that are ESBL-producers and in this way trends can be perceived by comparing yearly monitoring data. The third complementary method yields the most direct and therefore the most precise estimation of the percentage of ESBL-producing *E. coli*. This method requires either a high presence of these bacteria on the sample or a large number of samples. It will therefore be more suitable for poultry meat, and to a lesser extent for pork, than for beef.

**Table 11:** Advantages and disadvantages of various methods of isolation and enumeration of ESBL-producers in an EU monitoring scheme of ESBL carrying *E. coli*

| Method | Objective | Advantages | Disadvantages |
|---|---|---|---|
| **The current method (see EFSA, 2008a)** | | | |
| Examination of a single randomly- -selected *E. coli* isolate from non-selective culture plates | Estimation of the proportion of randomly selected ESBL-producing *E. coli* that are resistant | Simple<br>Comparable output across all MSs<br>The current method<br>Give access to complete (multi-)resistance profile through isolate-based data reporting | Low sensitivity, particularly where ESBL-producing *E. coli* constitute a small proportion of the total *E. coli* flora |
| **Complementary methods to monitor ESBL** | | | |
| 1. Use of selective medium that selects for ESBL-producing *E. coli* (with selective or non-selective enrichment) | Detection of ESBL within samples (determination of the proportion of samples contaminated with ESBL-producing *E. coli*) | Sensitive<br>Determine presence or absence of ESBL-producing *E. coli* within the limit of detection of the method<br>Useful where ESBL-producing *E. coli* are rare | Provide a qualitative result that may hide variability of situation and not reflect trends (towards a more dominant resistant *E. coli* population or conversely its reduction)<br>Less useful where ESBL-producing *E. coli* are common<br>This method cannot be used alone as the multi-resistance would be, in that case, investigated on a sub-population of *E. coli* |
| 2. Enumerate ESBL-producing *E. coli* and total *E. coli* colonies in the sample using dilution methods and subsequent plating onto selective media and non-selective media | Detection of ESBL + characterisation of importance within the whole *E. coli* population (enumeration) | Allows the proportion of ESBL-producing *E. coli* to be determined<br>Offer a quantitative result in addition to the detection of ESBL producers | Labour-intensive<br>Difficult to manage important numbers of samples<br>Counting methods are subject to a degree of variability<br>Rules of interpretation and reporting to be studied |
| 3. Enumerate ESBL-producing *E. coli* and total *E. coli* from direct culture onto selective media for *E. coli* | Detection of ESBL + characterisation of importance within the whole *E. coli* population (enumeration) | Allows the proportion of ESBL-producing *E. coli* to be determined<br>Offers a quantitative result in addition to the detection of ESBL producers | Bacterial load may be too high or insufficient to allow meaningful counts on selective plates<br>Less sensitive method |

### 5.2.3. Proposals for a harmonised monitoring approach

It will be important that all MSs adopt the same procedure intended to be appropriate for animals and production types of animals with a low prevalence of ESBL-producing *E. coli*, while also covering those sectors with a high prevalence of ESBL-producing *E. coli*. The monitoring scheme and sample size to be applied are the same as those suggested for indicator *E. coli* in section 3.

#### 5.2.3.1. Current procedure for *E. coli*, amended and updated

The current procedure of testing a randomly isolated commensal *E. coli* isolate recovered from non-selective media provides continuity with previous monitoring recommendations and is easily implemented by all MSs. It provides a lower degree of sensitivity than that obtained using selective media, but remains appropriate and has relevance for the assessment of risk to the consumer as it is presumed that *E. coli* will be transferred along the food chain in a random fashion (although their subsequent survival may differ). This procedure is essentially unchanged from the previous recommendations, apart from the differences relating to the antimicrobials which should be tested (cefotaxime, ceftazidime and meropenem, compared with cefotaxime only in the earlier 2007 and 2008 EFSA recommendations).

#### 5.2.3.2. Culture methods using enrichment and then selective medium for the detection of ESBL-producing *E. coli*

For animals (or production types of animals) with a low prevalence of ESBL-producing *E. coli* it is essential to use a selective medium to maximise sensitivity. There are differences in the relative importance of ESBL-producing *E. coli* and bacteria carrying AmpC beta-lactamases in the different MSs. It is considered that the method chosen should optimally detect both ESBL-producing *E. coli* and AmpC-producing *E. coli*. The advantage of this is that AmpC-producing *E. coli* are also included in the monitoring; a potential disadvantage is that where large numbers of AmpC-producing *E. coli* are present in samples they may obscure the concomitant presence of ESBL-producing *E. coli* in the same samples. For this reason, selective media have been developed for ESBL-producing *E. coli*, to ensure that they can be detected even in the presence of AmpC-producing bacteria (Randall et al. 2009). Therefore, it is suggested to use selective media in parallel to capture both ESBL- and AmpC-producing *E. coli* from the sample:

- A selective ESBL plates should be used after the enrichment stage, using a culture medium which is selective for ESBL-producing *E. coli* and inhibits AmpC-producing *E. coli*.
- A selective plate containing cefoxitin only should be used in parallel (and without AmpC inhibitors) to look for AmpC-producing *E. coli*.

The agar medium chosen may be selective for Enterobacteriaceae, and MacConkey medium has been used in previous European studies (see, for example, Girlich et al., 2007). Chromogenic media provide some advantages as *E. coli* may be presumptively identified from primary culture plates, which can be cost-effective in reducing the tests required for bacterial identification.

There are also issues regarding the cephalosporin which is chosen as the selective agent in the culture media. The same considerations regarding the susceptibility testing of *E. coli* isolates against both cefotaxime and ceftazidime, apply in relation to the inclusion of both compounds in the selective medium, because some ESBLs are ceftazidimases and some are cefotaximases. There are cost implications relating to the selective procedure which is adopted. Cefotaxime (1 mg/mL) alone has shown a good potential to detect all kind of ESBLs (including SHV and TEM variants) in different countries (i.e. in Germany, the RESET National Project www.reset-verbund.de, in The Netherlands, MARAN).

The EFSA opinion on extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals (EFSA, 2011a) recommended a selective enrichment broth to be used prior

plating on selective agar in detection of ESBL- producing *E. coli*. This selective enrichment will increase the sensitivity of the method and will enable detection of very low levels of such bacteria in the farm environment and in targeted food-production animals.

There may be some implications of using preliminary enrichment broths containing a cephalosporin *versus* non-selective enrichment using, for example, buffered peptone water in the isolation procedure. There is currently controversy concerning the relative merits of using enrichment broth containing a selective cephalosporin or non-selective broth without a cephalosporin.

It has been suggested that the use of liquid enrichment broths containing cephalosporins may enhance bacterial conjugation and exchange of resistance plasmids between bacteria. It may also be considered that allowing the susceptible background *E. coli* flora to proliferate along with the ESBL producers in a non selective broth will allow for an increased risk of conjugational transfer both in the enrichment broth (due to the high concentrations of recipient bacteria at the late stage of growth) and on the subsequent agar plates containing cephalosporins in low concentrations. There are therefore a series of options and permutations relating to the selective isolation of ESBL-producing *E. coli* which may be considered. These options are outlined in Table 12, with an indication of the rationale.

**Table 12:** Possible options on the selective isolation procedure and related rationale for the detection of ESBL-producing *E. coli*

| Procedure | Options | Rationale |
|---|---|---|
| Enrichment in liquid broth | Buffered peptone water | Non-selective broth<br>Buffered peptone water enables clones of *E. coli* initially present in low numbers in the sample to be recovered, by helping them to withstand any inhibitory effects attributable to the selective medium. |
| | Buffered peptone water plus cephalosporin | Selective liquid broth: increases sensitivity |
| Selective medium | MacConkey | Allows basic discrimination of *Enterobacteriaceae* to level of "coliforms"; further typing required to presumptively identify *E. coli.* Relatively inexpensive. Does not prevent overgrowth of, for example, AmpC-producing *E. coli.* |
| | Chromogenic agar | Presumptive identification of *E. coli*. Does not prevent overgrowth of, for example, AmpC-producing *E. coli.* |
| | Commercial chromogenic agar inhibitory to AmpC-producing *E. coli* | Enables preferential isolation of ESBL-producing *E. coli* from samples containing both ESBL-producing and AmpC-producing *E. coli* which may be important in some circumstances. |
| | The cephalosporin antimicrobial added to the selective medium - ceftazidime or cefotaxime. | Ceftazidime added to detect ESBLs which are primarily ceftazidimases, cefotaxime added to detect ESBLs which are primarily cefotaximases. |

#### 5.2.3.3. Detection methods for ESBL-carrying *E. coli*

The following two detection methods are considered the best options when monitoring of ESBL- and AmpC-producing *E. coli* in animals and food.

