

Foodborne antimicrobial resistance as a biological hazard¹

Scientific Opinion of the Panel on Biological Hazards

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SUMMARY

The European Food Safety Authority (EFSA) asked its Panel on Biological Hazards to identify, from a public health perspective, the extent to which food serves as a source for the acquisition, by humans, of antimicrobial-resistant (AMR) bacteria or bacteria-borne antimicrobial resistance genes, to rank the identified risks and to identify potential control options for reducing exposure.

The present extent of exposure to AMR bacteria was found to be difficult to determine, and the role of food in the transfer of resistance genes insufficiently studied. Nevertheless, foodborne bacteria, including known pathogens and commensal bacteria, display an increasing, extensive and diverse range of resistance to antimicrobial agents of human and veterinary importance, and any further spread of resistance among bacteria in foods is likely to have an influence on human exposure. By way of an example, a qualitative ranking of food (ending at point of purchase) as a vector of an AMR bacterium demonstrated the complexity of the problem and the extensive data requirements for a formal risk ranking.

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In all cases where antimicrobial treatment in humans is indicated, resistance to the antimicrobials of choice is of clinical importance. Resistant *Salmonella* and *Campylobacter* involved in human disease are mostly spread through foods. With regards to *Salmonella*, contaminated poultry meat, eggs, pork and beef are prominent in this regard. For *Campylobacter*, contaminated poultry meat is prominent. Cattle are a major verotoxigenic *Escherichia coli* (VTEC) reservoir and resistant strains may colonize humans via contaminated meat of bovine origin more commonly than from other foods. Animal-derived products remain a potential source of meticillin-resistant *Staphylococcus aureus* (MRSA). Food-associated MRSA, therefore, may be an emerging problem. Food is also an important source for human infections with antimicrobial resistant *Shigella* spp. and *Vibrio* spp.

The principles that are applied to the prevention and control of the spread of pathogenic bacteria via food will also contribute to the prevention and the spread of antimicrobial-resistant pathogenic bacteria. As antimicrobial resistance in foodborne pathogens and commensals represents a specific public health hazard, additional control measures for antimicrobial-resistant bacteria may therefore be necessary. There are few examples of control programmes that directly control AMR as the hazard, using measures that specifically address food. In terms of impact, controls operated at the pre-harvest phase, for example, those aimed at the control and limitation of antimicrobial usage, are potentially the most effective and as such are capable of playing a major role in reducing the occurrence of AMR bacteria in food as presented for sale.

The development and application of new approaches to the recognition and control of food as a vehicle for AMR bacteria and related genes based on epidemiological and source attribution studies directed towards fresh crop-based foods, raw poultry meat raw pigmeat and raw beef are recommended.

Specific measures to counter the current and developing resistance of known pathogenic bacteria to fluoroquinolones as well as to 3rd and 4th generation cephalosporins found in a variety of foods and in animals in primary production now require to be defined and put in place as a matter of priority.

A major source of human exposure to fluoroquinolone resistance *via* food appears to be poultry, whereas for cephalosporin resistance it is poultry, pork and beef that are important, these food production systems require particular attention to prevent spread of such resistance from these sources.

If a full risk assessment for a specific food-bacterium-combination, in respect of AMR, should be undertaken, methodologies currently available for the risk assessment of foods require to be modified for uniform adaptation at both MS and EU level for the risk assessment of those combinations (including foods originating from food animals, fish, fresh produce (e.g. lettuce etc.) and water, as a vehicle for the transmission of AMR bacteria and related genes).

Overall, control of all the routes by which AMR bacteria and their related genes can arise in the human patient, of which food is but one such route, requires a response from all stakeholders to acknowledge their responsibilities for preventing both the development and spread of AMR, each in their own area of activity including medicine, veterinary medicine, primary food animal production, food processing and food preparation, as well as in the regulation of food safety.

Key words: Antimicrobial resistance, food, *Salmonella*, *Campylobacter*, VTEC, MRSA, *Shigella*, *Enterococcus*, *Escherichia coli*, *Listeria monocytogenes*.

