Article

Antimicrobial resistance monitoring and surveillance in the meat chain: A report from five countries in the European Union and European Economic Area

Mc Nulty, Karl, Soon, Jan Mei, Wallace, Carol Anne and Nastasijevic, Ivan

It is advisable to refer to the publisher's version if you intend to cite from the work.
/10.1016/j.tifs.2016.09.010

For more information about UCLan's research in this area go to
http://www.uclan.ac.uk/researchgroups/ and search for <name of research Group>.

For information about Research generally at UCLan please go to
http://www.uclan.ac.uk/research/

CLoK
Central Lancashire online Knowledge
www.clok.uclan.ac.uk

Accepted Manuscript

Antimicrobial resistance monitoring and surveillance in the meat chain: A report from five countries in the European Union and European Economic Area

Karl Mc Nulty, Jan Mei Soon, Carol Anne Wallace, Ivan Nastasijevic

Please cite this article as: Nulty, K.M., Soon, J.M., Wallace, C.A., Nastasijevic, I., Antimicrobial resistance monitoring and surveillance in the meat chain: A report from five countries in the European Union and European Economic Area, *Trends in Food Science & Technology* (2016), doi: 10.1016/ j.tifs.2016.09.010.

1 **Antimicrobial resistance monitoring and surveillance in the meat chain: A report from**

2 **five countries in the European Union and European Economic Area**

3 Karl Mc Nulty[a], Jan Mei Soon[b], Carol Anne Wallace[b], Ivan Nastasijevic[c]

4 [a]School of Tourism, Letterkenny Institute of Technology, Shore Road, Killybegs, Co,

5 Donegal, Ireland

6 [b]International Institute of Nutritional Sciences and Applied Food Safety Studies, School of

7 Sport and Wellbeing, University of Central Lancashire, Preston, Lancashire PR1 2HE, United

8 Kingdom

9 [c]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

10 **Abstract**

11 Background

12 The emergence of antimicrobial resistance (AMR) in zoonotic foodborne pathogens

13 (*Salmonella, Campylobacter*) and indicator microorganisms (*E. coli*, enterococci) is a major

14 public health risk. Zoonotic bacteria, resistant to antimicrobials, are of special concern

15 because they might compromise the effective treatment of infections in humans.

16 Scope and approach

17 In this review, the AMR monitoring and surveillance programmes in five selected countries

18 within European Union (EU) and European Economic Area (EEA) are described. The

19 sampling schemes, susceptibility testing for AMR identification, clinical breakpoints (clinical

20 resistance) and epidemiological cut-off values (microbiological resistance) were considered

21 to reflect on the most important variations between and within food-producing animal

22 species, between countries, and to identify the most effective approach to tackle and manage

23 the antimicrobial resistance in the food chain.

24 Key findings and conclusions

25 The science-based monitoring of AMR should encompass the whole food chain, supported

26 with public health surveillance and should be conducted in accordance with `Zoonoses

27 Directive` (99/2003/EC). Such approach encompasses the integrated AMR monitoring in

28 food animals, food and humans in the whole food (meat) chain continuum, e.g. pre-harvest

29 (on-farm), harvest (in abattoir) and post-harvest (at retail). The information on AMR in

30 critically important antimicrobials (CIA) for human medicine should be of particular

31 importance.

32 **Keywords: antimicrobial resistance, foodborne pathogens, monitoring, surveillance,**

33 **public health.**

34

35 **1. Introduction**

36 In the last decade the antimicrobial resistance (AMR) associated with zoonotic foodborne

37 pathogens of bacterial origin is recognized as a major public health concern. Zoonotic

38 foodborne bacteria are infectious agents which may be transferred from animals to humans

39 via food ingestion (WHO, 2015). Zoonotic agents are believed to be responsible for up to

40 75% of infectious diseases in humans (Heymann, 2004; Behravesh et al., 2012). Therefore,

41 food-producing animals (cattle, sheep, pigs and poultry) are of particular importance for

42 emergence and transfer of AMR through the food consumption taking into consideration the

43 intensive, on-farm production practice frequently associated with misuse/overuse of

44 antimicrobials (Bischt et al., 2009).

45 It is well known that from 1940's, introduction of antibiotics to treat infectious diseases in

46 humans and animals revolutionized medicine. When it comes to food animals, antibiotics are

47 used not only to treat them against infectious diseases but also to prevent disease

48 development (metaphylaxis) and to promote their growth. However, the overuse and misuse

49 of antibiotics in food animals can lead to selective pressure on microorganisms and may

50 result in development and spread of antibiotic resistance (Cogliani et al., 2011). The first

51 integrated analysis on antimicrobial consumption in veterinary and human medicine at the

52 level of European Union (EU) and European Economic Area (EEA) was conducted in 2015

53 (ECDC/EFSA/EMA, 2015); the report aimed to provide better insight to the occurrence of

54 antimicrobial resistance in bacteria originated from humans and food animals. The excessive

55 veterinary use of antimicrobials applicable mainly for food-producing animal species,

56 including horses, in 26 European Union and European Economic Area countries was

57 estimated to be in total 7,982 tonnes per year, with the highest level of antimicrobial

58 consumption in pigs, cattle and poultry; additionally, the overall quantity of antibiotic

2

59 consumption in humans was in total 3,399.8 tonnes per year. Evidently, a significant amount

60 of antimicrobial agents per year are consumed in both, food animals and humans in EU and

61 EEA countries. Such practice may have important consequences for public health, as it may

62 promote development of antibiotic-resistant bacteria and transfer of resistance genes to

63 humans (WHO, 2011a). Nowadays, this causes serious treatment failures or necessitate the

64 use of second-line antimicrobials for therapy, more severe and longer-lasting disease,

65 increased hospitalization rates, including increased mortality, sequelae, and ultimately, higher

66 costs to society (WHO, 2011a). Having in mind the complexity of the international food trade

67 characterized with a longer food supply chain, as well as possibility for transfer of foodborne

68 pathogens from one country/continent to another within a short period of time, antibiotic

69 resistance became a growing international health issue; it deserves immediate attention by

70 health, veterinary, food and environmental authorities on the global scale.

71 Antimicrobial resistance associated with major zoonotic foodborne pathogens (*Salmonella*,

72 *Campylobacter*) occurring in food animals can spread to people via food/water consumption

73 and direct animal-human contact. In addition, commensal bacteria (e.g. *E. coli*, enterococci),

74 can also form a reservoir of resistance genes in environment, farm and food animals (Barton,

75 2000). This may facilitate transfer between bacterial species, including the transfer to

76 pathogens capable of causing disease in both humans and animals which may be difficult to

77 cure (EFSA, 2008).

78 In the European Union (EU) and European Economic Area (EEA), AMR became a very

79 serious public health challenge. The magnitude of the problem is highlighted by the fact that

80 more than 25 000 people die each year from infections caused by antibiotic resistant bacteria

81 (ECDC/EMEA, 2009). The resistance rate to antibiotics is high among both, Gram-positive

82 and Gram-negative bacteria that cause serious infections in humans and reaches 25% or more

83 in several EU Member States (ECDC/EMEA, 2009). The ineffective antibiotic treatments

84 result in extra healthcare costs and productivity losses of at least EUR 1.5 billion each year.

85 In addition, there is a gap between the burden of infections due to multidrug-resistant bacteria

86 and the development of new, effective antibiotics to tackle the problem. There are numerous

87 studies to highlight the problem related to AMR and to identify the sources and causes for

88 development of this phenomenon, but it is still uncertain how much it can be contributed to

89 the food chain, in particular meat chain.

90 The aim of this paper was to review the AMR monitoring and surveillance schemes in five
91 selected EU and EEA countries with focus on the contribution of the meat chain to
92 emergence, development and spread of antimicrobial resistance to humans. An overview of
93 the sampling schemes, susceptibility testing for AMR profile identification, clinical
94 breakpoints (clinical resistance) and epidemiological cut-off values (microbiological
95 resistance) were considered, including the most important differences between and within
96 food-producing animal species, between countries, and identification of the most effective
97 risk mitigation strategies to tackle the antimicrobial resistance in the meat chain.

98   Table 1. Summary of selected studies linking AMR to the meat chain

Authors	Type of article	Research focus	Module in the meat chain				
			1	2	3	4	5
Andersen et al. (2006)	Journal Article	Antimicrobial resistance among *Campylobacter* jejuni from raw poultry meat in retail in Denmark				X	
DANMAP (2014)	Scientific Report	Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark	X	X	X	X	X
ECDC (2014)	Summary Report	The European Union Summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014	X	X	X		X
EFSA (2014)	Scientific Report	Technical specifications on randomised sampling for harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria	X	X	X	X	
Gallay et al. (2007)	Journal Article	*Campylobacter* antimicrobial resistance among humans, broiler chickens and pigs. France		X			X
Leegard et al. (2000)	Journal Article	Emerging antimitotic resistance in *Salmonella*					X

typhimurium in Norway

			1	2	3	4	5
Lindmark et al. (2004)	Journal Article	Genetic characterisation and antimicrobial resistance of *Campylobacter jejuni* isolated from meats, water and humans in Sweden				X	X
MARAN (2013)	Scientific Report	Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2012	X	X	X	X	
NORM-VET (2013)	Scientific Report	Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway	X	X	X		X
RESAPATH (2012)	Scientific Report	French Surveillance network for antimicrobial resistance in pathogenic bacteria from animal origin	X				
SVARM (2014)	Scientific Report	Consumption of antibiotics and occurrence of antibiotic resistance in Sweden	X	X	X		X

99   Modules: 1 = Farm; 2 = Abattoir; 3 = Meat Processing; 4 = Retail; 5 – Consumers

6

100 A literature review was performed by analysing published scientific papers and the major

101 sources of information originated from the scholarly databases such as Web of Science,

102 EBSCO and ScienceDirect. The official web-sites of selected national monitoring and

103 surveillance schemes were also analysed, including the European Antimicrobial Resistance

104 Surveillance Network (EARS-Net) and Antimicrobial Consumption Interactive database

105 (ESAC-Net). This review identified relevant articles (research and review papers, technical

106 reports by international organizations) and databases, published in domains of zoonotic

107 foodborne pathogens and related antimicrobial resistance, including the public health impact.

108 The selection criteria chosen to identify the relevant articles within the scope of this review

109 and the objectives of this paper were as follow: 1) focus on the specific AMR monitoring and

110 surveillance programmes with well-established databases regarding meat chain-associated

111 antimicrobial resistance; 2) focus on the potential for improvement of harmonization of

112 national monitoring and surveillance systems and future research. However, some

113 geographical restrictions were taken, by including selected countries with intensive

114 experience and well-established AMR monitoring and surveillance programmes. Therefore,

115 monitoring and surveillance programmes on antimicrobial usage and antimicrobial resistance

116 of the major zoonotic foodborne pathogens with public health importance (*Salmonella,*

117 *Campylobacter*) and indicator bacteria (*E. coli*, *Enterococcus* spp.) were reviewed in four EU

118 Member States (MSs) (Denmark, Sweden, France and Netherlands) and one EEA country

119 (Norway) (Table 2).