- Use of selective enrichment broth containing a cephalosporin followed by plating on selective agar, as proposed in the scientific opinion on ESBL-/AmpC-producing bacteria (EFSA, 2011a).

- Use of non-selective enrichment broth (buffered peptone water) followed by plating on selective agar.

In both options the enrichment is followed by inoculation to a chromogenic[14] agar plate containing cefotaxime incorporated at the ECOFF with AmpC inhibitors to look for ESBL-producing *E. coli*. Inoculation to a chromogenic agar plate containing cefoxitin incorporated at the ECOFF without AmpC inhibitors is to be performed in parallel to specifically look for AmpC-producing *E. coli*. In addition, a chromogenic agar plate containing ceftazidime at the ECOFF may be also used in option to detect enzymes which are primarily ceftazidimases. Use of cephalosporin concentrations slightly higher than the ECOFF may be recommended to reduce the numbers of non-significant organisms able to grow on the selective medium and in view of the high MICs often obtained for organisms producing ESBL enzymes. A single *E. coli* colony is selected from every plate for further characterisation, including confirmation that the organism is *E. coli* using simple biochemical tests.

The proposed options were deemed as the most adequate of the methods to be used for detection of ESBL-/AmpC-producing *E. coli*, in the light of available information. Both have disadvantages and advantages, as described earlier in this chapter. It will be important that all MSs use the same harmonised procedure so that the outputs are comparable between MSs. This will necessitate agreement on the use of the same media and isolation procedures.

The methods could be evaluated through experimental studies looking at: (1) the issue of selective and non-selective enrichment with buffered peptone water and the subsequent recovery of ESBL-producing *E. coli* (sensitivity) and (2) the effects on the degree of plasmid transfer that may occur between strains during the isolation process. It is strongly suggested that such an experimental trial should be done before the technical specifications for harmonised AMR monitoring are finalised and before the protocol of a possible EU-wide baseline survey on ESBL-/AmpC-producing *E. coli* is drafted.

#### 5.2.3.4. Quantification methods for assessing the proportion of ESBL-producing *E. coli* present

The determination of the proportion of *E. coli* which are ESBL-producing *E. coli* is recommended as an optional measure for those MSs which have detected a high prevalence of ESBL-producing *E. coli* in samples using selective media. In addition, enumeration allows the follow-up of the situation over time and enables the circumvention of any saturation effect issue associated with the use of a highly sensitive method in a high prevalence context. Such enumeration will enable assessment of temporal trends, in particular possible decreasing numbers of ESBL-producing *E. coli* after implementation of mitigation measures. Two possible enumeration methods are described in Table 11. Quantification of the AmpC-producing *E. coli* in positive samples may be also envisaged as optional.

### 5.3. Potential usefulness of conducting an EU baseline survey on ESBL-mediated resistance

In addition to the proposals presented for routine monitoring of ESBLs, it would be considered of particular relevance to obtain a clear estimate on the actual prevalence of this type of resistance, since the objective of monitoring would change according to this variable. A possible way forward could be represented by an EU baseline survey with the major objective to determine the prevalence of ESBL-/AmpC-producing *E. coli* in food producing animals at slaughterhouse and in food samples at retail. Of additional interest would be the determination of prevalence also in the environment. Additional objectives of the baseline survey were identified as follows:

- to investigate the potential risks of transfer along the food chain by typing the strains for source attribution. To do so, ideally data coming from humans (commensal and pathogenic strains) should also be used.
- to assess the relationship between data on consumption of antimicrobials at animal population level and the occurrence of ESBL-mediated resistance.
- to assess the diffusion of genes.

[14] There may be different views regarding the use of selective chromogenic media (i.e. a chromogenic medium plus additional cephalosporin) and whether this is preferable to the use of the MacConkey medium as described above.

Some data are available on the relative sensitivity of the methods that are available for use, but there are also considerable data gaps. A baseline survey would enable these questions to be addressed and an optimal procedure to be designed for monitoring at the EU level. For this reason, a baseline survey would be useful and also allow investigation of the relative merits of the methods discussed above. Moreover, regardless of the above mentioned objectives, conducting a EU-wide baseline survey wouldhave the added value of building capacity in those countries that have no or very limited experience with the monitoring of ESBLs. Moreover, it may be also desirable to utilise the opportunity provided by a baseline survey for assessment of the presence or absence of ing the carbapenemase-producing *Enterobacteriaceae* (CPE) in animals, by including a parallel selective pre-enrichment step using a carbapenem for the samples gathered in the framework of the survey.

## 6. Recommendations on the test format for the collection and reporting of data

### 6.1. Minimum requirements of Directive 2003/99/EC

The requirements for the monitoring of antimicrobial resistance by the MSs are laid down in Directive 2003/99/EC. In particular, as regards the information that must be collected by the MSs, the following categories are listed in Annex II of the Directive:

1. animal species included in monitoring;
2. bacterial species and/or strains included in monitoring;
3. sampling strategy used in monitoring;
4. antimicrobials included in monitoring;
5. laboratory methodology used for the detection of resistance;
6. laboratory methodology used for the identification of microbial isolates;
7. methods used for the collection of the data.

### 6.2. Current reporting of AMR aggregated data

The provisions laid down in EU legislation have been retained in the current technical specifications (EFSA, 2007, 2008a) issued by EFSA for the monitoring of AMR in *Salmonella* and *Campylobacter* and indicator *E. coli* and enterococci, where the seven categories mentioned above have been expanded to a full description of the elements to be included in both qualitative and quantitative tables for the reporting of aggregated data. These elements have also been implemented in the EFSA web reporting application tool that has been developed and is being used for the electronic transmission of the data from the MSs to EFSA. The data are in fact submitted in the format of tables for each given combination of bacterial species/study population (animal or food category).

In the qualitative AMR tables, for each antimicrobial tested, the following information is reported: the number of isolates tested, the number of resistant isolates, the number of fully-susceptible and number of isolates resistant to 1, 2, 3, 4 or >4 antimicrobials. In the quantitative AMR tables, for each antimicrobial tested, the relative MIC distributions are reported, as the number of inhibited isolates at the corresponding values of antimicrobial concentration. For data obtained through diffusion method, the different inhibition zone diameters are reported.

### 6.3. Collection and reporting of AMR isolate-based data

The information on multipleresistance is not accessible with the aggregated data currently reported by MSs. AMR may occur in association, meaning that an isolate may be resistant to different classes of antimicrobials simultaneously (multiresistant). Many patterns of multiresistance may be encountered within the same bacterial subtype (e.g. serovar/serotype/phagetype and biotype). Analyses on multiple resistance, specific co-resistance patterns and association between resistance traits cannot be performed on the currently available dataset deriving from aggregated data. In order to perform such

analyses information needs to be collected with a greater level of granularity, and data must be reported at the level of each isolate tested for antimicrobial susceptibility.

The collection and reporting of AMR data at the isolate level enables more in-depth scientific analysis. In particular, it would be beneficial for detecting new multi-resistance patterns and performing analysis of the known co-resistance ones, evaluating geographical progression over time, conducting retrospective analysis and assisting in source attribution. In addition, the evaluation of phenotypic resistance patterns can give insight into resistance selection, since use of one antimicrobial can select for resistance to other unrelated antimicrobials (co-selection). Therefore, the collection of data on multi-resistance is of the utmost importance for investigating the relationship between antimicrobial use and resistance.

To this end, an AMR isolate-based data model has been specifically designed and published by EFSA recently (EFSA, 2012b) and tested during a pilot phase with 12 volunteering reporting countries (EFSA, 2012c). In the pilot, which proved to be technically successful, isolate based data were used to generate XML/Excel files that were submitted by the MSs as part of their national reports. The conclusions of the pilot were that the model used would improve the collection of AMR isolate-based data and other relevant epidemiological information.

The model used for the pilot has been slightly revised and it is currently being used for the collection and transmission of isolate based data from the reporting year 2011. The components of this model are shown in Table 13.

It is also expected that transmission of data at the level of the isolates would facilitate the reporting of detailed epidemiological information, such as the serovar of the *Salmonella* strains, the geographical area and production type/food category of origin. This should also ensure consistency with the detailed recommendations recently issued by EFSA (EFSA, 2012a) as regards the way data are presented in the EU SR on AMR.

Given the public health relevance of the emergence of multi-resistant bacteria, it is strongly recommended that antimicrobial resistance data collection is performed at isolate level. Moreover, it should be noted that, in case of a switch to reporting at isolate-based level, transmission of both quantitative and qualitative data at aggregated level would become redundant since it would not provide any information in addition to that obtainable through the isolate-based data.