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BACKGROUND AS PROVIDED BY EFSA

Antimicrobial resistant bacteria are biological hazards² associated with increased human morbidity and mortality and are of public health concern. The use of antimicrobial agents in animals, plant production and the production of other sources of food and feed has adverse public health consequences by creating a reservoir of resistant bacteria and of bacteria-borne resistance genes that can be passed on to humans, both directly or indirectly. Such resistance respects neither phylogenetical, geographical nor ecological borders. Mobile genetic elements harbouring resistance determinants can readily be transferred horizontally between bacteria from terrestrial animals, fish and humans; furthermore, such transfer can take place in natural environments such as the kitchen.

The use of antimicrobial agents for the treatment and control of infectious diseases in animals and crops continues because of considerations regarding animal health and welfare, and plant health. Consequently the transfer of antimicrobial-resistant bacteria and bacteria-borne resistance genes from animals or crops to humans via food remains a matter of public health concern.

The use of antimicrobials at subtherapeutic levels in food producing animals has long been viewed as undesirable e.g. the Swann report, 1969³. Since January 2006 the use of all antimicrobial feed additives has been banned within the EU in order to reduce the numbers of resistant bacteria in farm animals (Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition)⁴. The effect of this ban on the extent of bacterial antimicrobial resistance both within farm animals, and with regard to human health, however, is unclear.

Use of antimicrobial agents is the main driver for the development and spread of antimicrobial resistance. In addition, spontaneous mutation in foodborne bacteria or the spread of resistant bacteria in the absence of selective pressure may also contribute to the antimicrobial resistance burden in food.

Antimicrobial-resistant bacteria and bacteria-borne resistance genes can be spread to humans *via* food by different routes and mechanisms, for example:

- By foodborne spread of resistant zoonotic bacteria, e.g. *Salmonella* and *Campylobacter*. These bacteria may originate from various sources, including animals, the environment and humans.
- By foodborne spread of resistant non-zoonotic human pathogenic bacteria e.g. *Shigella* spp. and *Vibrio* spp. These bacteria do not have a primary reservoir in food animals, but can be spread from humans to food directly or indirectly through the environment, including water.
- By foodborne spread of resistant commensal bacteria carrying transferable antimicrobial resistance genes that can be passed on to human pathogenic bacteria. These resistant commensal bacteria may originate from various sources, including animals, the environment and humans.

² Hazard- “a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect”

³ Swann MM. Report of the Joint Committee on the use of antibiotics in animal husbandry and veterinary medicine. London: Her Majesty’s Stationary Office; 1969.

⁴ More precisely: Since January 2006 the use of antibiotics other than coccidiostats and histomonostats as feed additives has been banned within the EU (Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition)

The foodborne route of transfer of antimicrobial resistance is in addition to direct zoonotic spread resulting from contact with animals, e.g. livestock, pets and their excreta. Meanwhile, foods other than those originating from animals can also be vectors for the transmission of antimicrobial-resistant bacteria and bacteria-borne resistance genes to the consumer. In addition, food handlers can contaminate food during preparation, as has happened, for example, in the case of both meticillin-resistant *Staphylococcus aureus* (MRSA) and resistant *Shigella* spp. Finally, as already mentioned, the presence of antimicrobial-resistant bacteria in food may be the result of environmental contamination, e.g. from water sources, in the case of aquacultural and horticultural produce in particular. The extent and relative importance of the contribution of each of these pathways to the risk of antimicrobial resistance in microorganisms of human health concern is unknown.

On 17 April 2008, EFSA published the draft opinion of the BIOHAZ Panel on the self-tasking mandate on foodborne antimicrobial resistance as a biological hazard and invited comments. The closure date of the consultation was 27 May 2008.

Twelve submissions of public comments were received from individuals, food processors, member states food safety authorities, European Community agencies and associations representing sectors of the European food industry. EFSA and the Panel on Biological Hazards (BIOHAZ) wish to acknowledge and thank those who provided comments. EFSA and the BIOHAZ Panel took into consideration all the received comments and, where appropriate, modified the draft opinion.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Scientific Panel on Biological Hazards is asked, from a public health perspective,

1. To identify in terms of qualitative risk⁵, the extent to which food serves as a source for the acquisition, by humans of antimicrobial-resistant bacteria or bacteria-borne antimicrobial resistance genes.
2. To rank the identified risks.
3. To identify potential control options for reducing exposure.

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⁵ Risk is defined as “a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food”, Codex Alimentarius Commission, Procedural Manual.