7

120 Table 2. Monitoring and surveillance programmes of four selected EU member states and 1 EEA country

Programme	Type of surveillance			Country	Source
	animals	food	humans		
Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)	x	x	x	Denmark (EU)	www.danmap.org
French surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin (RESAPATH)	x		x	France (EU)	www.resapth.org https://www.anses.fr/en/thematique/veterinary-medicine-anmv
Monitoring of Antimicrobial Resistance and Antibiotic Usage in the Netherlands (MARAN)	x	x	x	The Netherlands (EU)	http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/Central-Veterinary-Institute.htm
Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM)	x		x	Sweden (EU)	http://www.sva.se/en/antibiotika/svarm-reports
Norwegian Surveillance System for Antimicrobial Drug Resistance (NORM/NORM-VET)	x	x	x	Norway (EEA)	www.vetinst.no/eng/Research/Publications/Norm-Norm-Vet-Report

2. AMR status in the EU and EEA

Antimicrobial resistance is a serious public health threat in Europe. For invasive bacterial infections, prompt treatment with effective antimicrobial agents is especially important and this is usually the single most effective intervention to reduce the risk of fatal outcome. Ongoing increase of antimicrobial resistance in invasive bacterial isolates according to the report by European Antimicrobial Resistance Surveillance Network / EARS-Net (EARS, 2014) to a number of key antimicrobial groups (3rd and 4th generation of cephalosporins, fluoro- and other-quinolones, glycopeptides, macrolides and ketolides), as well as penicillin's and aminoglycosides to a certain degree (WHO, 2011b), is of great concern and should be considered as the highest priority. The antimicrobial resistance situation in Europe shows large variations depending on the bacterium, antimicrobial group and geographical region (ECDC, 2014). These variations between the EU Member States (MSs) and EEA countries (Norway, Iceland and Switzerland) might be also due to the lack of uniformity in sampling schemes, laboratory methods used for identification of AMR profile, approach regarding clinical breakpoints (clinical resistance) and epidemiological cut-off values (microbiological resistance), as well as defined priorities regarding public health impact. To overcome this issue, the `Zoonoses Directive` (EU, 2003a) was issued, to support the harmonization of national monitoring and surveillance schemes for foodborne diseases, including AMR. The importance of protecting human health against diseases and infections transmissible directly or indirectly between animals and humans (zoonoses) was stressed, including foodborne zoonoses. It implies that EU MSs shall ensure that integrated data on the occurrence of zoonoses and zoonotic agents and related antimicrobial resistance in animals, food and humans are collected, analysed and published without delay (Figure 1). However, up to the time of writing this article, substantial differences exists between the MSs regarding specific aspects in implementation of national monitoring and surveillance systems for zoonotic foodborne pathogens and AMR, which create certain difficulties in interpreting and extrapolating data between MSs.

148
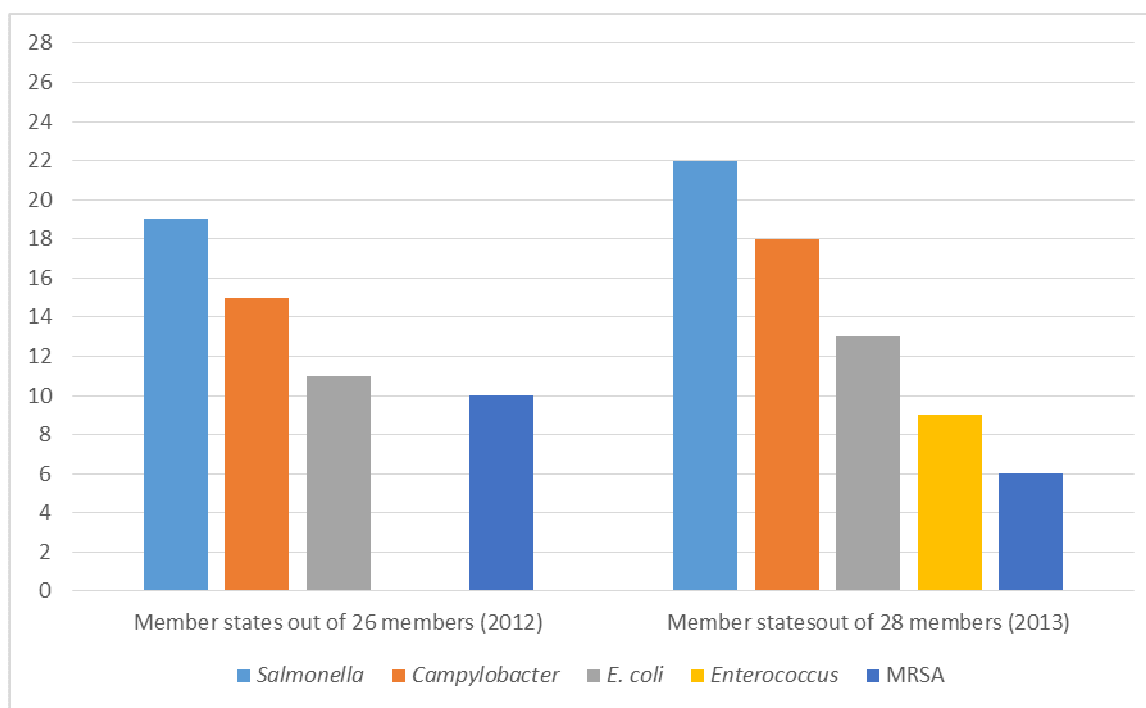
Figure 1. Number of Member States' submissions of antimicrobial resistance in zoonotic (*Campylobacter*, *Salmonella,* MRSA) and indicator bacteria (*E. coli*, *Enterococcus*) in animals, food and humans (Adapted from EFSA/ECDC, 2015)

2.1. AMR in Humans

The first concerns about antimicrobial resistance in humans were raised in Denmark, in 1994 and 1995, due to usage of the growth promoting antimicrobial (avoparcin). This led to the occurrence of vancomycin resistant *Enterococcus faecium* in humans (Bates et al., 1994; Aarestrup, 1995). During this period of time, there was also a growing awareness and a general public concern about overuse of antibiotics in Danish pig and poultry production and the effects on antimicrobial resistance.

In France, a comprehensive study was conducted to define the antimicrobial profiles and patterns related to *Campylobacter*-associated infections in humans and to compare this with *Campylobacter* isolated from broiler chicken and pigs (Gallay et al., 2007). The database originated from 1986-1990 was compared with trends from 1999-2004; it was reported that resistance to nalidixic acid increased dramatically (3 fold), while the patterns of resistance to quinolones and fluoroquinolones for *C. jejuni* were similar between 1999 and 2004, in human

166 and broiler isolates. Skurnik et al. (2006) carried out a study to determine the level of

167 antimicrobial resistance in *E. coli* of animal faecal origin in several animal populations with

168 different exposure to human contact (wild animals, farm animals and pets). It was proven that

169 occurrence of antimicrobial resistance in *E. coli* isolated from animal faecal material

170 happened due to anthropogenic influence. Obviously, the emergence, development and

171 spread of antimicrobial resistance is a dynamic process flowing into both directions -

172 zoonotic impact (animal/food-human) and anthropogenic (humans-animals). French Agency

173 for Food, Environmental and Occupational Health & Safety released a report on the usage of

174 colistin (ANSES, 2015), an antibiotic used in veterinary medicine (in livestock), which is also

175 of the highest importance in human medicine. Due to its toxicity, colistin is only prescribed

176 for the treatment of severe human infections involving bacteria resistant to all other

177 therapeutic options (including bacteria resistant to last-generation cephalosporins and

178 carbapenems). Initially, it was considered that colistin, because of the absence of any

179 mechanism for transferring resistance to this antibiotic between bacteria, shouldn`t be

180 included in the list of critically important antibiotics used in veterinary medicine. However,

181 in 2015, the first transferable mechanism for resistance to colistin (the *mcr-1* gene) was

182 described in China in pigs and chickens, in meat sold at retail, and also among bacterial

183 strains isolated in humans. European Medicines Agency recommended additional monitoring

184 of off-label use of colistin and restrictions on indications to therapy or metaphylaxis and

185 removing all indications for prophylactic to minimise any potential risk associated with a

186 broader use (EMA, 2016); consequently ANSES revised its risk assessment and included the

187 colistin in the list of veterinary antibiotics of critical importance.

188 In Netherlands, the epidemiological link of antimicrobial resistance between animals and

189 humans was investigated in an integrated study carried out by van den Boggard and

190 Stobberingh (2000); it was concluded that use of antibiotics in food animals may provoke the

191 emergence and dissemination of resistant bacteria. It is observed that the level of resistance of

192 pathogenic foodborne bacteria (*Salmonella*, *Campylobacter*) and commensal bacteria (*E. coli*,

193 *Enterococcus*) increases after the introduction of antibiotic. It is known that commensal

194 bacteria are a reservoir of resistance genes for pathogenic (foodborne) bacteria. Their level of

195 resistance may serve as a good indicator for selection pressure from antibiotic usage and for

196 prediction of resistance in pathogens. Monitoring of resistance in indicator bacteria

197 (*Escherichia coli* and *enterococci*) in different ecological compartments, e.g. in environment

198 (manure, water, feed), animals, food of animal origin (meat), patients and healthy humans,

11

199 should provide valuable data on resistance prevalence and facilitate the understanding of the

200 resistance transfer from animals to humans and vice versa.

201 In a study carried out in Sweden from 2000-2004, genetic characterization of *Campylobacter*

202 isolates associated with antimicrobial resistance was conducted to provide better

203 understanding of epidemiological link between AMR in humans, meats and water. This study

204 confirmed the link between meat consumption and antimicrobial resistance in humans and

205 also enabled focusing on identification and eradication of the major reservoirs with common

206 clones of the public health importance (Lindmark et al., 2004).

207 In Norway, the study carried out from 1975-1998, revealed the emergence of multi-resistant

208 *Salmonella Typhimurium* DT104 isolates collected from humans; the first multi-resistant

209 isolate appeared in 1994, while in 1998 already 23% of domestically acquired isolates were

210 multi-resistant (Leegard et al., 2000).

211 Significant increase in the rate of gram-negative microorganisms isolated from humans

212 (blood and cerebrospinal liquor), as well as foodstuffs had been observed in EU, from 2011-

213 2014 (ECDC, 2014). Additionally, a possible relationship between antimicrobial usage in

214 food animals and the occurrence of AMR in humans was conducted (ECDC, 2015).

215 It is estimated that 11,381.8 tonnes of active substance with antimicrobial effect was used in

216 humans and food animals in 26 EU/EEA countries in 2012 (ECDC/EFSA/EMEA, 2015)

217 (Figure 2).
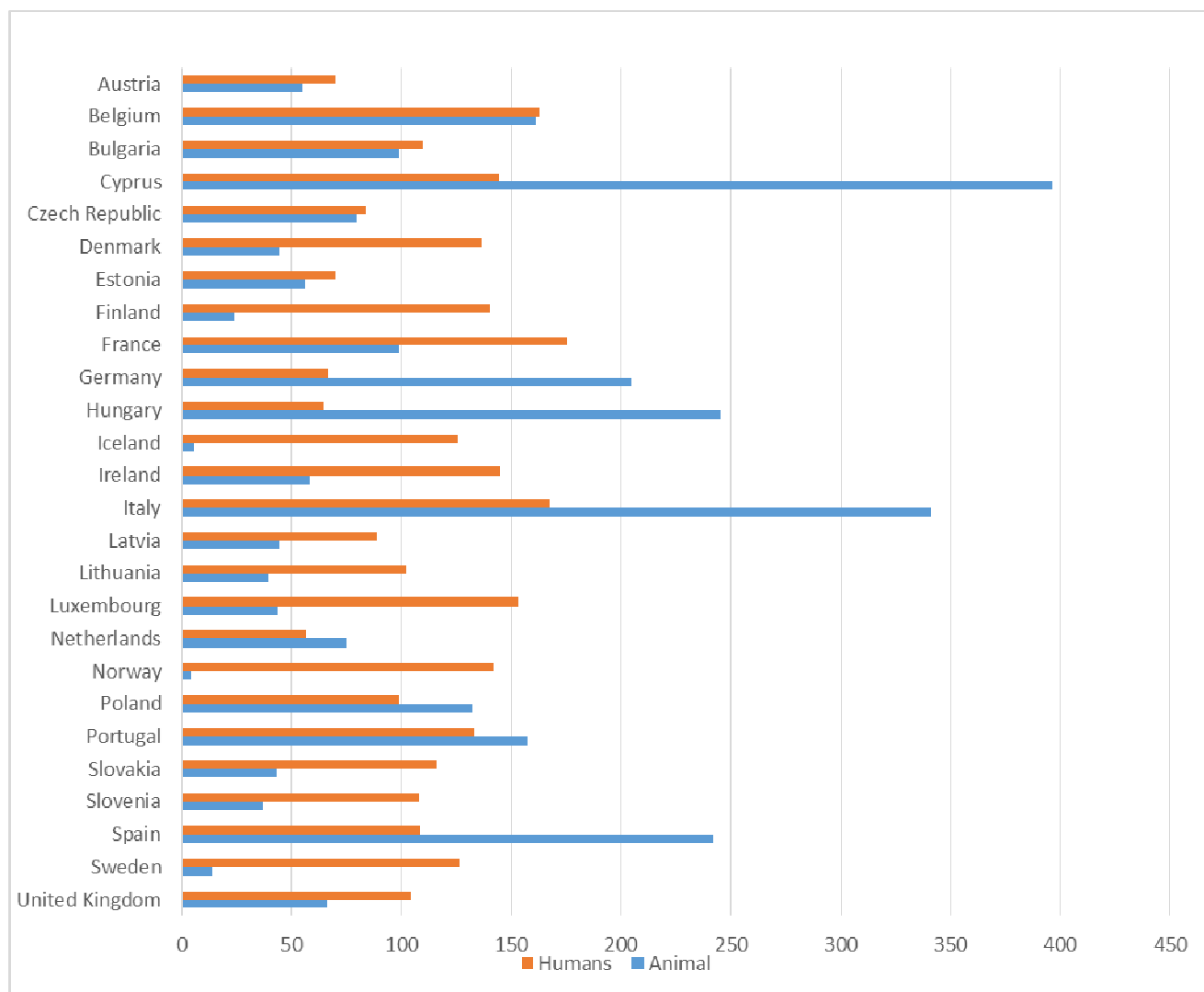
Figure 2. Comparison of biomass-corrected consumption of antimicrobials (mg/kg) in humans and food-producing animals by 26 EU/EEA countries in 2012 (Adapted from ECDC/EFSA/EMEA, 2015)