**Table 13:** Variables included in the data model for the transmission of isolate-based AMR data for the reporting year 2011

| |
|---|
| **General information and identification of the isolate** |
| Result Code |
| Reporting Year |
| Reporting Country |
| Language |
| **Information about type and source of samples and isolates** |
| Zoonotic agent |
| Matrix |
| **Information about the sampling performed** |
| Total number of units tested |
| Sampling stage |
| Sample type |
| Sampling context |
| Sampler |
| Program Code |
| Sampling strategy |
| Sampling details |
| Area of Sampling |
| **Information about the laboratory** |
| Laboratory Identification Code |
| Laboratory Isolate Code |
| Total number of isolates available in the laboratory |
| **Information about the sampling and testing for antimicrobial susceptibility** |
| Sampling Year/month/day |
| Isolation Year/month/day |
| Susceptibility Test Year/month/day |
| **Information about the method and the antimicrobial** |
| Method |
| Antimicrobial substance |
| Cut-off value |
| **Information about dilution method** |
| Lowest |
| Highest |
| MIC value |
| **Synergy testing** |
| Synergy testing with clavulanic acid for ceftazidime |
| Synergy testing with clavulanic acid for cefotaxime |
| **Information about diffusion method** |
| Disc content |
| Disc diameter |
| IZD value |
| **Additional information** |
| Comment |

## CONCLUSIONS AND RECOMMENDATIONS

**ToR 1. Provide detailed guidance on the monitoring of bacterial species, food animal species and/or food products and methodologies which should be considered as most relevant for antimicrobial resistance (AMR) monitoring from a public health perspective, taking into account AMR mechanisms;**

- It is acknowledged that mandatory monitoring of AMR in *Salmonella* and *Campylobacter* is already foreseen by the existing European Union (EU) legislation. Amendments are therefore proposed to include also a consistent yearly monitoring of the indicator *E. coli* (non-pathogenic) and indicator enterococci distinguishing between the species *E. faecalis* and *E. faecium*. Monitoring of indicator bacterial species is considered to be particularly useful because of the steady decline in the prevalence of *Salmonella* (thanks to the success of the existing monitoring and control programmes implemented in poultry) leading to decreased availability of *Salmonella* to be used for the monitoring of AMR, in particular in anticipation of the future analyses of the relationship between antimicrobial use and resistance.

- In setting up priorities for the monitoring of AMR from a public health perspective, the potential exposure of the consumers has been considered as the first variable to be taken into account. As a consequence of this approach, a list of combinations of bacterial species, food-producing animal populations (mainly corresponding to different production types) and food categories has been drawn up for which a consistent yearly monitoring is recommended. This list comprises:
    - *Salmonella*: in laying hens, broilers and fattening flocks of turkeys (in the framework of existing national control programmes); in fattening pigs and calves under 1 year of age and in broiler, turkey, pig and bovine meat (fresh).
    - *Campylobacter*: *C. jejuni* in broilers and broiler meat (fresh); *C. coli* in fattening pigs. Existing requirements for mandatory monitoring of *Campylobacter* in calves under 1 year of age are no longer deemed necessary on the basis of the available evidence on its actual prevalence. Similarly, monitoring in bovine meat is not recommended.
    - Indicator *E. coli* (non- pathogenic): in broilers, fattening pigs, calves under 1 year of age and in broiler, pig and bovine meat(fresh).
    - Indicator enterococci: in broilers, fattening pigs, calves under 1 year of age and in broiler, pig and bovine meat (fresh).

- Bearing in mind potential consumers' exposure, for some animal populations and their derived meat, for which consumption is typical of certain MSs but not in others, the concept of a threshold (based either on the tons of animal slaughtered or meat consumption) should be introduced to establish mandatory monitoring.

  On the basis of this approach, a list of combinations of bacterial species, food-producing animal populations and food products is proposed for which a consistent yearly (i.e. mandatory) monitoring is recommended when animal production exceeds 10 000 tons/year slaughtered. This list comprises:
    - *Campylobacter*: in fattening flocks of turkeys.
    - Indicator *E. coli* (non pathogenic): in in fattening flocks of turkeys, sheep and goats.
    - Indicator enterococci: in fattening flocks of turkeys.

  Similarly, recommendations for mandatory monitoring on a yearly basis are provided when meat consumption exceeds 10.000 tons/year. This list comprises:
    - *Salmonella*: in veal, ducks and geese meat (fresh).
    - *Campylobacter*: in turkey meat (fresh).

  - Indicator *E. coli* (non-pathogenic): in veal, turkey, sheep, goats, ducks and geese meat (fresh).
  - Indicator enterococci: in veal, turkey, sheep, goats, ducks and geese meat (fresh).

- A second list of combinations of bacterial species and food-producing animal populations has also been drawn up for which potential consumers' exposure is considered not a priority and therefore monitoring of resistance can be performed on a less intensive schedule (e.g. every third year). This list comprises:
  - *Salmonella*: in breeding flocks of *Gallus gallus* from egg and meat sectors, in breeding flocks of turkeys, in dairy cows and in young bovines (1-2 years).
  - *Campylobacter:* in calves under 1 year of age.
  - Indicator *E. coli (non-pathogenic):* in breeding flocks of *Gallus gallus* from egg and meat sectors, in breeding flocks of turkeys, in dairy cows and in young bovines (1-2 years).
  - Indicator enterococci*:* in breeding flocks of *Gallus gallus* from egg and meat sectors, in breeding flocks of turkeys, in dairy cows and in young bovines (1-2 years).

- It is acknowledged that the proposed list is not exhaustive as regards the bacterial species and that it should be broadened to accommodate recommendations on the monitoring of methicillin resistant *Staphylococcus aureus* (MRSA). This will be the focus of a separate EFSA report in the near future.

- With regard to the food categories to be included in the monitoring, the focus of this report is on fresh meat. Consideration was given to the need for inclusion of other foods, such as vegetables, but was not deemed necessary at this stage.

- For the most sensitive detection of emerging resistance and monitoring trends, the sampling would occur annually. As resources often are limited, a sampling interval of 3 years, for example, can be applied for each study population.

- In providing recommendations regarding the sampling stage, careful consideration has been given to the possibility of using samples deriving from other existing monitoring schemes, whenever possible. Thus, it is recommended that isolates from the mandatory *Salmonella* control programmes in poultry are used for the AMR monitoring. Sampling at the slaughterhouse is proposed as the recommended option for other sampling since this is considered the most cost-effective for the Member States. With regard to the sampling of food, a greater level of flexibility is offered, leaving it up to each individual MS to decide whether this should be performed at retail or at processing/cutting plant level.

- With regard to the sample size, the current value of 170 samples per year included in the existing specifications was re-assessed and confirmed after being re-calculated using a logistic regression model with bias reduction and profile likelihood confidence intervals as an improved method. Of note, the sample size of 170 refers to each individual study population.

- As regards the analytical methods to be used, it is acknowledged that molecular typing techniques are the gold standard and that their use is becoming more and more common. At this stage, however, it is deemed too premature for the Member States to abandon the currently used phenotypic monitoring to favour molecular methods.

- Standardised dilution methods are therefore recommended as the preferred methods for AMR testing as they are able to provide a semiquantitative measurement of the susceptibility in the shape of an antimicrobial concentration (expressed in mg/L) that is reproducible between different laboratories with an acceptable uncertainty (+ or – one dilution step).

- Analyses based on molecular typing techniques could be piloted in more focused settings, such as EU baseline surveys. This would allow these techniques to be applied to a small subset of selected and targeted isolates rather than in the routine monitoring.

**ToR 2. Reconsider the antimicrobials, epidemiological cut-offs values and recommended optimum concentration range to be tested at least for the combination selected under Terms of Reference 1;**

- The harmonised panel of antimicrobials, currently included in the EFSA's technical specifications in use should be retained to provide continuity of surveillance data and allow epidemiological tracing of isolates with particular patterns of resistance (particularly in relation to certain *Salmonella* serovars).

- The panel should be expanded to accommodate new substances selected on the basis of their critical role in human health, such as last resorts antimicrobials in human medicine. On the basis of this approach, the following substances are proposed for inclusion in the existing harmonised panels of antimicrobials for susceptibility testing of the following bacterial species:
    - *Salmonella* and indicator *E. coli* (non-pathogenic): colistin, ceftazidime and meropenem;
    - Indicator enterococci*:* teicoplanin, tigecycline, daptomycin;
    - *Campylobacter:* no changes are deemed necessary.

- There is growing concern about the occurrence of ESBL-mediated resistance and emergence of bacterial strains bearing carbapenemases. To this end, a strategy for phenotypic testing has been devised that would allow better characterisation of the mechanisms of resistance involved. For *Salmonella* and *E. coli* a two-step approach is proposed that foresees a further testing of those isolates showing resistance to the extended spectrum cephalosporins included in the first panel of antimicrobial substances. It is recommended that isolates resistant to either cefotaxime or ceftazidime or both are further tested for antimicrobial susceptibility with cefoxitin and cefepime to define whether they have the ESBL, pAmpC and/or carbapenemase phenotype.