ASSESSMENT

1. Introduction

Many scientific reviews have focussed on antimicrobial resistance in zoonotic bacterial pathogens and the possible link between the use of veterinary antimicrobials, prophylactics and growth promoters and resistance issues in human medicine (ACMSF, 1999; Anderson, 2003). If strategic prevention and controls are to be effective, it is important to better understand the ecology, epidemiology and extent of such resistance among food-borne pathogens.

The consequences of the use of antimicrobials⁶ in primary animal production and to a lesser extent in other areas of food production including aquaculture and horticulture have been reviewed elsewhere. Notwithstanding this, to date the contribution of food in all its processed and non-processed forms has not been studied in detail. In particular, the relative contribution of food to the occurrence of antimicrobial resistance to critically important antimicrobials in bacteria causing disease in humans has not been the subject of scientific opinions. In this Opinion the ways in which food serves as a vehicle for the acquisition of antimicrobial-resistant bacteria or bacteria-borne antimicrobial resistance genes causing infections in humans is addressed with a view to conducting an initial ranking of the identified risks and identifying potential control options.

The issue of antimicrobial resistance (AMR) is of world wide concern. The ECDC in its review of 2005 data on communicable diseases in Europe, identified AMR as a major problem in European health care, and one that undoubtedly prolongs patient suffering, costs money and is responsible for the death of thousands of European citizens each year (ECDC, 2007). The WHO, FAO, including Codex, and OIE have each (individually or jointly) reviewed the area and provided guidelines, recommendations and lists of clinically important antimicrobials (e.g. FAO/OIE/WHO, 2003; WHO, 2007). The latest activity in this area is the Codex Ad Hoc Intergovernmental Task Force on Antibiotic Resistance (Codex, 2007), which aims to assess the risks to human health associated with the presence in food and feed of antimicrobial-resistant organisms, and genes, and to develop risk management advice based on that assessment to reduce such a risk.

The most important factor influencing the emergence and spread of AMR is the use of antimicrobial agents in different hosts with spread of resistant bacteria and resistance genes between hosts of the same or of different species (SSC, 1999). In the human, veterinary and horticultural spheres there is a variety of ways in which antimicrobials come to be dispensed and applied. In human medicine, antimicrobials are widely used for therapy and prophylaxis both in hospitals and in the community, under varying levels of supervision. Likewise, as already mentioned in the Background to this Opinion, the same antimicrobial agents continue to be widely used in animals and aquatic species bred for food production, for therapeutic treatment, prophylaxis and growth promotion (no-longer in the EU), and also in companion animals, for therapy and prophylaxis, also under varying degrees of supervision. Oral medication of large groups of animals is particularly likely to favour emergence of and selection for AMR. Also, in primary production, conditions exist that facilitate the spread of bacteria, such as high density and/or poor infection control.

⁶ Antimicrobial: A drug, not a disinfectant, which, at low concentrations, exerts an action against microbial pathogens and exhibits selective toxicity towards them (EFSA, 2004a)

Whilst of relevance to the development of antimicrobial resistance in the microflora of humans, consideration of the effects of residues of antimicrobial substances in food are not within the scope of this Opinion. The development of and selection for resistance, as well as links between resistance to biocides⁷ and antimicrobials (Gilbert and McBain, 2003), are briefly discussed; as these issues are the subject of study elsewhere they are outside the terms of reference of this mandate. Decontamination of fresh poultry carcasses with decontamination substances are dealt with in other opinions of the respective panels of EFSA, including a specific mandate on related antimicrobial issues (EFSA, 2006, 2008a).

Figure 1 illustrates ways in which AMR can arise in food as consumed, against a background that includes the continued use of antimicrobials in human medicine and in food production (see, for example, Aarestrup (2006) for other pathways of transmission of AMR bacteria to humans). Because of their complexity, the factors that can lead to the contamination of food in the final stages of its preparation, as in the kitchen, are not addressed in detail here. The direct relevance of the application of good hygienic practices in this and other phases of the food chain in preventing and controlling such contamination is emphasised.