A resistance to third-generation cephalosporin's in *Klebsiella pneumoniae* and *Escherichia coli* increased significantly at EU/EEA level as well as in many of the individual MSs. 3rd generation cephalosporin resistance was often associated with fluoroquinolone and aminoglycoside resistance. Resistance trends in gram-positive bacteria showed a more diverse pattern across Europe. The percentage of EU/EEA population from which the methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated, continued to decrease over the last four years, from 18.6 % to 17.4 % in 2011 and 2014, respectively. The significantly increasing four-year trend for vancomycin resistance in *Enterococcus faecium* (commensal microorganism) was observed from 2013. EU data regarding AMR for *Salmonella* in humans indicated increased resistance associated with ampicillin, cefotaxime, chloramphenicol,

13

232   ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulphonamides,

233   tetracycline's and trimethoprim. The AMR reported for *Campylobacter* was mainly

234   connected with amoxicillin, ampicillin, ciprofloxacin, erythromycin, gentamicin, nalidixic

235   acid and tetracycline (EFSA, 2011). These findings are closely related to the prevailing use of

236   certain class of antibiotics in selected EU and EEA countries (Table 3).

237 Table 3. Most commonly used antimicrobials in selected EU/EEA countries

Country	Programme	Cattle	Pigs	Poultry	Combined cattle, pigs and poultry	Food producing animal consumption in tonnes active (ECDC/EFSA/EMEA, 2015)
Denmark	DANMAP	1. Penicillin's b-Lactase sensitive 2. Tetracycline's 3. Sulphonamides and Trimethoprim	1. Tetracycline's 2. Penicillin's b-Lactase sensitive 3. Macrolides	1. Tetracycline's 2. Macrolides 3. Penicillin's (others)	N/A[**]	107 tonnes
France	RESAPATH	N/A[*]	N/A[*]	N/A[*]	1. Tetracycline's, 2. Sulphonamides, 3. Penicillin's,	761.5 tonnes
The Netherlands	MARAN	1. Penicillin's 2. Combinations 3. Tetracycline's	1. Tetracycline's 2. Penicillin's 3. Trimethoprim/ Sulphonamides	1. Macrolides / lincosamides 2. Quinolones 3. Polymixins	N/A[**]	245.7 tonnes
Sweden	SVARM	N/A[*]	N/A[*]	N/A[*]	1. Benzyl penicillin 2. Sulphonamides 3. Tetracycline's	10.6 tonnes
Norway	NORM-VET				**1.** Penicillin's **2.** Sulphonamides **3.** Aminoglycosides	7.1 tonnes

238 * Breakdown of antimicrobials for individual species unavailable
239 **Breakdown of antimicrobials for combined species unavailable

240

241   2.2. AMR in Food (Meat) Animals

242 Development and increase of AMR in humans has a connection with antibiotic use in another

243 ecological compartment – food animals. Therefore, the Member States (MSs) of the EU

244 followed a monitoring system since 2003 (EU, 2003a; Directive 2003/99/EC that sets rules for

245 monitoring on AMR and provides Member States, a. to ensure that monitoring provides

246 comparable data on the occurrence of AMR in zoonotic agents and b. to assess the trends and

247 sources of AMR in their territory). In 2013, based on the proposals issued by EFSA, the

248 European Commission put forward and discussed with the MSs a new legislation on the

249 harmonised monitoring of antimicrobial resistance in zoonotic (*Salmonella*, *Campylobacter)*

250 and commensal bacteria (*Escherichia coli* and *Enterococcus* spp.) in food-producing animals

251 and food; a list of combinations of bacterial species, food producing animal populations and

252 food products was defined, panel of antimicrobials and tests to be used are recommended and

253 priorities for the monitoring of antimicrobial resistance from a public health perspective were

254 set up (EU, 2013; Commission Decision 2013/652/EC on the monitoring and reporting of

255 antimicrobial resistance in zoonotic and commensal bacteria). Such approach should provide

256 better consistency between EU MSs, regarding sampling, method of susceptibility testing and

257 reporting, as well as improve the comparability of the data generated among MSs.

258 A comprehensive study of AMR in bacteria isolated from food animals to antimicrobial

259 growth promoters and therapeutic agents was carried out in Denmark, in 90's (Aarestrup et

260 al., 1998). The acquired resistance to all used growth promoting antimicrobials was

261 confirmed, with most frequent occurrence of resistance observed to avilamycin, avoparcin,

262 bacitracin, flavomycin, spiramycin, tylosin and virginiamycin. The occurrence of resistance

263 varied according to animal origin and bacterial species. The highest levels of resistance were

264 observed among indicator bacteria (enterococci), while less resistance was observed among

265 pathogenic zoonotic bacteria (*Salmonella, Campylobacter*). Similarly like in other EU MSs,

266 the thermo-tolerant *Campylobacter* was the most commonly reported pathogen associated

267 with gastrointestinal bacterial infections in humans. Broilers are identified as the primary

268 source of infection, though other sources may also exist, e.g. water from untreated water

269 sources and other infected animals. The particular resistance found in *C. jejuni* isolates was to

270 ciprofloxacin and nalidixic acid. Among the *Salmonella* isolates (*S. Typhimurium* and *S.*

271 *Derby*) from healthy Danish pigs, relatively high levels of resistance (34% - 49%) were

272 observed to ampicillin, sulphonamide, and tetracycline (DANMAP, 2014). In indicator

bacteria (enterococci), a high level of resistance in *Enterococcus faecalis* isolated from broilers was observed to tetracycline (49%), followed by erythromycin (27%) and chloramphenicol (2%). Parallel to that, a very high occurrence of resistance to tetracycline (83%) and moderate to high occurrence of resistance to erythromycin (49%) and chloramphenicol (24%) was found in *E. faecalis* isolates from pigs. The occurrence of resistance to tetracycline has increased over the last five years, which may lead to the increase of potential risk of spreading the antimicrobial resistance, via horizontal gene exchange, to other pathogenic bacteria (DANMAP, 2014).

In France (RESAPATH, 2012) it is estimated that the resistance level in *S. Typhimurium* isolated from cattle is very high, especially to amoxicillin (89%), tetracycline (92%) and sulphonamides (72%). The resistance level in *E. coli* isolated from pigs was extremely high to amoxicillin (97%), gentamycin (94%), tetracycline (98%), enrofloxacin (94%) and trimethoprim-sulphonamides (97%). In hens and broilers, the extreme level of resistance in *E. coli* was confirmed to amoxicillin (98%), ceftiofur (97%), gentamycin (96%), tetracycline (98%), flumequine (97%), enrofloxacin (97%) and trimethoprim-sulphonamides (97%).

In Netherlands, the antimicrobial resistance detected in *S. Typhimurium* was predominantly associated with pigs, but was also found (although less predominant) in cattle and poultry. Resistance of *S. Enteritidis* was mainly present in poultry and more specifically in laying hens and contaminated eggs, while resistance in *S. Dublin* was observed mainly in cattle (MARAN, 2013).The highest resistance levels of *C. jejuni* isolated from poultry were observed for tetracycline and the quinolones (ciprofloxacin and nalidixic acid) raising a public health concern, and much lower in isolates from laying hens. However, resistance to macrolides, e.g. erythromycin, the first choice antibiotic in human infections (critically important antibiotic), was still low. This is in line with finding that macrolide resistance was not detected in *C. coli* from pig meat. Surveillance in indicator bacteria (*E. coli*) showed resistance to ampicillin, tetracycline's, sulphonamides and trimethoprim and it was commonly detected in broilers, turkey, pigs and veal calves. Although resistance to fluoroquinolones decreased, it was still commonly present in indicator *E. coli* from poultry sources. The promising results were reported regarding resistance to 3rd generation cephalosporins (critically important antibiotics) which was low in most animal species. Susceptibility testing of enterococci is considered of lesser priority than *E. coli* and from 2013 and onwards poultry, pigs and cattle are sampled every three years instead of annually (MARAN, 2013).

17

306 In Sweden, the majority of submissions for testing on antimicrobial resistance originated
307 from clinical samples associated with diseased animals. Therefore, data may be biased taking
308 into consideration the samples from treated animals or from herds where antibiotic treatment
309 is common, versus clinically healthy animals where antimicrobial treatments were rare.
310 Isolates are classified as susceptible or resistant by Epidemiological Cut Off Values
311 (ECOFFs) issued by European Committee of Antimicrobial Susceptibility Testing
312 (EUCAST). In *E. coli*, clinical samples from pigs, taken on-farm (faeces) or post-mortem
313 (faecal material from intestines), the resistance to streptomycin (50%), trimethoprim-
314 sulphamethoxazole (46%), ampicillin (40%) and tetracycline (25%) was the most common
315 trait. Multi-resistance occurred in 42% (50/118) of the isolates in 2014, which is higher than
316 in previous years (38% in 2013, 24% in 2012, 25% in 2011, 15% in 2010, 19% in 2009 and
317 14% in 2008). The reason for this increase remained uncertain. In *E. coli* samples obtained
318 from cattle (calves no more than a few weeks old, when the resistance in enteric bacteria is
319 usually high) during the period 2012-2014, resistance was higher than in previous years for
320 streptomycin (42%), tetracycline (31%) and ampicillin (24%). Multi-resistance occurred in
321 76% (22/29) of the isolates from 2014, compared to 70% in 2013, 50% in 2012 and 40% in
322 2007-2011. In broilers, laying hens and turkeys, the occurrence of ESBL-producing *E. coli*
323 from faeces and environment is monitored and the epidemiology of this resistance is studied.
324 The majority of isolates (75%) were susceptible to all antibiotics tested (SVARM, 2014).
325 In Norway, the situation regarding antimicrobial resistance to *Salmonella* spp. in food
326 animals is very good since those animal populations are almost free from *Salmonella* spp.
327 To maintain this favourable situation, Norway runs an extensive surveillance programme that
328 covers both live animals (cattle, pigs and poultry) and meat samples (NORM-VET, 2013).
329 However, in 2013, the resistance to fluoroquinolones was found in *S. Virchow* from pig,
330 while the multi-resistant *S. Typhimurium* was isolated from one pig herd (resistance to
331 tetracycline, ampicillin, sulfamethoxazole and streptomycin). The isolates of *Campylobacter*
332 *jejuni* in broilers were obtained from caecal samples and all broiler flocks slaughtered before
333 50 days of age were tested for the presence of *Campylobacter* spp. In 2013, one *C. jejuni*
334 isolate per positive flock (total of 96 flocks) was submitted for susceptibility testing. The
335 highest rate of resistance was detected for fluoroquinolones (ciprofloxacin [5.2%], nalidixic
336 acid [5.2%]), tetracycline (3.1%) and streptomycin (2.1%). These findings confirmed that the
337 prevalence of antimicrobial resistance among *C. jejuni* isolates from Norwegian broilers is
338 low. This is also in line with common practice in Norwegian poultry flocks where therapeutic
339 use of antimicrobial agents in broilers is relatively low and the products applicable for such

340 use contain either amoxicillin or phenoxymethylpenicillin; nalidixic acid is not used in

341 poultry at all. *Escherichia coli* and *Enterococcus* spp. are monitored as indicator bacteria. *E.*

342 *coli* isolates were obtained from samples from a total of 204 layer flocks and 131 turkey

343 flocks; the highest resistance was found to tetracycline (12.8% and 7%, respectively),

344 ampicillin (9.2% and 12.8%, respectively), sulfamethoxazole (11.3% and 9.2%, respectively),

345 trimethoprim (5.9% and 3.7%, respectively) and streptomycin (4.3% and 4.6%, respectively).