- Additionally, testing of azithromycin, florfenicol and tigecycline in *Salmonella* and indicator *E. coli* is suggested as of potential scientific and public health interest, but inclusion of these two substances in the mandatory panel is not warranted at this stage.

- In the interpretation of resistance, the use of the European Committeee on Antimicrobial Suscetibility testing (EUCAST) epidemiological cut-off values is recommended, whenever available, and should be included in the EU legislation for harmonisation purposes. A periodic revision of the corresponding legislation should therefore be envisaged to ensure that updates to the values are adequately reflected in the legislation.

- When proposing amendments to the existing panel of antimicrobials, the room available in a 96-well plate has been considered. Accordingly, the proposed increase in the number of substances to be tested had to be offset by a reduction in the range of concentrations tested for some of these substances.

- To this end, proposals have been made defining optimal, advised and minimum concentration ranges to be tested. Both the EUCAST epidemiological cut-off values and the clinical breakpoints are, however, included in the minimum range, so that the data can still be analysed and also compared with human isolates. It was considered that testing concentrations corresponding to the left side of wild-type distributions (those lower than the modal concentrations) would be desirable but does not provide any additional information, since

these values are meant to be constant over time. Rather it was considered more relevant to thoroughly encompass the minimum inhibitory concentration (MIC) distributions of isolates with acquired reduced susceptibility.

- An exception would be represented by ciprofloxacin, since in the case of fluoroquinolones there was a consensus for following evolution on the fullscale of concentration ranges.

**ToR 3. Assess the need and, if considered relevant, propose harmonised parameters for the specific monitoring of Shiga toxin-producing *Escherichia coli* (STEC/VTE) and extended-spectrum beta-lactamases ESBLs;**

- Monitoring AMR in STEC/VTEC was considered a study of a particular subpopulation of intestinal bacteria in animals, and it is thought that it would not give additional information to the monitoring of randomly selected generic indicator *E. coli*.

- One reason for monitoring AMR in STEC/VTEC could be to investigate linkage of virulence (i.e. shiga-toxin production) to certain resistance phenotypes. Such information could give insight in possible co-selection of virulence and resistance whereby use of a specific antimicrobial in a certain category of animals could select for STEC/VTEC. However, it was considered that methods other than phenotypic monitoring are better suited for this purpose.

- There are several options for improved analytical methods for ESBL-/AmpC-producing *E. coli* monitoring. Each has advantages and disadvantages, and some are more suitable when the expected ESBL-/AmpC-producing *E. coli* prevalence is low whereas others are better when the prevalence is expected to be relatively high.

- Two alternative options are suggested for detection of ESBL-/AmpC-producing *E. coli* in animals and food are suggested, which differ regarding the use of selective or non-selective enrichment. It is recommended that a study comparing the usefulness of these methods for monitoring purposes should be carried out.

- It is recommended as an optional measure to carry out a parallel quantification (enumeration) of the ESBL-/AmpC-producing *E. coli* in animal populations and food sectors in which a high prevalence has been observed. Moreover, enumeration allows the circumvention of any saturation effect issue in a high-prevalence context and thus assessing temporal trends, in particular decreasing ones.

- It might be desirable to perform an EU-wide baseline survey with the major objective to assess the prevalence of ESBL-/AmpC-producing *E. coli* in animals and food.

**ToR 4. Indicate the best format for the collection and reporting of data;**

- Analyses on multi-resistance, specific co-resistance patterns and association between resistance traits cannot be performed on the currently available dataset deriving from reporting of aggregated AMR data. In order to perform such analyses, information needs to be collected with a greater level of granularity, and data must be reported at the level of each bacterial isolate tested for antimicrobial susceptibility.

- For the reporting year 2010, EFSA has successfully piloted a model for the generation of isolate-based data as XML/Excel files that were submitted by the MSs as part of their national reports. The conclusions of the pilot were that the model used would improve the collection of AMR isolate-based data and other relevant epidemiological information.

- The data model used for the pilot has been slightly revised and is currently being used on a voluntary basis by Member States for the reporting of data from year 2011.

- It is expected that transmission of data at the level of the isolates would facilitate the reporting of detailed epidemiological information and would consequently allow performance of more detailed analyses for inclusion in the EU Summary Report on AMR.

- Given the public health relevance of the emergence of multi-resistant bacteria, it is therefore strongly recommended that AMR data collection is performed at isolate level by the Member States and other reporting countries.

- Moreover, in case of a switch to reporting at isolate-based level, transmission of both quantitative and qualitative data at aggregated level would become redundant since it would not provide any information in addition to that obtainable through the isolate-based data.

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## APPENDICES

### A. OVERVIEW OF MSS REPORTING QUANTITATIVE AMR DATA FOR INCLUSION IN THE EU SUMMARY REPORTS OVER THE PERIOD 2004–2010

**Table 1A:** Overview of the number of MSs reporting quantitative data on *Salmonella*, *Campylobacter*, indicator enterococci and *E. coli* for inclusion in the EU Summary Report on Antimicrobial Resistance over the years 2004–2010

| Data on *Salmonella* | *Salmonella* spp. | | | | *S.* Typhimurium | | | | *S.* Enteritidis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2004–2007 | 2008 | 2009 | 2010 | 2004–2007 | 2008 | 2009 | 2010 | 2004–2007 | 2008 | 2009 | 2010 |
| **Diffusion methods** | | | | | | | | | | | | |
| Meat from broilers | 5 | 10 | 9 | 11 | 2 | 4 | 3 | 2 | 3 | 9 | 6 | 6 |
| Meat from turkeys | 3 | 4 | 2 | 7 | | 3 | 2 | 1 | | | 2 | 1 |
| Meat from bovine animals | 6 | 3 | 9 | 8 | 3 | 2 | 5 | 6 | 2 | 2 | 2 | 4 |
| Meat from pigs | 7 | 9 | 11 | 11 | 6 | 6 | 9 | 8 | | | 3 | 1 |
| *Gallus gallus* | 11 | 18 | 22 | 18 | 10 | 12 | 13 | 15 | 7 | 15 | 14 | 15 |
| Turkeys | 7 | 3 | 1 | 11 | 5 | 3 | 2 | 6 | 4 | | 1 | 4 |
| Pigs | 11 | 16 | 14 | 13 | 11 | 14 | 13 | 12 | 5 | 5 | 4 | 4 |
| Cattle | 9 | 10 | 10 | 13 | 8 | 8 | 10 | 10 | 2 | 1 | 4 | 1 |
| **Dilution method** | | | | | | | | | | | | |
| Meat from broilers | 7 | 2 | 2 | 1 | 4 | | | – | 7 | 2 | 1 | 1 |
| Meat from turkeys | 2 | 1 | | 1 | 1 | 1 | | | 1 | | | |
| Meat from bovine animals | 6 | 1 | | 1 | 5 | – | 1 | 1 | 2 | | | |
| Meat from pigs | 5 | 2 | 1 | 2 | 4 | 2 | 1 | 2 | 2 | | 1 | 1 |
| *Gallus gallus* | 11 | 4 | 2 | 4 | 6 | 1 | 1 | 2 | 10 | 4 | 1 | 4 |
| Turkeys | 6 | 1 | | 3 | 3 | | – | 2 | 4 | 1 | | |
| Pigs | 11 | 2 | 1 | 2 | 10 | 1 | | 2 | 6 | 1 | 1 | |
| Cattle | 11 | 2 | | 1 | 9 | 1 | | 1 | 5 | | | 1 |

**Table 1A (continued):** Overview of the number of MSs reporting quantitative data on *Salmonella*, *Campylobacter*, indicator enterococci and *E. coli* for inclusion in the EU Summary Report on Antimicrobial Resistance over the years 2004–2010

| **Data on *Campylobacter*** | ***C. jejuni*** | | | | ***C. coli*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **2004–2007** | **2008** | **2009** | **2010** | **2004–2007** | **2008** | **2009** | **2010** |
| **Dilution method** | | | | | | | | |
| Meat from broilers | 6 | 7 | 6 | 9 | 5 | 2 | 10 | 7 |
| Meat from turkeys | – | 1 | – | – | – | – | 1 | – |
| Meat from bovine animals | – | – | – | – | – | – | – | – |
| Meat from pigs | 1 | – | 1 | 1 | 1 | 1 | – | 1 |
| *Gallus gallus* | 11 | 22 | 6 | 9 | 7 | 16 | 8 | 5 |
| Turkeys | 1 | 1 | – | – | 1 | 1 | – | – |
| Pigs | 2 | 1 | 5 | 2 | 8 | 5 | 3 | 6 |
| Cattle | 6 | 5 | 4 | 6 | 5 | 2 | 7 | 5 |

**Table 1A (continued):** Overview of the number of MSs reporting quantitative data on indicator *E. faecium*, *E. faecalis* and *E. coli* for inclusion in the EU Summary Report on Antimicrobial Resistance over the years 2004–2010

| **Data on indicator bacteria** | ***E. faecium*** | | | | ***E. faecalis*** | | | | ***E. coli*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2004–2007** | **2008** | **2009** | **2010** | **2004–2007** | **2008** | **2009** | **2010** | **2004–2007** | **2008** | **2009** | **2010** |
| **Dilution method** | | | | | | | | | | | | |
| Meat from broilers | – | 1 | 1 | 2 | – | – | 1 | 2 | 2 | 3 | 3 | 2 |
| Meat from turkeys | – | – | – | – | – | – | – | – | 1 | 2 | 2 | – |
| Meat from bovine animals | – | 2 | 1 | 1 | – | 1 | 1 | 1 | 2 | 4 | 2 | 1 |
| Meat from pigs | – | 2 | 1 | 1 | – | 2 | 1 | 1 | 2 | 4 | 3 | 1 |
| *Gallus gallus* | 7 | 5 | 5 | 5 | 5 | 3 | 5 | 5 | 9 | 6 | 7 | 6 |
| Turkeys | – | 1 | – | – | – | – | – | – | – | 1 | 1 | – |
| Pigs | 6 | 7 | 6 | 6 | 5 | 4 | 5 | 6 | 10 | 7 | 7 | 6 |
| Cattle | 6 | 5 | 6 | 3 | 4 | 3 | 6 | 3 | 9 | 7 | 9 | 5 |

## B. EUROSTAT DATA ON FOOD-PRODUCING ANIMALS

**Table 1B:** Livestock in EU MSs, 1 000 heads (animals), 2011 Eurostat data

| EU MSs | Total cattle population | Bovine animals <1 year | Calves for slaughter | Other calves | Bovine animals 1–2 years | Bovine animals >2 years | Heifers | Cows | Dairy cows | Pigs | Laying hens[(a)] | Sheep | Goats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Belgium | 2 471.6 | 678.5 | 166.0 | 512.4 | 494.2 | 1 298.9 | 264.6 | 999.3 | 510.6 | 6 327.9 | :(b) | :(b) | :(b) |
| Bulgaria | 567.4 | 139.8 | 66.5 | 73.2 | 58.1 | 359.7 | 25.1 | 329.9 | 306.8 | 608.3 | 6 217.0 | 1 454.6 | 341.4 |
| Czech Republic | 1 339.5 | 392.4 | 17.3 | 375.1 | 299.3 | 647.8 | 76.2 | 556.7 | 374.1 | 1 487.2 | :(b) | :(b) | :(b) |
| Denmark | 1 612.0 | 548.0 | 268.0 | 280.0 | 306.0 | 758.0 | 67.0 | 681.0 | 579.0 | 12 348.0 | 3 900.0 | :(b) | :(b) |
| Germany | 12 527.8 | 3 851.2 | 221.1 | 3 630.1 | 2 930.6 | 5 746.0 | 783.9 | 4 873.9 | 4 190.1 | 27 402.5 | 34 036.0 | 1 657.8 | 160.0 |
| Estonia | 239.4 | 63.2 | 3.0 | 60.2 | 47.8 | 128.4 | 15.7 | 110.2 | 95.5 | 362.2 | 674.0 | :(b) | :(b) |
| Ireland | 5 925.3 | 1 868.0 | 0.0 | 1 868.0 | 1 441.9 | 2 615.4 | 287.1 | 2 118.6 | 1 055.3 | 1 552.9 | :(b) | 3 321.3 | :(b) |
| Greece | 627.0 | 169.0 | 88.0 | 81.0 | 128.0 | 330.0 | 38.0 | 272.0 | 130.0 | 1 109.0 | 11 151.9 | 8 956.0 | 4 791.0 |
| Spain | 5 923.2 | 2 078.0 | 1 378.4 | 699.7 | 774.8 | 3 070.4 | 308.7 | 2 618.8 | 798.0 | 25 634.9 | :(b) | 17 002.7 | 2 693.1 |
| France | 19 142.0 | 5 521.0 | 827.0 | 4 694.0 | 3 295.0 | 10 326.0 | 2 104.0 | 7 786.0 | 3 678.0 | 13 950.0 | 51 310.0 | 7 644.0 | 1 383.0 |
| Italy | 5 897.5 | 1 782.8 | 509.9 | 1 272.9 | 1 393.9 | 2 720.8 | 505.8 | 2 145.0 | 1 755.0 | 9 350.8 | :(b) | 7 942.6 | 959.9 |
| Cyprus | 56.9 | 21.1 | 9.5 | 11.5 | 9.9 | 26.0 | 1.7 | 24.1 | 24.1 | 438.9 | 507.4 | 355.9 | 290.3 |
| Latvia | 380.6 | 103.9 | 50.4 | 53.5 | 66.7 | 210.0 | 20.9 | 186.1 | 164.1 | 375.2 | :(b) | :(b) | :(b) |
| Lithuania | 752.4 | 200.7 | 61.4 | 139.3 | 144.1 | 407.6 | 33.3 | 367.8 | 349.5 | 790.3 | 3 823.1 | 60.4 | 15.0 |
| Luxembourg | 188.1 | 51.9 | 5.5 | 46.4 | 42.7 | 93.4 | 16.0 | 74.7 | 44.5 | 91.3 | 87.0 | :(b) | :(b) |
| Hungary | 701.0 | 189.0 | 60.0 | 129.0 | 138.0 | 372.0 | 37.0 | 328.0 | 251.0 | 3 032.0 | 12 544.0 | 1 095.0 | 81.0 |
| Malta | 15.1 | 4.6 | 0.0 | 4.6 | 3.5 | 7.1 | 0.5 | 6.4 | 6.3 | 46.3 | 314.7 | 11.9 | 4.9 |
| Netherlands | 3 912.0 | 1 581.0 | 919.0 | 662.0 | 591.0 | 1 740.0 | 112.0 | 1 611.0 | 1 504.0 | 12 103.0 | :(b) | 1 113.0 | 392.0 |
| Austria | 1 976.5 | 623.4 | 164.8 | 458.6 | 429.9 | 923.2 | 121.8 | 784.2 | 527.4 | 3 004.9 | 5 724.5 | 361.2 | 72.4 |
| Poland | 5 500.9 | 1 361.6 | 77.9 | 1 283.7 | 1 256.2 | 2 883.1 | 229.9 | 2 568.0 | 2 446.1 | 13 056.4 | 49 040.0 | 212.7 | 111.8 |
| Portugal | 1 519.1 | 462.2 | 133.1 | 329.1 | 209.5 | 847.4 | 120.3 | 683.4 | 242.0 | 1 984.5 | 8 452.0 | 2 172.5 | 417.5 |
| Romania | 2 002.4 | 482.3 | 150.9 | 331.4 | 235.6 | 1 262.1 | 105.6 | 1 131.8 | 1 118.5 | 5 404.2 | 35 602.8 | 8 498.0 | 1 313.3 |
| Slovenia | 462.3 | 146.2 | 13.3 | 132.9 | 119.1 | 197.0 | 21.2 | 170.7 | 109.1 | 347.3 | :(b) | :(b) | :(b) |
| Slovakia | 463.4 | 133.3 | 18.7 | 114.6 | 91.4 | 238.7 | 33.6 | 201.3 | 154.1 | 580.4 | 6 266.0 | 393.9 | 34.1 |
| Finland | 902.7 | 303.6 | 3.6 | 299.9 | 222.4 | 376.7 | 29.1 | 337.3 | 281.5 | 1 289.7 | :(b) | :(b) | :(b) |
| Sweden | 1 449.7 | 479.9 | 21.3 | 458.6 | 330.2 | 639.6 | 87.5 | 529.6 | 347.6 | 1 567.7 | :(b) | 622.7 | 0.0 |
| United Kingdom | 9 675.0 | 2 857.0 | 0.0 | 2 857.0 | 2 344.0 | 1 298.9 | 732.0 | 3 442.0 | 1 800.0 | 4 326.0 | :(b) | :(b) | 0.0 |

(a): 2010 data.
(b): Not available.