346 It is known that acquired resistance to cephalosporins among gram negative bacteria (e.g. *E.*

347 *coli*) has called on special attention in recent years. Production of extended-spectrum beta-

348 lactamases (ESBLs) or transferable AmpC are major mechanisms behind such resistance

349 (Babic et al., 2006). ESBL producing *E. coli* were not detected in any of the 204 samples

350 taken from layer flocks, indicating prevalence below 1.8%. However, the results from the

351 broiler production revealed very high resistance to 3rd generation cephalosporin's (43%). In

352 *E. faecalis*, the resistance was determined from samples taken from layers and turkey; the

353 highest level of resistance was found in tetracycline (31.5% and 41.5%, respectively),

354 erythromycin (10.1% and 18.2%, respectively), bacitracin (3.3% and 18.2%, respectively)

355 and narasin (1.1% and 12.1%, respectively).

356 *2.2.1. Meat/Meat products.*

357 The occurrence of antimicrobial resistance associated with bacteria found on/in meat/meat

358 products was investigated in many studies carried out in European countries.

359 In Denmark, Andersen et al. (2006) conducted a study to determine the antimicrobial

360 resistance of *Campylobacter jejuni* in raw poultry meat at retail level. The highest level of

361 resistance was reported to tetracycline, nalidixic acid and ciprofloxacin, while low resistance

362 was observed to macrolides (antibiotics important for human health). Wielinga et al. (2014)

363 conducted a study to evaluate the evidence-based policy to control antimicrobial resistance in

364 the food chain. They investigated the conflict of interest between the major stakeholders from

365 agriculture, veterinary, health and commercial level and concluded that success of the

366 national surveillance and monitoring programmes can be only achieved if all stakeholders,

367 from farm-to-fork, are involved.

368 In France, Granier et al. (2011) conducted a review to assess AMR in *Listeria*

369 *monocytogenes*, in food and environmental isolates, from 1996 to 2006. More than two

370 hundred strains were collected and selected on the basis of a unique pulsed-field gel

electrophoresis (PFGE) profile. Half of the strains were isolated from food samples and a quarter from food processing plants. Out of the total number of isolates, 20% belonged to meat (pork, 10%; poultry, 5%; and beef, 5%) while other originated from dairy and sea products. Resistance to erythromycin, tetracycline-minocycline, and trimethoprim was reported. Further, a comprehensive one-year study was carried out to establish prevalence and characterization of *Campylobacter jejuni* in retail chicken meat in French outlets (Guyard-Nicodeme et al., 2015). *Campylobacter* was detected in 76% of collected samples and resistance to tetracycline was the most common (53.6%), followed by ciprofloxacin (32.9%) and nalidixic acid (32%). All tested isolates were sensitive to erythromycin, chloramphenicol and gentamycin.

In Netherlands, Bruin et al. (2010) reported on prevalence and quantity of highly resistant *Enterobacteriaceae* (HRE), including ESBLs, in retail meat. The tested retail meat samples were chicken (52%), beef (29%), pork (9%), and other sources (9%). The ESBL producing *E. coli* was recovered from 18% of tested samples and all ESBL positive samples were chicken (34% positive). Resistance levels were very high to ampicillin (98%) and amoxicillin/clavulonic acid (80%), and low to cotrimoxazole (7%), gentamicin (5%), while resistance wasn`t observed to piperacillin/tazobactam, meropenem and ciprofloxacin. Since majority of tested chicken meat samples were ESBL positive it is concluded that chicken meat is a potential source of pandemic ESBL producing *E. coli* in the community and hospitals. Overdevest et al (2011) also confirmed the high prevalence of ESBL producing *E. coli* in retail chicken meat (79.8%). Genetic analysis showed that the predominant ESBL genes in chicken meat and human rectal swab specimens were identical. These findings implied that the role of ESBLs in chickens and its possible transmission to humans should be further investigated and clarified. Since it is well-known that restrictive use of antibiotics may result in lower resistance rates, Van der Broucke-Grauls (2014) speculated how powerful restrictive use should be to minimize the rise of antimicrobial resistance? The author gives an opinion that the resistance to antimicrobials in the future will slowly continue to rise, in spite of restricted use of antimicrobials since recently. It was concluded that the emergence of antimicrobial resistance is clearly of multi factorial nature and it is still uncertain what are the main contributors leading to this phenomenon. In Netherlands, a movement toward lower antibiotic use in animal husbandry already started. The use of 3rd generation cephalosporins was completely stopped in broilers and pigs, in March 2010. The promising results were reduction in resistance in *E. coli* from chicken, pigs, and calves. The future will bring the

404 answer whether this change is sufficient to slow down the rising resistance in humans (Van
405 der Broucke-Grauls, 2014).

406 In Sweden, Ge et al. (2003) conducted a study to determine antimicrobial resistance in retail
407 chicken meat. They reported that around 94% of tested meat samples were contaminated with
408 *Campylobacter* strains that were resistant to at least one of seven antimicrobials in the panel.
409 The resistance to tetracycline was the highest (82%), followed with doxycycline (77%),
410 erythromycin (54%), nalidixic acid (41%) and ciprofloxacin (35%). Egervarn et al. (2014)
411 studied the prevalence of *E. coli*, with transferable ESBL and AmpC beta-lactamases, and
412 *Salmonella* on meat imported into Sweden (imported pork, beef and broiler meat). The
413 authors highlighted that increased occurrence of *Enterobacteriaceae* (including *E. coli*) with
414 transferable ESBL/AmpC beta-lactamases in humans may be linked with food (meat)
415 producing animals. The prevalence of ESBL/AmpC-producing *E. coli* was 2-13% in pork
416 meat, 0-8% in beef and 15-95% in broiler meat. Interestingly, the highest prevalence of
417 ESBL/AmpC-producing *E. coli* was reported in South American broiler meat (95%),
418 followed by broiler meat from Europe, (excluding Denmark) (61%) and from Denmark
419 (15%). The results of the study implicated that meat imported into Sweden may present a
420 significant source of human exposure to ESBL/AmpC-producing *E. coli*. This is particularly
421 important since the ingestion of this organism by consumers may lead to transfer of resistance
422 genes (bla$_{CTX-M-2}$ and bla$_{CTX-M-8}$), via conjugation, to another bacterium, including those with
423 human pathogenic potential. Yavari (2012) carried out a comprehensive review in Sweden,
424 selected European countries and USA on antibiotic resistance in *Salmonella enterica*,
425 emphasizing the role of food animal control. A success of national monitoring and
426 surveillance programme for control of AMR in Sweden is a consequence of efficient policy
427 towards controlling the antibiotic resistance by effective management and regular prevention
428 programs, and controlling different ecological/production compartments such as feed, food
429 animals and humans. Such policy also resulted in effective collaboration of different
430 organization in Sweden and led to decrease in the consumption of antibiotic in animals.
431 Subsequently, low consumption of antibiotics in animals and humans led to the low
432 prevalence of *Salmonella*. The success of any disease control program lies in the
433 effectiveness and intensity of inter-sectoral cooperation. The communication between
434 veterinary organizations and health care providers is essential to exchange the knowledge and
435 relevant information. The international collaboration is also needed to achieve more effective
436 control over spread of salmonellosis and to target antibiotic resistance (Yavari, 2012).

437 In Norway, Mo et al. (2016a) reported that *E. coli* resistant to extended-spectrum

438 cephalosporins was found in broiler production and consequently in broiler meat, in spite of

439 the restrictive policy indicated that the usage of antimicrobials is rare. The isolates from

440 intestinal microbiota of broilers and from chicken meat in retail were compared to establish

441 the epidemiological link via clones and resistance plasmids. Interestingly, it was revealed that

442 clonal expansion via horizontal transfer, supported with stability of plasmid containing

443 bla$_{CMY-2,}$ is maintained and disseminated within the broiler farms in Norway despite the

444 absence of selective pressure due to low use of antimicrobials. In subsequent study Mo et al.

445 (2016b) investigated the risk factors for occurrence of cephalosporin-resistant *E. coli* in

446 Norwegian broiler flocks. The authors concluded that implementation of a high level of

447 biosecurity is of crucial importance for decrease in the occurrence of cephalosporin-resistant

448 *E. coli* in broiler flocks. The most important biosecurity risk factors were to minimize the

449 number of people entering the broiler house during production cycles, as well as rigorous

450 cleaning and disinfection routines between production cycles. These measures could result

451 with decrease of resistance only if there is no selection pressure from antimicrobial use in the

452 broiler production.

453

454 2.3. Sampling plans

455 Monitoring of antimicrobial resistance in EU MSs should be based on isolates obtained from

456 clinical samples regularly submitted to a diagnostic laboratory or on actively collected

457 isolates from healthy or diseased animals and meat products in all production stages: 1) pre-

458 harvest (farm), 2) harvest (abattoir) and 3) post-harvest (retail) (EFSA, 2008, 2014b). The

459 selection of isolates from clinical infections usually depends on the submission of samples

460 taken on farm from local veterinarian, while sampling at slaughterhouse and retail will

461 usually depend on regular visits by competent authority according to the national plan for

462 AMR monitoring and surveillance.

463 *2.3.1. Pre-harvest (on farm)*

464 The objective of AMR monitoring is to collect and test for antimicrobial susceptibility of at

465 least 170 representative *Salmonella* spp. isolates obtained respectively from the populations

466 of laying hen flocks, broiler flocks and fattening turkey flocks in the MS, on a yearly basis

467 (*Salmonella* National Control Programme/NCP); the sampling should be carried out either by

468 the Competent Authority (CA) or under its supervision, by the Food Business Operator

469 (FBO). In addition, FBO should take the responsibility to submit for susceptibility testing the

470 *Salmonella* strains which are randomly selected and originate from different (positive) flocks

471 and, optimally, from different farms.

472 Two sampling approaches are suggested: 1) a stratified sampling strategy, e.g. proportional

473 allocation within a sampling frame of *Salmonella* spp. strains deriving from the isolate

474 collections available from the official laboratories and/or other laboratories designated by the

475 CA, and 2) a simple random sampling (SRS), e.g. within the sampling frame of flocks

476 involved in the NCP and which have tested positive for *Salmonella*. It is suggested to design

477 the sampling plan as a quarterly SRS of the flocks tested positive for *Salmonella*.

478 *2.3.2. Harvest (at abattoir)*

479 The objective is to collate and test for antimicrobial susceptibility of at least 170

480 representative isolates of *Salmonella* spp. obtained respectively from carcasses of broilers,

481 fattening turkeys, fattening pigs and bovines under 1 year of age. A collection of

482 representative caecal samples (the number to be determined in each MS according to the

483 estimation of the annual production) should be conducted to obtain isolates as follows: *E. coli*

484 from broilers, fattening turkeys, fattening pigs and bovines under 1 year of age;

485 *Campylobacter jejuni* from broilers and fattening turkeys; and isolates of Extended Spectrum

486 Beta-Lactamase (ESBL)-/AmpC-/carbapenemase-producing *E. coli* from broilers, fattening

487 turkeys, fattening pigs and bovines under 1 year of age. Under voluntary basis, the isolates of

488 *E. faecium* and *E. faecalis* (indicator organisms) may be also taken from broilers, fattening

489 turkeys, fattening pigs and bovines under 1 year of age, as well as isolates of *Campylobacter*

490 *coli* from broilers and fattening pigs.