**Table 2B:** Slaughtered animals in MSs, 1000 tonnes carcasses, 2011 Eurostat data

| | Bovines total | Adult cattle | Bullocks | Bulls | Cows | Heifers | Calves | Young cattle | Pigs | Sheep | Lambs | Goats |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **EU MSs** | | | | | | | | | | | | |
| Belgium | 272.286 | 219.404 | 0.190 | 85.650 | 129.472 | 4.092 | 51.119 | 1.763 | 1 108.255 | 2.384 | 1.846 | 0.054 |
| Bulgaria | 5.006 | 4.096 | 0.121 | 1.123 | 2.316 | 0.633 | 0.198 | 0.712 | 48.222 | 2.392 | 2.335 | 0.022 |
| Czech Republic | 72.124 | 71.334 | 0.100 | 35.948 | 29.510 | 5.774 | 0.508 | 0.282 | 262.944 | 0.159 | 0.112 | 0.002 |
| Denmark | 133.000 | 102.200 | 3.500 | 27.700 | 59.000 | 12.000 | 0.000 | 30.800 | 1 718.400 | 1.500 | 1.300 | 0.000 |
| Germany | 1 158.337 | 1 106.108 | 9.235 | 550.882 | 396.750 | 149.242 | 44.947 | 7.282 | 5 563.640 | 20.717 | 16.024 | 0.450 |
| Estonia | 7.617 | 7.414 | 0.146 | 1.890 | 4.741 | 0.637 | 0.065 | 0.138 | 30.961 | 0.093 | 0.038 | 0.000 |
| Ireland | 546.807 | 545.489 | 209.825 | 87.419 | 106.385 | 141.862 | 0.270 | 1.048 | 233.714 | 48.124 | 41.102 | 0.000 |
| Greece | 59.231 | 47.562 | 0.174 | 33.397 | 7.171 | 6.820 | 2.206 | 9.463 | 115.121 | 71.196 | 53.884 | 33.564 |
| Spain | 605.598 | 374.790 | 2.551 | 192.902 | 94.727 | 84.611 | 15.102 | 215.706 | 3 479.470 | 131.717 | 118.434 | 10.402 |
| France | 1 559.350 | 1 333.311 | 92.867 | 386.642 | 690.747 | 163.058 | 193.787 | 32.252 | 1 998.317 | 85.324 | 70.531 | 7.359 |
| Italy | 1 009.212 | 880.434 | 6.400 | 555.311 | 139.572 | 179.154 | 117.127 | 11.651 | 1 570.225 | 32.525 | 20.971 | 1.203 |
| Cyprus | 4.816 | 3.923 | 0.000 | 2.067 | 1.404 | 0.452 | 0.028 | 0.865 | 55.213 | 2.575 | 1.952 | 2.354 |
| Latvia | 17.131 | 16.088 | 0.010 | 4.594 | 8.824 | 2.660 | 0.311 | 0.732 | 23.451 | 0.217 | 0.084 | 0.000 |
| Lithuania | 41.079 | 40.608 | 0.000 | 16.997 | 17.902 | 5.709 | 0.257 | 0.214 | 58.856 | 0.097 | 0.031 | 0.000 |
| Luxembourg | 8.880 | 8.676 | 0.336 | 3.934 | 2.614 | 1.787 | 0.104 | 0.100 | 9.504 | 0.038 | 0.032 | 0.003 |
| Hungary | 25.979 | 25.549 | 0.016 | 4.654 | 18.163 | 2.720 | 0.131 | 0.299 | 387.304 | 0.162 | 0.053 | 0.000 |
| Malta | 1.115 | 1.113 | 0.000 | 0.675 | 0.372 | 0.068 | 0.002 | 0.000 | 7.262 | 0.064 | 0.001 | 0.011 |
| Netherlands | 381.558 | 162.839 | 0.000 | 19.446 | 140.428 | 2.967 | 180.949 | 37.770 | 1 347.165 | 12.862 | 9.538 | 1.870 |
| Austria | 217.111 | 213.382 | 10.957 | 110.674 | 61.928 | 29.824 | 7.277 | : (a) | 543.771 | 7.520 | 5.100 | 0.826 |
| Poland | 379.929 | 370.411 | 0.043 | 198.614 | 124.671 | 47.083 | 9.210 | 0.308 | 1 810.778 | 0.554 | 0.288 | 0.054 |
| Portugal | 95.999 | 73.040 | 0.835 | 41.591 | 18.418 | 12.194 | 9.812 | 13.147 | 383.750 | 10.054 | 8.626 | 0.900 |
| Romania | 27.525 | 21.240 | 1.202 | 3.971 | 13.804 | 2.263 | 1.084 | 5.201 | 253.546 | 4.109 | 1.019 | 0.022 |
| Slovenia | 35.571 | 33.260 | 0.251 | 23.355 | 6.005 | 3.649 | 1.969 | 0.341 | 22.954 | 0.115 | 0.111 | 0.003 |
| Slovakia | 11.281 | 11.186 | 0.000 | 4.496 | 5.813 | 0.877 | 0.059 | 0.036 | 56.908 | 0.526 | 0.465 | 0.000 |
| Finland | 82.654 | 82.280 | 0.000 | 48.899 | 24.217 | 9.167 | 0.080 | 0.294 | 201.755 | 0.890 | 0.682 | 0.000 |
| Sweden | 147.779 | 133.448 | 14.342 | 63.518 | 42.026 | 13.562 | 4.427 | 9.904 | 256.085 | 5.068 | 4.113 | 0.008 |
| United Kingdom | 936.561 | 932.945 | | 117.847 | 192.457 | 251.971 | 3.146 | 0.470 | 806.021 | 289.318 | 237.583 | 0.188 |

(a): Not available.

**Table 2B (continued):** Slaughtered animals in MSs, 1 000 tonnes carcasses, 2011 Eurostat data

| | Poultry | Chickens | Turkeys | Ducks |
|---|---|---|---|---|
| **EU MSs** | | | | |
| Belgium | 495.795 | 487.050 | 8.528 | 0.118 |
| Bulgaria | 98.448 | 73.428 | 0.043 | 21.216 |
| Czech Republic | 170.084 | 166.636 | 0.245 | 3.123 |
| Denmark | 186.300 | 185.700 | 0.000 | 0.000 |
| Germany | 1 423.187 | 853.785 | 467.714 | 57.310 |
| Estonia | 17.415 | 17.415 | 0.000 | 0.000 |
| Ireland | 121.690 | 111.889 | 3.310 | 4.108 |
| Greece | 175.233 | 173.050 | 1.751 | 0.089 |
| Spain | 1 387.089 | 1 111.906 | 173.322 | 5.781 |
| France | 1 733.000 | 1 060.000 | 384.000 | 243.000 |
| Italy | 1 219.882 | 894.744 | 309.484 | 3.858 |
| Cyprus | 27.400 | 27.220 | 0.180 | 0.000 |
| Latvia | 22.808 | 22.807 | 0.000 | 0.000 |
| Lithuania | 75.631 | 67.943 | 4.099 | 0.001 |
| Luxembourg | 0.000 | 0.000 | 0.000 | 0.000 |
| Hungary | 383.491 | 219.828 | 82.678 | 57.224 |
| Malta | 4.155 | 4.155 | 0.000 | 0.000 |
| Netherlands | 857.248 | 840.922 | 0.000 | 16.284 |
| Austria | 110.867 | 95.064 | 15.784 | 0.017 |
| Poland | 1 384.837 | 1 046.247 | 204.150 | 14.441 |
| Portugal | 292.106 | 245.633 | 36.256 | 8.736 |
| Romania | 293.858 | 288.183 | 5.675 | 0.000 |
| Slovenia | 58.284 | 52.903 | 5.382 | 0.000 |
| Slovakia | 56.688 | 56.667 | 0.021 | 0.000 |
| Finland | 101.508 | 92.493 | 7.930 | 0.006 |
| Sweden | 119.796 | 111.528 | 3.711 | :(a) |
| United Kingdom | 1 560.122 | 1 357.004 | 170.115 | 33.002 |

(a): Not available.

# C. TECHNICAL NOTE ON THE METHOD LOGISTIC REGRESSION WITH BIAS REDUCTION AND PROFILE LIKELIHOOD CONFIDENCE INTERVALS

## 1. THE BIAS REDUCTION METHOD AND THE PROFILE LIKELIHOOD METHOD

### 1.1. Improved maximum-likelihood estimation for binomial-response data

The R function *brglm( )* in the R package *brglm* fits binomial-response generalised linear models (including logit models) using the bias reduction method developed in Firth (1993) for the removal of the leading O(1/*n*) term from the asymptotic expansion of the bias of the maximum likelihood estimator. The bias reduction method is an improvement over traditional maximum likelihood because:

- the bias-reduced estimator is second-order unbiased and has smaller variance than the maximum likelihood estimator (so smaller confidence intervals and higher power tests), and
- the resultant estimates and their corresponding standard errors are always finite while the maximum likelihood estimates can be infinite (in situations where complete or quasi-separation occurs).

In the setting retained the improved maximum-likelihood estimation on the logistic regression model with a time trend linear on the logit scale was applied.

### 1.2. Profile likelihood confidence intervals

A $(1-\alpha)100$ % confidence interval for $\theta$ can be defined by inverting the likelihood ratio test: the $(1-\alpha)100$ % confidence interval equals the set of values $\theta_0$ for $\theta$ that cannot be rejected as a null hypothesis $H_0$: $\theta = \theta_0$ at significance level $\alpha$.

In the setting applied, the parameter $\theta$ of interest is the slope parameter in the logistic regression model, and the test of interest is the test whether there is a specific increase or decrease over time (alternative hypothesis $H_1$: slope not equal to 0) or not (null hypothesis $H_0$: slope equals 0).

Profile likelihood (PL) confidence intervals have better coverage characteristics than the typical Wald type 95 % confidence intervals, the latter having the well-known generic form point estimate $\pm$ 1.96 $\times$ standard error. PL confidence intervals take asymmetry in the likelihood curve into account, whereas the Wald-type intervals are by definition symmetrical (and, as a consequence, could lead to intervals getting outside the [0,1] interval for proportions). More details are available from literature, e.g. Hudson (1971).

## 2. SAMPLE SIZE CALCULATIONS AND ALTERNATIVE DESIGNS FOR DETECTION OF TRENDS IN THE PROPORTION OF RESISTANCE

The sample size calculation leading to the sample size 170 and recommended by the technical specifications issued in 2007 and 2008 was based on the standard formulas for comparing two proportions. Also, the detection of trend calculations were based on comparison of the proportions at the start and the end of the 3-year period.

The alternative approach using a logistic regression model also allows investigation of other designs to detect trend over time, such as optimal sample size determination for every year throughout the envisaged period, rather than only having two sampling moments (at the start and at the end of the period). Within the framework of generalised linear models, such an approach allows more extensions, such as the inclusion of other covariates.

### 2.1. Methodology

The required sample size for a specific design, for a given level of significance and power for a specific design effect, is determined on simulations rather than on analytic formulas. Analytic formulas are available only for simple designs and are often based on asymptotic results. Here the simulation approach was chosen as it allows application of the improved estimation procedure based

on logistic regression with bias reduction in combination with profile likelihood confidence intervals (as described in section 1) and as it simulates the real finite sample distributional properties rather than using asymptotic formulas. More specifically:

- Power is computed for different sample sizes by simulating 1000 samples according to a particular true trend. The relative number of times the null hypothesis of no trend is rejected gives the power of the test.
- By simulating 1000 samples according the null hypothesis of no trend, it can be checked whether the test procedure respects the level of significance. It should only reject the null hypothesis of no trend in approximately 50 samples (for a 5 % level of significance).

### 2.2. Results

The same situations as those detailed in the Annex 1 of the EFSA Journal (2007), issue 16, pp. 1–46 ("Report including a proposal for a harmonized monitoring scheme of antimicrobial resistance in Salmonella in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers") were examined. They are recalled below:

- Trend 1: Starting from an initial proportion of resistance of 50 %: a detection of 5 % decrease in proportion of resistance per year, over a period of 3 years.
- Trend 2: Starting from an initial proportion of resistance of 0.1 %: an increase by 2 % per year can be detected, over a period of 3 years.

In a third section, some further alternative situations, with gradually increasing starting proportions of resistance, were also investigated.

#### 2.2.1. Trend 1: Starting from an initial proportion of resistance of 50 %: a detection of 5 % decrease in proportion of resistance per year, over a period of 3 years

The power to detect time trend 1 is calculated for different (total) sample sizes *n* and different designs. The three alternative designs shown in Table C1 were considered: a first design where half of the total number of observations are taken in year 1 and the other half 3 years later and two further designs with a growing proportion of the total number of observations taken in the two intermediate years. For illustration, Figure 1C shows a typical dataset generated under design 1.

**Table C1:** Different designs under consideration, to detect a particular time trend after a period of 3 years

| | **Year 1** | **Year 2** | **Year 3** | **Year 4** |
|---|---|---|---|---|
| **Design 1** | *0.5n* | *0* | *0* | *0.5n* |
| **Design 2** | *0.4n* | *0.1n* | *0.1n* | *0.4n* |
| **Design 3** | *0.3n* | *0.2n* | *0.2n* | *0.3n* |

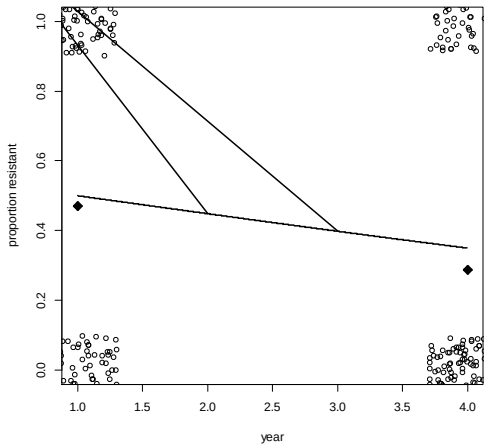


**Figure C1:** A typical dataset as generated under design 1. Half of the samples are tested in year 1 and the other half 3 years later. The 0–1 indicator values (1 for resistance) have been jittered somewhat to show the data more clearly. The resulting proportions resistant for both years are plotted as a solid dot. The decreasing line shows the true time trend which we aim to detect with power 0.80 and level of significance 0.05

Figure C2 shows, for the three designs considered, the power of the design as a function of the sample size. The horizontal dashed line indicates the envisaged power of 80 %. The sample size for which the power curve crosses the power 80 % line indicates the required sample size. It is clear that higher power is reached, and consequently a lower total sample size is needed for design 1, than for designs 2 and 3. The more evenly spread over years (from designs 2 to 3), the less power for the same total sample size. From that statistical perspective the best option is design 1. The black curve for design 1 crosses the power 80 % line at a total sample size of 340, which corresponds to a sample size of 170 at year 1 and 3 years later.

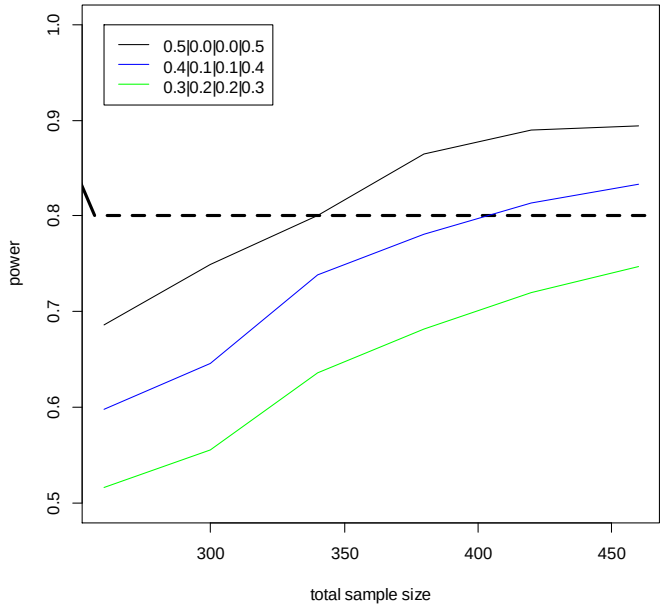


**Figure C2:** Power curves for the three designs to detect time trend 1

**Further discussion**

- Power is highest, and consequently the sample size needed to achieve a power of 80 % is smallest, for the design that maximises the variance in the scheduled time points, i.e. for the

design that puts half of the observations in year *t* and half of them in year *t + 3*. For design 1, a total of 340 observations (170 in year *t* and 170 in year *t + 3*) guarantees a power of 80 % to detect a linear decreasing trend of 5 %, from an initial proportion of 50 %. That means that in 80 % of the simulated samples with this decreasing trend, the null hypothesis of no trend was rejected.

- For design 2 with the same total sample size of 340 but consecutive sample sizes 136 (year *t*), 34 (year *t + 1*), 34 (year *t + 2*), 136 (year *t + 3*), the power decreases to 74 %. It decreases further to 64 % for design 3 with the successive yearly sample sizes of 102, 68, 68, 102.
- Roughly, for each 10 % transferred from the outer time points to the intermediate time points, the corresponding loss in power is 8–9 %.
- For design 2 a total sample size of about 400 is needed to reach a power of 80 %. For design 3 more than 500 observations are needed (a total sample size of 500 gave a power of 79 %).
- The decrease in power is moderate to substantial when sampling also in the intermediate years.
- Design 2 is to be preferred to design 3. A sampling design with sizes 160, 40, 40, 160 is sufficient to guarantee a power of 80 %, as an alternative to design 1 with sizes 170, 0, 0, 170.