491 *2.3.3 Post-harvest (retail meat)*

492 The objective is to collect 300 representative random samples of fresh meat of broilers, pig

493 meat and bovine meat, respectively and to test them for the presence of ESBL-/AmpC-

494 /carbapenemase-producing isolates of *E. coli*. In case a MS has a lower level of meat

495 production on a yearly basis, e.g. production of less than 100 000 tonnes of poultry meat per

496 year, less than 100 000 tonnes of pig meat per year and less than 50 000 tonnes bovine meat

497 per year, 150 samples of fresh pig, bovine and broiler meat should be tested at retail, instead

498 of 300 samples. A `retail` means an outlet selling directly to the final consumer for domestic

499 consumption, e.g. outlets/supermarkets, specialist shops and markets, but excluding catering

500 activities, restaurants and wholesalers.

501 The sampling design is based on a proportionate stratified sampling scheme at the MS level.

502 The samples are allocated proportionally to the size of the human population in the regions

23

503 accounting for at least 80 % of the national population. At the second level, the sampling

504 should be conducted at retail outlets. At the third level, samples within the different meat

505 categories should be selected. The 300/150 samples (of each meat category) should be

506 allocated in proportion to the size of the human population.

507 In Denmark, sampling for *Salmonella* spp. includes isolates from healthy pigs (caecum

508 samples) and pork (carcass swabs) collected at abattoirs as part of national surveillance and

509 control programmes, as well as from human cases. The structured surveillance programme of

510 antibiotic resistance to *Salmonella* in Danish pigs and pork started from 2011. *Salmonella*

511 isolates from broiler, layer hens and cattle farms, as well as isolates from other types of meat

512 (Danish and imported) are not presented. Interestingly, the monitoring and surveillance plan

513 include only resistance among *S. Typhimurium* since the numbers of poultry flocks and meat

514 samples infected or contaminated with *S. enteritidis* decreased over the last ten years

515 (DANMAP, 2014). For *Campylobacter*, randomly collected samples are taken from broilers

516 and cattle at slaughter and from fresh broiler meat ready for retail. Isolates from human cases

517 originate from three out of five geographical regions in Denmark. The results for resistance

518 profile of *Campylobacter jejuni* in Denmark indicated that 85-95% of the human

519 campylobacteriosis cases are caused by *C. jejuni*. For Enterococci, a random collection of

520 *Enterococcus* isolates from healthy pigs and broilers at slaughter (*E. faecalis* only*)* and from

521 domestic fresh broiler meat, pork and beef sold at wholesale and retail outlets (both *E.*

522 *faecalis* and *E. faecium*) was conducted. Enterococci (*E. faecalis)* from imported broiler

523 meat, beef and pork were also included. Only one isolate per farm or meat sample is included

524 in the final report. There are no specific sampling plans for testing of Extended Spectrum

525 Beta-Lactamase (ESBL)-/AmpC-/carbapenemase-producing *E. coli* from broilers, fattening

526 turkeys, fattening pigs and bovines.

527 In France, the collection of samples for AMR survey in bacteria isolated from the food chain

528 is carried out by the French Agency for Food Safety (AFSSA, Paris). To assess a risk for

529 emergence and dissemination of antimicrobial resistance between ecological compartments,

530 and consumers, the sampling is conducted in animals, food and environment. The collection

531 of samples is carried out in such a way that data may be compared between these

532 compartments, at national and international level. Two types of epidemiological surveillance

533 networks have been set up. The first type is based on gathering *Salmonella* zoonotic strains in

534 AFSSA where they are systematically tested for their antimicrobial susceptibility (Martel et

24

535 al., 2000). *Salmonella* strains isolated from environment, food producing animal and food are

536 collected under the `*Salmonella* Network` programme, which is targeted national

537 epidemiological surveillance system set up to monitor non-human *Salmonella* throughout the

538 food chain. The network was officially created in 1997 and today includes nearly 150 public

539 and private veterinary laboratories in 94 departments across France. The second type of

540 surveillance is managed by AFSSA and serves as a multi-centric system to collect antibiotic

541 susceptibility data on pathogenic strains isolated in local public veterinary diagnostic

542 laboratories. Each network has been designed for one particular type of investigation. Data on

543 AMR are summarized in French surveillance network for antimicrobial resistance in

544 pathogenic bacteria of animal origin which started from 1982 (firstly in bovines) and

545 nowadays in called `RESAPATH`. From 2000, the surveillance system was expanded to pigs

546 and poultry and, in 2007, to other animal species such as small ruminants, companion

547 animals or horses (RESAPATH, 2012). However, there is no specific information on

548 sampling plans employed in this national programme, except that sampling will encompass

549 harvesting of faeces or caeca from diseased animals, on farm and/or abattoir. Commensal

550 bacteria (*E. coli*, *Enterococcus faecium*) and zoonotic strains (*Campylobacter* spp. and some

551 *Salmonella* isolates) are isolated according to type: bovine, porcine, or avian.

552 In Netherlands, sampling is implemented according to national plan for monitoring of AMR

553 and antibiotic usage in animals (MARAN, 2013). Sampling strategy has a goal to obtain

554 annual collections of *E. coli* and *Salmonella enterica*, representative of the Dutch food-

555 producing animal bacterial populations, including isolates obtained from retail. The samples

556 are regularly taken from poultry populations on farm (the faecal samples) and/or abattoir

557 (caecal samples), as well as poultry meat at retail (Leverstein-van Hall et al., 2011).

558 Additional data on sampling plan were not available in Dutch national plan. Further, the

559 Dutch approach to AMR encompasses all ecological compartments where human health is

560 threatened by antibiotic resistant bacteria, e.g. healthcare sector, food producing animals,

561 food and environment. This is an integrated approach based on the `One Health` concept. The

562 main focus lies in healthcare and food-producing animals because the emergence and spread

563 of antibiotic resistant bacteria starts from food-producing animals and subsequent transfer to

25

564 humans; the healthcare settings may be also environments where the transfer of resistance

565 genes due to excessive use of antibiotics may be facilitated.

566 In Sweden, the sampling is carried out to cover all respective sectors - animal, food and

567 humans. The collected samples are tested in designated public health laboratories coordinated

568 by the Public Health Agency of Sweden and veterinary/food laboratories coordinated by the

569 National Veterinary Institute. The results are jointly interpreted and reported in an integrated

570 manner by both institutions (SVARM, 2014). Clinical isolates are taken from food-producing

571 animals (on farm), e.g. pigs, cattle and sheep, and from humans (isolates from blood culture).

572 Information on the indication for sampling was not available for many samples and the

573 majority of submissions were likely from animals with disease. Therefore, data may be

574 biased towards samples from treated animals or from herds where antibiotic treatment is

575 common.

576 In Norway, the sampling of indicator organisms (*Escherichia coli* and *Enterococcus* spp.),

577 which form the normal enteric microbiota, is carried out to determine the prevalence of

578 acquired antimicrobial resistance. This can be used as an indicator of the selective pressure

579 from use of antimicrobial agents in various populations. These bacteria may form a reservoir

580 of transferable resistance genes from which antimicrobial resistance can be spread to other

581 bacteria, including those responsible for infections in animals or humans. Faecal samples are

582 taken via boot swabs from layer flocks and from turkey, including ESBL-producing *E. coli*

583 from turkey fillets at retail. The sampling of isolates of zoonotic food borne pathogens, e.g.

584 *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and non-zoonotic pathogen -

585 *Shigella* spp. is also conducted. Human clinical isolates are collected from blood, urine and

586 cerebrospinal fluid. The sampling plan is carried out according to provisions given in

587 Regulation 652/2013/EC on the monitoring and reporting of antimicrobial resistance in

588 zoonotic and commensal bacteria. *Salmonella* spp. isolates are taken from each population of

589 laying hens, broilers and fattening turkeys sampled in the framework of the national control

590 programmes (EU, 2003b); carcasses of broilers, fattening turkeys, fattening pigs, and bovines

591 under one year of age, are also collected. *Campylobacter jejuni* isolates are collected from

592 caecal samples gathered at slaughter from broilers and from fattening turkeys.

593

594 2.4. Antimicrobial susceptibility testing

595 Susceptibility testing aims to quantify drug potency against specific pathogenic bacteria and
596 to establish what measures can be taken to safely formulate the drug so it is a viable option
597 for therapeutic treatments. It is also used to establish if changes in pathogenic behaviour
598 against already tested drugs is occurring due to microbial resistance. When EUCAST defines
599 a microorganism as "s*usceptible"* this generally means that the microorganism is susceptible
600 to the therapy and that success when this specific antimicrobial agent is used is high. The
601 opposite is defined when the microorganism is resistant to selected antimicrobial agent.
602 When determining the ability of antimicrobials to be successful against a specific pathogen,
603 the following information should be taken into consideration, e.g. the site of infection, ability
604 of antimicrobial to reach infection site, as well as formulations available and dosage regimes
605 (EFSA/ECDC, 2016).

606 Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing (AST)
607 and remains one of the most widely used AST methods in routine clinical microbiology
608 laboratories; it is very suitable for application and almost all antimicrobial agents can be
609 tested since it requires no special equipment (Matuschek et al., 2014). Disk diffusion proved
610 to be a reproducible and accurate method for AST if performed according to
611 recommendations (Woods, 1995). European Committee on Antimicrobial Resistance Testing
612 (EUCAST), with assistance from the European Society of Clinical Microbiology and
613 Infectious Diseases (ESCMID) suggested a disc diffusion test with diameter breakpoints
614 correlated with the EUCAST minimum inhibitory concentration (MIC) breakpoints
615 (Matuschek et al., 2014), defined by inhibition zone diameters (IZD) expressed in mm. The
616 MIC is used to describe the effect a new drug has on a specific organism. It identifies the
617 minimum concentration required by an antimicrobial to inhibit the growth of an organism
618 visually, after an overnight incubation period. It is the most widely used method for
619 antimicrobial susceptibility testing (AST) in clinical laboratories throughout the EU/EEA
620 (EUCAST, 2015). The disk diffusion method is widely used in France (L'Observatoire
621 National de l'Epidémiologie de la Résistance Bactérienne aux antibiotiques/ONERBA) and
622 Sweden (Swedish Veterinary Antimicrobial Resistance Monitoring/SVARM).

623 Although disk diffusion is the most widely used method for measurement of antimicrobial
624 activity against *Salmonella* in routine clinical laboratories, since it is inexpensive and
625 relatively easy to perform, the dilution method (where the MIC is determined in mg/L) is a
626 more accurate measurement than disk diffusion; it is considered as the gold standard for AST.

27

627 Therefore, for monitoring purposes the micro-broth dilution is recommended as the preferred

628 testing method. However, there is a good to excellent correlation between the values obtained

629 in mm and in mg/L. Validated methods of gradient strip diffusion or disk diffusion according

630 to EUCAST protocols are also accepted. The dilution method is routinely used by Danish,

631 Dutch and Norwegian national monitoring systems for antimicrobial resistance – DANMAP,

632 MARAN and NORM-VET, respectively.

633 *2.4.1. Clinical breakpoints*

634 Clinical breakpoints are developed for laboratory testing on antimicrobials to determine

635 therapeutic value against new and already developed antimicrobials. Organisms may be

636 graded as susceptible (s) - when a micro-organism is defined as susceptible by a level of

637 antimicrobial activity associated with a high likelihood of therapeutic success; intermediate

638 (I) - when a level of antimicrobial agent activity is associated with uncertain therapeutic

639 effect; and resistant (R) - when a level of antimicrobial activity is associated with a high

640 likelihood of therapeutic failure (EUCAST, 2012). Regardless of the method used to

641 determine susceptibility, the purpose is to assimilate drug potency required to inhibit or kill a

642 pathogen within the body, by using pharmacokinetics and pharmacodynamics.

643 *2.4.2. Epidemiological cut-off values (ECOFFs)*

644 Standardised epidemiological cut off values (ECOFFs) are described by the EU Reference

645 Laboratory for Antimicrobial Resistance (EURL-AR, 2013) as "*essential for the comparison*

646 *of antimicrobial susceptibility monitoring results*". For the purpose of monitoring, EURL-AR

647 recommend the use of EUCAST ECOFFs which allows categorisation of bacteria as follows:

648 (i) wild type (for a species with the absence of acquired and mutational resistance

649 mechanisms to the drug in question) or (ii) non-wild type (for a species with the presence of

650 an acquired or mutational resistance mechanism to the drug in question).

651 When bacteria are identified as having resistance, the MIC and IZD displays two major sub-

652 populations: i) one is a fully susceptible set of isolates, and ii) the other is a fully resistant

653 population. The change to being resistant may be due to changes in the cell walls, which

654 make it permeable and there may be the possibility of isolates to fall between resistant and

655 susceptible. MIC testing of the isolates, after culturing, can verify the reduction in

656 susceptibility of the pathogen to antimicrobial agents. ECOFFs are derived by testing a

657 suitable number of isolates from a wild-type population, to ensure that an identified organism

658 can be treated in order to determine the likelihood of success or failure of a specific

659 antimicrobial for clinical purposes. Accordingly, the epidemiological cut off values

660 recommended by the EURL-AR for interpretation of AST results are defined for *Salmonella*

661 spp., *Campylobacter coli*, *Campylobacter jejuni*, *Escherichia coli*, *Staphylococcus aureus*,

662 *Enterococcus faecium* and *E. faecalis* (EURL-AR, 2013).

663

3. Harmonization of national AMR monitoring and surveillance programmes

665 Surveillance of antimicrobial resistance at targeted intervals or ongoing monitoring of the

666 prevalence of resistance in bacteria from environment, food animals, food and humans is of

667 utmost importance for food safety in the context of public health (OIE, 2016). Monitoring of

668 bacteria from food products of animal origin intended for human consumption should be

669 collected in different stages along the food (meat) chain, i.e. pre-harvest (on farm), harvest (at

670 abattoir) and post-harvest (processing, packaging, storage, distribution and retail).
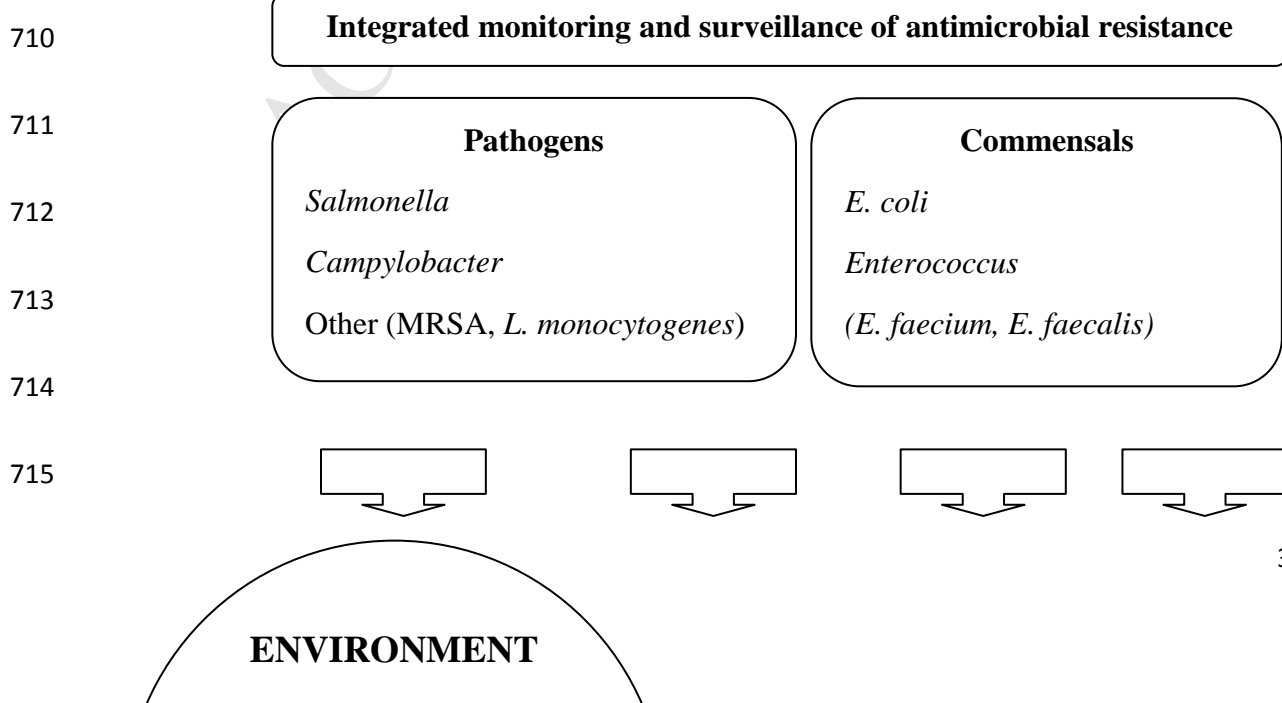
671 National antimicrobial resistance monitoring and surveillance programmes should be science-

672 based and may include the following components: a) statistically based surveys (veterinary

673 practitioners, farmers), b) sampling and testing of food animals on farm, at live animal

674 markets and, at slaughter, c) an organized sentinel programme, e.g. targeted sampling of food

675 animals, herds, flocks and vectors (birds, rodents), d) analysis of veterinary practice and

676 diagnostic laboratory records, e) sampling and testing of products of animal origin intended
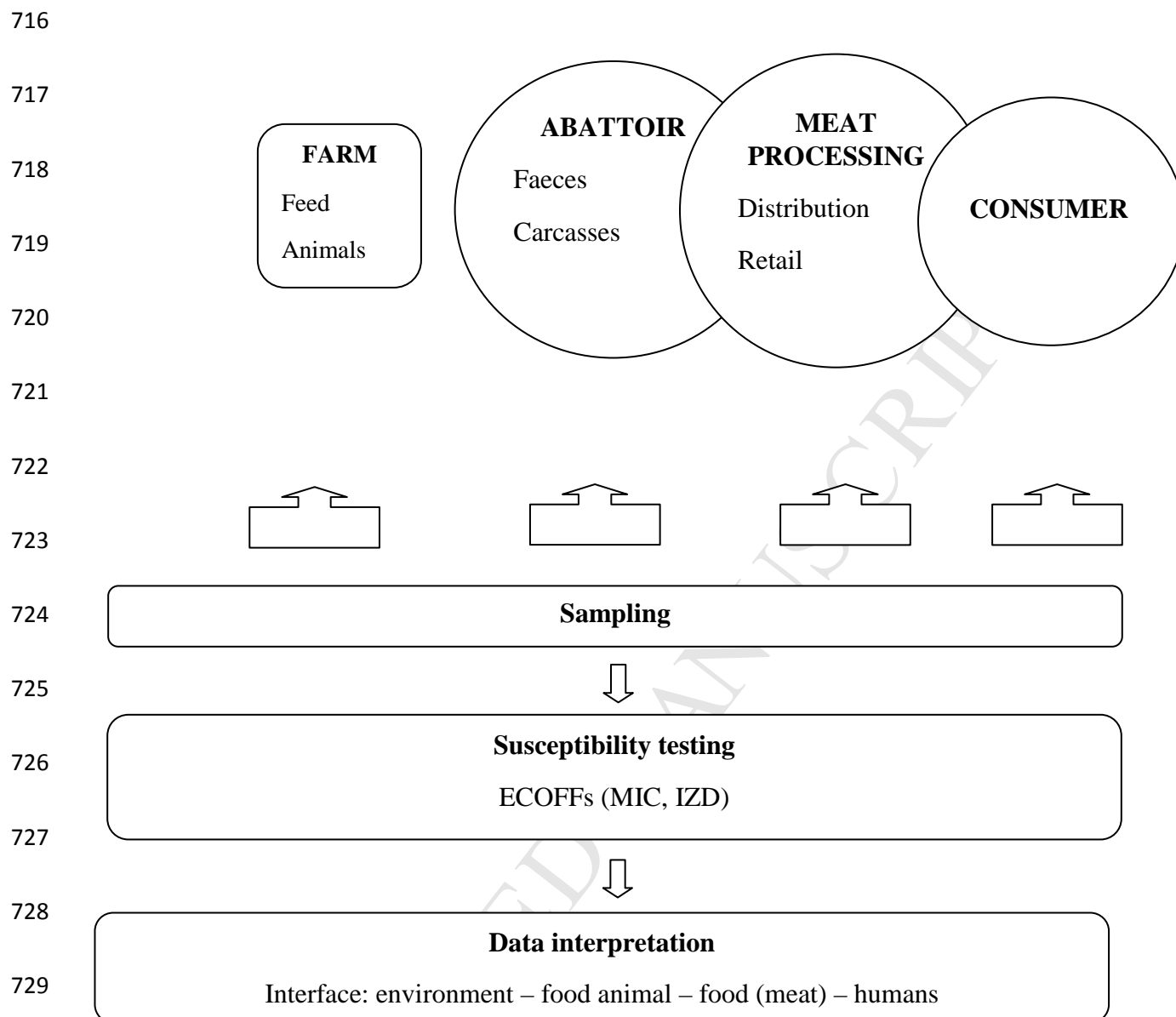
677 for human consumption (OIE, 2016).

678 Sampling strategy should be based on the characteristics of the national livestock production

679 systems, on the basis of available information and to assess which sources are likely to

680 contribute most to a potential risk to animal and human health. For example, sampling at pre-

681 harvest level (on farm) may encompass feed and composite faecal sample, at harvest level (at

682 abattoir) the faecal content from the gut (ampulla recti for pigs/bovine and caecal samples for

683 broilers), as well as swabs from carcasses to assess the overall hygiene at slaughter and the

684 level of microbiological contamination of carcass/meat. Post-harvest level (processing,

685 packaging, distribution and retail) should include sampling of food to assess the overall

686 microbiological contamination from slaughter to consumer.

687 The monitoring of bacterial microorganisms should be focused on animal bacterial pathogens
688 relevant to national priority to detect the emerging resistance that may pose a threat to animal
689 and human health and to guide veterinarians in their prescribing decisions (minimizing the
690 use of critically important antibiotics for human health). Major zoonotic foodborne pathogens
691 (*Salmonella*, *Campylobacter*) should be monitored in food animals and feed, food of animal
692 origin and humans. For *Salmonella*, serovars of public health importance should be included
693 (*S. Typhimurium* and *S. Enteritidis*); other serovars should be also included based on the
694 epidemiological situation in country. For *Campylobacter*, the most important serovars for
695 public health should be monitored (*C. jejuni and C. coli*) and they should be monitored
696 primarily from poultry and derived food products. Both, *Salmonella* and *Campylobacter*
697 isolates should be identified to the species level and serotyped according to internationally
698 standardised procedures, preferably at the nationally designated laboratories.

699 Other, emerging, zoonotic pathogens may be also included in the national resistance
700 monitoring and surveillance plan, such as methicillin-resistant *Staphylococcus aureus*
701 (MRSA) and *Listeria monocytogenes*.

702 The monitoring of commensal bacteria, such as *E. coli* and enterococci (*Enterococcus
703 faecium* and *Enterococcus faecalis*) should be carried out in environment (farm surroundings;
704 manure, soil, water), because they represent the natural reservoir for transfer of antimicrobial
705 resistance genes to pathogenic bacteria, feed and food animals (the samples of gut content
706 should be taken preferably at abattoir), food of animal origin, as well as humans; this is
707 important in order to establish a possible epidemiological link between food animals and
708 humans and to provide a better overview to the use and misuse of specific antimicrobial
709 agents (Figure 3).

710

Integrated monitoring and surveillance of antimicrobial resistance

711

Pathogens	**Commensals**
Salmonella	*E. coli*
Campylobacter	*Enterococcus*
Other (MRSA, *L. monocytogenes*)	*(E. faecium, E. faecalis)*

712

713

714

715

30

ENVIRONMENT

716

717

718

719

720

721

722

723

724

725

726

727

728

729

730 Figure 3. A framework of integrated monitoring and surveillance of antimicrobial resistance
731 in the meat chain

732 A bacterial isolate should be always preserved until the reporting is completed. Preferably,
733 selected isolates should be permanently preserved and stored. The maintenance of database of
734 isolates originated from the previous years may also enable the epidemiological retrospective
735 studies.

736 Overall, a consistency in sampling (target number of isolates per animal population and per
737 module in the food chain, e.g. farm, abattoir, retail), method of susceptibility testing, the
738 panel of antimicrobials and tests to be included, as well as reporting system, is of essential
739 importance to improve the comparability of data generated between EU MSs and EEA

740 countries. This should be achieved by the vigorous implementation of recommendations

741 issued by EFSA on randomised sampling for harmonised monitoring of antimicrobial

742 resistance in zoonotic and commensal bacteria (EFSA, 2014b). Currently, a substantial

743 differences exists between five selected EU and EEA countries regarding design and

744 implementation of the national AMR monitoring and surveillance system (Table 4).