**Note:** All tests are performed at the significance level of 5 %. So the probability is 0.95 that one concludes that there is no trend, in the event that there really is no trend. A simulation from a generating model with no trend confirmed that the test procedure reached the right level (e.g. 49 out of 1 000 samples falsely led to rejection in case of design 1).

### 2.2.2. Trend 2: Starting from an initial proportion of resistance of 0.1 %: an increase by 2 % per year can be detected, over a period of 3 years

For this second time trend we consider again the same three designs, as summarised in Table C1. For illustration, Figure C3 shows a randomly selected dataset generated under design 3. Note that the underlying increasing time trend (solid curve) corresponds to a total increase of 6 % (three times 2 %), but that the trend is not fully linear on the probability scale. This non-linearity is typical for very low or very high proportions. The time trend is, however, perfectly linear on the logit scale (log(proportion/(1 – proportion)), which is the most natural type of trend for binary data models.

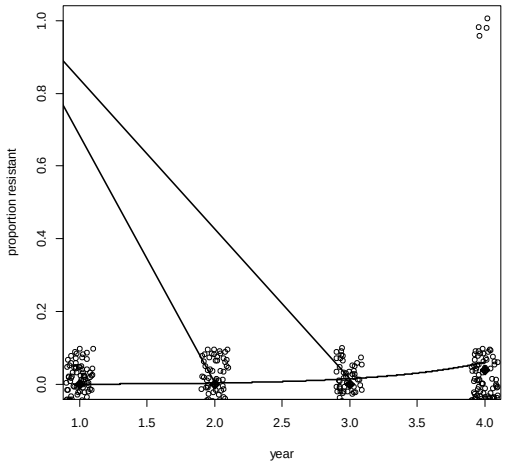


**Figure C3:** A typical dataset as generated under design 3 for time trend 2. Thirty per cent of the samples are tested in year 0, 20 % at year 1 and 2 and 30 % at year 3. The 0–1 indicator values (1 for resistance) have been jittered somewhat to show the data more clearly. The resulting proportions resistant for both years are plotted as a solid dot. The increasing line shows the true time trend which we aim to detect with power 0.80 and level of significance 0.05

Figure C4 shows the power as a function of the sample size for the three designs considered. The horizontal dashed line indicates again the envisaged power of 80 %. As for the first time trend, it is clear that higher power is reached, and consequently a lower total sample size is needed, for design 1 than for designs 2 and 3. The black curve for design 1 crosses the power 80 % line at a total sample size of 230, which corresponds to a sample size of 115 at the first year and 3 years later.

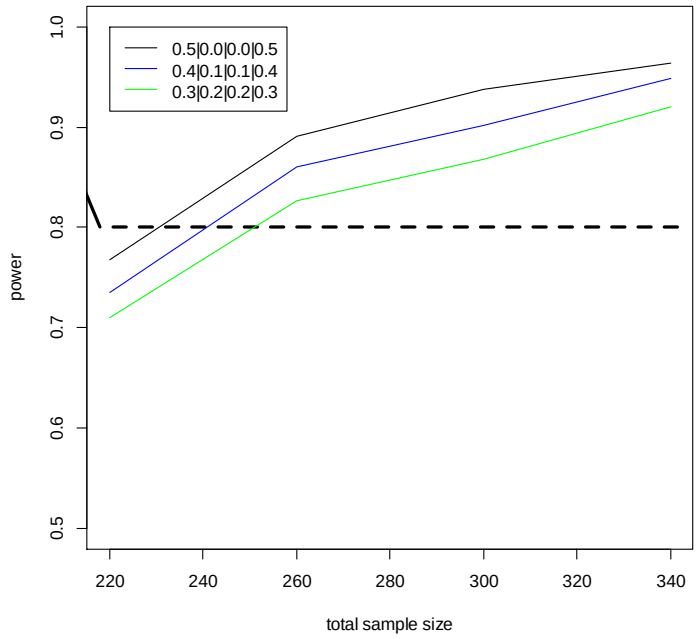


**Figure C4:** Power curves for the three designs to detect time trend 2

**Further discussion**

- Again, as in the first situation, power is highest, and consequently the sample size needed to achieve a power of 80 % is smallest, for the design that maximises the variance in the scheduled time points, i.e. for the design that puts half of the observations in year *t* and half of them in year *t* + 3. For design 1, a total of 230 observations (115 in year t and 115 in year *t* + 3) guarantees a power of 80 % to detect an increasing trend to 6.1 % after 3 years, from an initial proportion of 0.1 %.
- It was observed that the impact of the design is similar but less pronounced. Roughly speaking, for each 10 % transferred from the outer time points to the intermediate time points, the corresponding loss in power is 2–3 %.

### 2.2.3. Further alternative situations, with gradually increasing starting proportions of resistance

In this section two alternative designs are investigated in more detailed. In the first design, with a total samples size of 340, half of the samples are taken in year 1 and the other half 3 years later (earlier design 1). In an alternative design, 40 additional samples are taken in the two intermediate years, leading to a total size of 420. These designs are examined for two type of increasing trends (increase of 6 % and increase of 15 %) in combination with four levels of starting proportions.

**Table C2:** Power results for different total samples sizes corresponding to two different designs, and combinations of different starting proportions and different trends leading to different proportions after 3 years

| Starting proportion | Proportion after 3 years | Type of design | Total size | Power |
|---|---|---|---|---|
| 0.001 | 0.061 | 170\|0\|0\|170 | 340 | 0.96 |
| 0.001 | 0.061 | 170\|40\|40\|170 | 420 | 0.98 |
| 0.05 | 0.11 | 170\|0\|0\|170 | 340 | 0.52 |
| 0.05 | 0.11 | 170\|40\|40\|170 | 420 | 0.55 |
| 0.10 | 0.25 | 170\|0\|0\|170 | 340 | 0.95 |
| 0.10 | 0.25 | 170\|40\|40\|170 | 420 | 0.97 |
| 0.20 | 0.35 | 170\|0\|0\|170 | 340 | 0.89 |
| 0.20 | 0.35 | 170\|40\|40\|170 | 420 | 0.89 |

For the different levels of starting proportions and levels of increase, the power characteristics for both designs are quite similar. The design 170|40|40|170 is, however, less efficient than 170|0|0|170 as it needs a larger total size to reach the same power. This is what we expect from the results in sections 2.2.1 and 2.2.2.

**Further discussion**

- The power is very high (about 0.97 %) to detect a total increase of 6 % when the proportion resistant is very low (0.1 %) at year 1. The power drops considerably (below the desired 80 %) to detect that same increase of 6 % for the starting proportion of  %. Indeed, an increase of 6 % is *relatively* larger at the scale of 0.1 % (about 60 times) than at the scale of 5 % (about double).
- The power is very high to detect a total increase of 15 % in both starting proportions: about 96 % for starting proportion 10 % and 89 % for starting proportion 20 %.
- Both type of designs yield almost identical power results under all settings. So they can be considered as power-equivalent.

**ABBREVIATIONS**

| | |
|---|---|
| AMR | antimicrobial resistance |
| BIOHAZ | EFSA Panel on Biological Hazards |
| CIPARS | Canadian Integrated Program for Antimicrobial Resistance Surveillance |
| EC | European Commission |
| ECDC | European Centre for Disease Prevention and Control |
| ECOFF | epidemiological cut-off value |
| EFSA | European Food Safety Authority |
| EMA (or EMEA) | European Medicines Agency |
| ESBL | extended-spectrum beta-lactamase |
| ESC | extended-spectrum cephalosporin |
| ESVAC | European Surveillance of Veterinary Antimicrobial Consumption |
| EU | European Union |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| EURL | European Union Reference Laboratory |
| Eurostat | Statistical Office of the European Communities |
| EUSR | European Union Summary Report |
| IZD | inhibition zone diameter |
| MIC | minimum inhibitory concentration |
| MRSA | meticillin (or methicillin)-resistant *Staphylococcus aureus* |
| MS | Member State |
| NARMS | National Antimicrobial Resistance Monitoring System |
| NRL | National Reference Laboratory |
| PCR | Polymerase Chain Reaction |
| SCENHIR | European Commission's Scientific Committee on Emerging and Newly Identified Health Risks |
| STEC | Shiga toxin-producing *Escherichia coli* |
| ToR | Term of Reference |
| VRE | vancomycin-resistant *Enterococcus faecium* |
| VTEC | vero(cyto)toxigenic *Escherichia coli* |
| WG | Working Group |
| WGS | Whole Genome Sequencing |
| XML | eXtensible Markup Language |