745 Table 4. Comparative overview of the national AMR monitoring and surveillance systems in

746 five selected EU and EEA countries

Sampling and testing	[†]Zoonotic pathogens		[♯]Commensals		Susceptibility testing	
	*Food animal, matrix, module	Humans	Food animals	Humans	Disk diffusion	Dilution method
Country						
Denmark	*Salm* (P, c, A; P, cs, A) *Camp* (B, A; C, A; fb, R)	*Salm Typhimurium* (f) *Camp Jejuni* (f)	Ec (P, c, A; C, c, A; B, c, A; fb/fp/fc, R) En (P, c, A; B, c, A; fb/fp/fc, R)	na		x
France	*Salm* (P, f/c, F/A; C, f/c, F/A; B, f/c, F/A) *Camp* (P, f/c, F/A; C, f/c, F/A; B, f/c, F/A)	na	Ec (P, f/c, F/A; C, f/c, F/A; B, f/c, F/A) En (P, f/c, F/A; C, f/c, F/A; B, f/c, F/A)	na	x	
Netherlands	*Salm* (B, f, F; B, c, A; fb, R) *Camp* (B, f, F; B, c, A;	*Salm Typhimurium, Enteritidis* (f)	Ec (P, f, F; C, f, F; B, f, F; fb/fp/fc, R)	na		x

	fb, R)	*Camp*	En (P, c, A;		
		Jejuni (f)	fp, R)		
		STEC (f)			
Sweden	*Salm* (P, f, F; C, f, F; S, f, F)	*Salm* (bl),	Ec (P, f/c, F/A, C, f, A; B, c, A)	na	x
		Camp (bl)			
	Camp (P, f, F; C, f, F; S, f, F)		En (P, f/c, F/A, C, f, A; B, c, A)		
Norway	*Salm* (P, f/c, F/A; C, f/c, F/A; B, f/c, F/A)	*Salm Typhimurium, Enteritidis* (bl, u, cf),	Ec (B, f, F; fb, R)	na	x
	Camp (B, c, A)	*Camp Jejuni* (bl, u, cf),	En (B, f, F; fb, R)		
		Yer enterocoilitca (bl, u, cf),			
		‡*Shi* (bl, u, cf)			

747 *Food animal: P (pigs), C (cattle), S (sheep), B (broilers)

748 Matrix (sample): c (caecum), f (faeces), cs (carcass swabs), fb (fresh broiler meat), fp (fresh pork meat), fc
749 (fresh cattle meat), bl (blood), u (urine), cf (cerebrospinal fluid)

750 Module in the meat chain: F (farm), A (abattoir), R (retail)

751 †Zoonotic bacteria: *Salm* (*Salmonella*), *Camp* (*Campylobacter*), *Yer* (*Yersinia*), STEC (Shiga toxin producing *E.*
752 *coli*)

753 ‡Non-zoonotic bacteria: *Shi* (*Shigella*)

754 ♯Commensals: Ec (*Escherichia coli*), En (*Enterococcus faecium, Enterococcus faecalis*)

755 na: not applicable (the lack of data)

756

4. Conclusion

Over the last decade, the AMR associated with zoonotic foodborne pathogens is recognized as a major public health concern in Europe. Zoonotic foodborne bacteria are infectious agents which may be transferred from animals to humans via food consumption. Modern food-animal production uses large amounts of antibiotics not only for therapeutic purposes but also to prevent disease and promote animal growth. As a result, large numbers of healthy animals are routinely or often exposed to antibiotics. Such intensive, on-farm production practice, can trigger a development of bacterial resistance towards antimicrobials. Food-producing animals (cattle, sheep, pigs and poultry) are of particular importance for emergence and transfer of AMR which may be transferred to humans. The use of antibiotics in one sector or environmental compartment or country may influence the spread of resistance in others. The special importance should be given to commensal microbiota (*E. coli*, enterococci). These bacteria can also acquire antimicrobial resistance as a response to selective pressures and may form a reservoir of resistance genes in environment, farm and food animals, with the potential for transferring resistance to pathogenic bacteria which, in turn, may cause infection in humans difficult to cure. Infections with foodborne pathogens (*Salmonella*, *Campylobacter*), resistant to antimicrobials, may result in serious treatment failures or necessitate the use of second-line antimicrobials for therapy.

The review of available scientific and professional literature regarding contribution of the meat chain to development and transfer of AMR from meat animals to humans, revealed that in five selected countries – four EU MSs (Denmark, France, Netherlands and Sweden) and one EEA country (Norway), healthy or diseased food-producing animals (cattle, pigs and poultry) and derived meats are regularly sampled - on farm, at abattoir and retail. The differences between these five countries regarding sampling schemes and susceptibility testing were evident (Table 4). A substantial difference was observed regarding food animal category, sample matrix (faeces, caecum, fresh meat) and module in the meat chain (farm, abattoir, retail) where sampling was conducted. In all five countries, detection and susceptibility testing for *Salmonella* and *Campylobacter,* as well as *E. coli* and enterococci was included in the national plan, although the selection of food animal category, matrix and module in the meat chain differed. The susceptibility testing for major zoonotic foodborne pathogens in humans (samples from blood, urine, cerebrospinal fluid) was carried out regularly for *Salmonella typhimurium* and *Campylobacter jejuni* - in Denmark, Netherlands,

789 Sweden and Norway; data from France were scarce and mostly related to individual studies

790 regarding AMR profile of *L. monocytogenes*, *Campylobacter* and *Salmonella*. In Norway,

791 other pathogens were also regularly included in the national AMR monitoring plan (*Yersinia*

792 *enterocolitica* and *Shigella*, in humans). Data on susceptibility testing for commensals in

793 humans were not available in neither of the five selected countries. The disk diffusion method

794 is widely used in France and Sweden, while the dilution method is routinely used in

795 Denmark, Netherlands and Norway.

796 Integrated monitoring and surveillance of antimicrobial resistance in commensal and zoonotic

797 foodborne bacteria from humans, animals and food is an essential source of information when

798 formulating measures to improve food safety and protect consumers from exposure to

799 resistant bacteria from foods. To harmonise the sampling and susceptibility testing and

800 provide better consistency between EU MSs, the EFSA guidelines for the monitoring of

801 antimicrobial resistance (e.g. target number of isolates per animal population - on farm, at

802 abattoir and at retail; method of susceptibility testing; a panel of antimicrobials to be included

803 and test ranges) should be applied. Such approach is also needed to improve the

804 comparability of data generated among EU MSs.

805 The effective risk mitigation strategies to tackle the antimicrobial resistance in the food

806 (meat) chain context should be based on promotion of inter-sectoral cooperation at national

807 and international level. Veterinary, agricultural and pharmaceutical authorities at the national

808 level should give consideration to establishing a regulatory framework for authorizing and

809 controlling veterinary medicines, including critically important antibiotics for veterinary

810 medicine and human health. Integrating monitoring and surveillance in the environment-food

811 animal-food (meat)-humans continuum is of utmost importance to tackle successfully the

812 issue of antimicrobial resistance. The essential point is to reduce the need for antibiotics in

813 food animal production systems by improving animal health through biosecurity measures,

814 e.g. disease prevention (introduction of effective vaccines) and good hygiene and

815 management practices – on farm and at abattoir. Future research needs should be based on

816 knowledge gaps such as: securing comparable national data on the occurrence of antibiotic

817 resistance in relevant bacteria from environment, food animals, food products and humans,

818 including the use of various types of antibiotics in different categories of food animals;

819 actively using surveillance data in epidemiological research and risk assessment, including

820 the evaluation of interventions; improve the understanding of mechanisms of resistance

821 development and transfer; and development of new antibiotics and alternative approaches to

822 antibiotic therapy.

823

824 **5. References**

825 Aarestrup, F. M., 1995. Occurrence of glycopeptide resistance among Enterococcus faecium

826 isolates from conventional and ecological poultry farms. *Microbial Drug Resistance, 1*, 255-

827 257

828 Aarestrup, , Bager, F., Jensen, N. E., Madsen, M., Meyling, A., & Caspar, H. (1998).

829 Surveillance of antimicrobial resistance in bacteria isolated from food and animals to

830 antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS, 106*, 606-

831 622.

832 Andersen, S. R., Saadbye, P., Shukri, N. M., Rosenquist, H., Nielsen, N. L., & Boel, J.(2006).

833 Antimicrobial resistance among Campylobacter jejuni isolated from raw poultry meat at retail

834 in Denmark. *International Journal of Food Microbiology, 107(3),* 250-255.

835 ANSES (2015). French Agency for Food, Environmental and Occupational Health & Safety.

836 Resistance to antibiotics: new information concerning colistin.

837 https://www.anses.fr/en/lexique/antimicrobial-resistance (accessed on 23 September 2016).

838 Babic, M., Hujer, A. M., & Bonomo, R. A. (2006). What's new in antibiotic resistance?

839 Focus of beta-lactamase. *Drug Resistance Updates, 9*, 142-156.

840 Barton, M. D. (2000). Antibiotic use in animal feed and its impact on human health.

841 *Nutritional Research Review, 13*, 279-299.

842 Bates, J., Jordens, J. Z., & Griffiths, D. T., (1994). Farm animals as a putative reservoir for

843 vancomycin-resistant enerococcal infection in man. *Journal of Antimicrobial Chemotherapy,*

844 *34*, 507-14.

845 Behravesh, C. B., Williams, I. T., & Tauxe , R. V., (2012). Emerging fodborne pathogens and

846 problems: expanding prevention efforts before slaughter or harvest. In: *Institute of Medicine*

847 *(US). Improving Food Safety Through a One Health Approach: Workshop Summary.*

848 Washington DC: National Academies of Press, p. A14.

849 Bischt, R., Katiyar, A., Singh, R. & Mittal, P. (2009). Antibiotic resistance- A global issue of

850 concern. *Asian Journal of Pharmaceutical and Clinical Research, 2(2)*, 34-39.

851 Bruin, J., van der Aar, M., Overdevest, I., Kluytmans, J. & Diederen, B.(2010).Prevalence of

852 highly resistant Enterobacteriaceae including ESBLs in retail meat. *Journal of Hospital*

853 *Infection, 76(1)*, 69.

854 Bywater, R., Deluyker, H., Deroover, E., de Jong, A., Marion, H., McConville, M. et al.

855 (2004). A European survey of antimicrobial susceptibility among zoonotic and commensal

856 bacteria isolated from food producing animals. *Journal of Antimicrobial Chemotherapy,*

857 *54(4)*, 744-754.

858 Cogliani, C., Goossens, H. and Greko, C. (2011) Restricting Antimicrobial Use in

859 Food Animals: Lessons from Europe. *Microbe 6(6)*, 274-279.

860 DANMAP (2014). *Use of antimicrobial agents and occurrence of antimicrobial resistance in*

861 *bacteria from food animals, food and humans in Denmark.*

862 http://www.danmap.org/~/media/Projekt%20sites/Danmap/DANMAP%20reports/DANMAP

863 %202014/Danmap_2014.ashx (Accessed on 23 June 2016).

864 EARS (2014). Antimicrobial resistance surveillance in Europe 2014.

865 http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf

866 ECDC/EFSA/EMEA (2015). ECDC/EFSA/EMA first joint report on the integrated analysis

867 of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in

868 bacteria from humans and food producing animals. *EFSA Journal, 13(1)*, 4006.

869 ECDC/EMEA (2009). *European Centre for Disease Prevention and Contol/European*

870 *Medicines Agency) joint technical report. The bacterial challenge: time to react.*

871 http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challeng

872 e_Time_to_React.pdf (Accessed on 16 February 2016).

873 ECDC (2014). *The European Union summary report on antimicrobial resistance in zoonotic*

874 *and idicator bateria from humans, animals and food in 2014.*

875 http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-zoonotic-bacteria-

876 humans-animals-food-EU-summary-report-2014.pdf (Accessed on 13 July 2016).

877 ECDC (2015). *Antimicrobial consumption (AMC) reporting protocol 2016.*

878 http://ecdc.europa.eu/en/activities/surveillance/ESAC-

879   Net/publications/Documents/antimicrobial-consumption-ESAC-Net-reporting-protocol-

880   2014.pdf (Accessed on 24 June 2016).

881   EFSA/ECDC (2015). *EU Summary report on antimicrobial resistance in zoonotic and*

882   *indicator bacteria from humans, animals and food in 2013,* Parma,Italy. Stockholm, Sweden:

883   European Food Safety Authority.

884   EFSA (2007). Report on the task force on zoonoses data collection including a proposal for a

885   harmonised montoring scheme of antimicrobial resistance in Salmonella in fowl (*Gallus*

886   *gallus*), turkeys and pigs and, *Campylobacter jejuni* and *C. coli* in broilers. *EFSA Journal, 96*,

887   1-46.

888   EFSA (2008). Report from the task force on zoonoses data collection including guidance for

889   harmised monitoring and reporting of antimicrobial resiatnce in commensal Escherichia coli

890   and Enterococcus spp. from food and animals. *EFSA Journal, 141,* 1-44.

891   ECDC/EMEA (2009). The bacterial challenge: time to react A call to narrow the gap between

892   multidrug-resistant bacteria in the EU and the development of new antibacterial agents.

893   http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Tim

894   e_to_React.pdf (accessed on 19 September 2016)

895

896   EFSA (2011). Scientific Opinion on *Campylobacter* in broiler meat production: control

897   options and performance objectives and/or targets at different stages of the food chain. Panel

898   on Biological Hazards. *EFSA Journal, 9(4)*, 2105.

899   EFSA (2014a). The EU Summary Report on antimicrobial resistance in zoonotic and

900   indicator bacteria from humans, animals and food 2012. *EFSA Journal, 12(3)*, 3590.

901   EFSA (2014b). Technical specifications on randomised sampling for harmonised monitoring

902   of antimicrobial resistance in zoonotic and commensal bacteria. *EFSA Journal, 5(12)*, 3686.

903   EFSA & ECDC (2016). The European Union summary report on antimicrobial resistance in

904   zoonotic and indicator bacteria from humans, animal and foods in 2014. *EFSA Journal,*

905   *14(2),* 4380.

906   Egervarn, Borjesson, S., Byfors, S., Finn, M., Kaipe, C., Englund, S.et al.(2014). Esherichia

907   coli with extended-spectrum beta-lactamase or transferable AmpC beta-lactamase and

908 Salmonellla on meat imported into Sweden. *International Journal of Food Microbiology,*

909 *171*, 8-14.

910 EMA (2016). Updated advice on the use of colistin products in animals within the European
911 Union: development of resistance and possible impact on human and animal health. European
912 Medicines Agency.
913 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC5
914 00211080.pdf (accessed on 25 September 2016).

915 EU (2003a). *Commission Directive 2003/99 on the monitoring of zoonoses and zoonotic*

916 *agents.* http://eur-lex.europa.eu/legal-

917 content/EN/TXT/PDF/?uri=CELEX:32003L0099&from=EN

918 (Accessed on 12 July 2016).

919 EU (2003b). *Commission Regulation 2160/2003 on the control of Salmonella and other*

920 *specified food-borne zoonotic agents.* http://eur-

921 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF

922 (Accessed on 12 July 2016).

923 EU (2013). *Commission Decision 2013/652/EC on the monitoring and reporting of*

924 *antimicrobial resistance in zoonotic and commensal bacteria.* http://eur-

925 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:303:0026:0039:EN:PDF

926 (Accessed on 12 July 2016).

927 EUCAST (2012). *EUCAST Definitons of clinical breakpoints and epidemiological cut off*

928 *values.* http://www.srga.org/Eucastwt/eucastdefinitions.htm

929 (Accessed on 24 March 2016).

930 EUCAST (2015). *Breakpoints tables for interpretation of MICs and zone diameters.*

931 http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_B

932 reakpoint_table.pdf (Accessed on 24 March 2016).

933 EURL-AR (2013). *Cut off values recommended by the EU Reference Laboratory for*

934 *Antimicrobial Resistance (EURL-AR).* http://www.crl-ar.eu/data/images/faq/eurl-

935 recommended%20cut%20off%20values-29-11-2013.pdf

936 (Accessed on 10 July 2016).

937 Gallay, A., Prouzet-Mauleon, V., Kempf, I., Lehours, P., Labadi, L., Camou, C. et al. (2007).

938 Campylobacter antimicrobial drug resiatance among humans, broiler chickens and pigs,

939 France. *Emerging Infectious Diseases, 13(2)*, 259-266.

940 Ge, B., White, D. G., McDermott, P. F., Girard, W., Zhao, S., Hubert, S.et al., (2003).

941 Antimicrobial-resistance *Campylobacter* species from retail raw meats. *Applied and*

942 *Environmental Microbiology, 69(5)*, 305-3007.

943 Granier, S. A., Moubareck, C., Colaneri, C., Lemire, A., Roussel, S., Dao, T.-T., et al.

944 (2011). Antimicrobial resistance of *Listeria monoctytogenes* isolates from food and the

945 environment in France over a 10 year period. *Applied and Environmental Microbiology,*

946 *77(8)*, 2788-2790.

947 Guyard-Nicodeme, M., Rivoal, K., Houard, E., Rose, V., Quesne, S., Mourand, G. et al.

948 (2015). Prevalence and characterisation of *Campylobacter jejuni* from chicken meat sold in

949 French retail outlets. *International Journal of Food Microbiology, 203,* 8-14.

950 Heymann, D., 2004. *Control of communicable diseases manual.* (18th ed.). Washington:

951 American Public Health Association.

952 Leegard, T. M., Caugant, D. A., Froholm, L. O., Holby, E. A. & Lassen, J.(2000). Emerging

953 antibiotic resistance in *Salmonella* Typhimurium in Norway. *Epidemiology and Infection,*

954 *125(3)*473-80.

955 Leverstein-van Hall, M. A., Dierikx, C. M., Stuart, J. C., Voets, G. M.,van den Munckhof, M.

956 P., van Essen-Zandbergen, A. et al. (2011). Dutch patients, retail chicken meat and poultry

957 share the same ESBL genes, plasmids and strains. *Clinical Microbiology and Infection, 17(6)*,

958 873-880.

959 Lindmark, H., Harbom, B., Thebo, L., Andersson, L., Hedin, G., Osterman, B. et al. (2004).

960 Genetic characterization and antimicrobial resistance of *Campylobacter jejuni* isolated from

961 meats, water and humans in Sweden. *Journal of Clinical Microbiology, 42(2)*700-706.

962 MARAN (2013). Monitoring of antimicrobial resistance and antibiotic usage in animals in

963 the Netherlands in 2012. https://www.wageningenur.nl/upload_mm/7/8/9/52388c6c-858c-

964 483c-b57d-227029fe778a_005738_Nethmap_2013%20def_web.pdf

965 (Accessed on 25 June 2016).

966 Martel, J. L., Tardy, F., Brisabois, A., Lailler, R., Coudert, M. & Chaslus-Dancla, E. (2000).

967 The French antibiotic resistance monitoring programs. *International Journal of AntimIcrobial*

968 *Agents, 14*(4), 275-283.

969 Matuschek, E., Brown, D. F. and Kahlmeter, G., 2014. Development of EUCAST disk

970 diffusion antimicrobial susceptibility testing method and its implementation in routin

971 microbiology laboratories. *Clinical Microbiology and Infection, 20*, O255-O266.

972 Mo, S. S., Slettemeas, J. S., Berg, E. S., Norstrom, M. & Sunde, M.(2016a). Plasmid and host

973 strain characteristics of Escherichia coli resistant to extended-spectrum Cephalosporins in the

974 Norwegian broiler production. *PLoS One, 11(4)*, e0154019.

975 Mo, S. S., Kristoffersen, A. B., Sunde, M., Nodtvedt, A. & Norstrom, M. (2016b). Risk

976 factors for occurence of cephalosporin-resistant *Escherichia coli* in Norwegian broiler flocks.

977 *Preventive Veterinary Medicine, 130,* 112-118.

978 NORM-VET (2013). *Usage of antimimicrobial agents and occurrence of antmicrobial*

979 *resistance in Norway.* http://www.vetinst.no/eng/Publications/NORM-MORN-VET-

980 Report/NORM-NORM-VET-2013

981 (Accessed on 23 June 2016).

982 OIE (2015). List of antimicrobial agnets of veterinary importance, Resolution No. XXVIII.

983 http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/Eng_OIE_List_ant

984 imicrobials_May 2015.pdf (Accessed on 24 June 2016).

985 OIE (2016). Harmonisation of national antimicrobial resistance surveillance and monitoring

986 programmes.

987 http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_antibio_harm

988 onisation.pdf (Accessed on 24 June 2016).

989 Overdevest, I. Willesem, I., Rijnsburger, M., Eustace, A., Xu. L., Hawkey, P. et al. (2011).

990 Extended-spectrum β- Lactamase genes in E*scherichia coli* in chicken meat and humans, the

991 Netherlands. *Emerging Infectious Diseases, 17(2)*, 1216-1222.

992 RESAPATH (2012). French surveillance network for antimicrobial resistance in pathogneic

993 bacteria from animal origin. http://www.anses.fr/en/system/files/LABO-Ra-

994 Resapth2012EN.pdf

995 (Accessed on 23 June 2016).

996 Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B. et al. (2006).

997 Effect of vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*.

998 *Journal of Antimicrobial Chemotherapy,* 57, 1215-1219.

999 SVARM (2014). Consumption of anitibiotics and occurrence of antibiotic reistance in

1000 Sweden.

1001 http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/swedres_svarm2014.

1002 pdf (Accessed on 23 June 2016).

1003 van den Boggard, A. E., & Stobberingh, E. E., (2000). Epidemiology of resistance to

1004 antibiotics: Links between animal and humans. *International Journal of Antimicrobial*

1005 *Agents, 17(3)*, 502-5.

1006 Vanderbroucke-Grauls, C. M. J. E. (2014). Antimicrobial resistance in the Netherlands: a

1007 natural experiment. *Frontiers in Public Health, 2*, 1-2.

1008 WHO (2011a). *Tackling antibiotic resistance from a food safety perspective in Europe,*

1009 Copen Hagen: World Health Organisation Regional Office for Europe.

1010 WHO (2011b). *Critically important antibiotics for human medicine.*

1011 http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf

1012 (Accessed on 21 June 2016).

1013 WHO (2015). Neglected zoonotic diseases.

1014 http://www.who.int/neglected_diseases/diseases/zoonoses/en/ (Accessed on 21 March 2016).

1015 Wielinga, P. R., Hensen, V. F., Aarestrup, F. M. & Schlundt, J., 2014. Evidence based policy

1016 for controlling antimicrobial resistance in the food chain in Denmark. *Food Control, 40,* 185-

1017 192.

1018 Woods, G. L. (1995). In vitro testing of antimicrobial agents. *Infection Diseases Clinical*

1019 *North America, 9*, 463-481.

1020 Yavari, L. (2012). Antibiotic resistance in Salmonella enterica and the role animal and animal

1021 food control: A literature review of Europe and USA.

1022 http://www.phmed.umu.se/digitalAssets/104/104569_leila-yavari.pdf

1023 (Accessed on 25 June 2016).

1024

1025

1026

Antimicrobial resistance monitoring and surveillance in the meat chain: A report from five countries in the European Union and European Economic Area

Karl Mc Nulty[a], Jan Mei Soon[b], Carol Anne Wallace[b], Ivan Nastasijevic[c]

[a]School of Tourism, Letterkenny Institute of Technology, Shore Road, Killybegs, Co, Donegal, Ireland

[b]International Institute of Nutritional Sciences and Applied Food Safety Studies, School of Sport and Wellbeing, University of Central Lancashire, Preston, Lancashire PR1 2HE, United Kingdom

[c]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

Highlights

- The emergence of antimicrobial resistance (AMR) in zoonotic foodborne pathogens.
- Resistant zoonotic bacteria compromise the effective treatment in humans.

- AMR monitoring and surveillance programmes reviewed in 5 EU/EEA countries.
- Sampling, susceptibility testing, clinical and epidemiological cut-off values.
- Integrated AMR monitoring in food animals, food and humans in the whole meat chain.