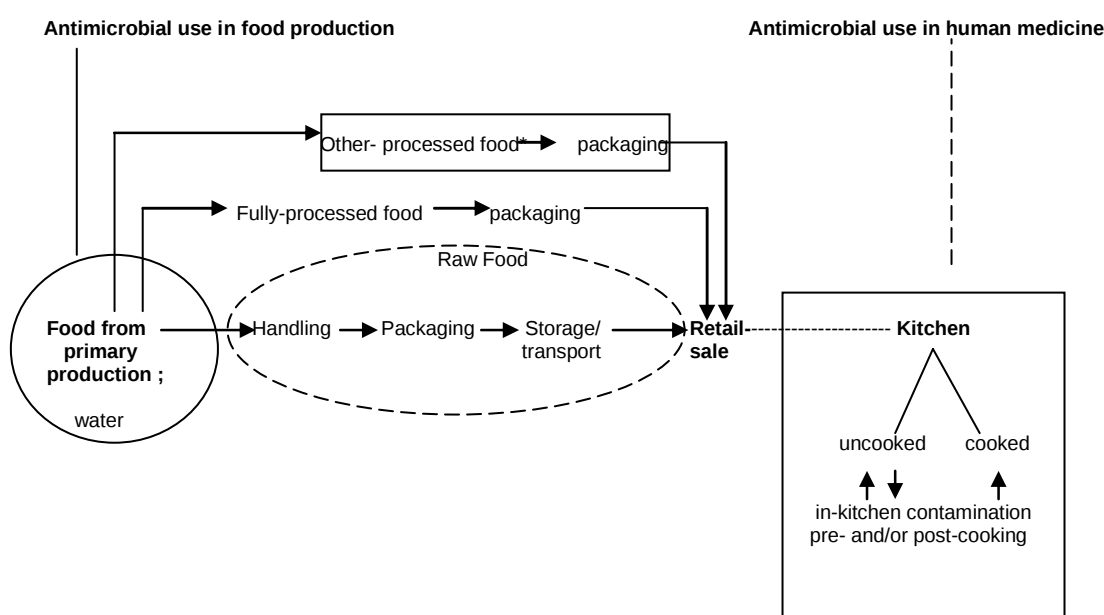


Figure 1. A schema for the possible transmission of antimicrobial resistance *via* food⁸.

Transfer of antimicrobial resistance can involve different kinds of microorganisms. Human bacterial pathogens can be acquired directly by person-to-person spread and from the environment, as well as from animals including both food producing animals and domestic

⁷ Biocides: "Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means." Directive 98/8/EC

⁸ Food from primary production includes fresh meat, fruit and vegetables. Water is also included in this category

pets, or as foodborne pathogens directly from food. Commensal bacteria, i.e. those bacteria belonging physiologically to the human or animal microflora and which are not primarily considered as pathogenic for their host, can likewise be acquired by the consumer through contaminated food or from the environment. Bacteria deliberately introduced into the food chain for manufacturing purposes, e.g. fermentation cultures, and probiotics, likewise require to be considered in the context of antimicrobial resistance transfer through the agency of food. In a wider sense also, bacteria belonging to the natural food microflora belong to the latter group of microorganisms.

In accordance with Directive 2003/99/EC, EFSA is responsible for preparing the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the EU. Further to this, EFSA's Task Force on Zoonoses Data Collection has also published proposals for harmonised monitoring schemes for antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers (EFSA, 2007a), and has proposed technical specifications for a planned baseline survey on MRSA in breeding pigs (EFSA, 2007b). Proposed harmonisation for monitoring of antimicrobial resistance in other bacteria is under consideration.

2. Relevant antimicrobials and definition of antimicrobial resistance

Antimicrobials encompass antibacterial, antiviral, antifungal and antiparasitic agents. In this document, the term will be limited to antibacterial agents classically used for therapy, prophylaxis or until recently (in the EU), growth promotion. As explained above, the effect of disinfectants and other biocides on antimicrobial resistance are not addressed in this document.

Antimicrobial susceptibility or resistance is generally defined on the basis of *in vitro* parameters. The terms reflect the capacity of bacteria to survive exposure to a defined concentration of an antimicrobial agent, but different definitions are used depending on whether the objective of the investigation is clinical diagnostics (see 2.2.1) or epidemiological surveillance (see 2.2.2).

2.1. Antimicrobials of human and veterinary importance

Antimicrobials are grouped into classes on the basis of chemical structure and mode of action. Most antimicrobials used for the treatment of animals belong to classes that are also used in human medicine. A list of antimicrobial classes, examples of substances used for the treatment of infections in humans and animals, along with comments on cross-resistance within and between classes and examples of resistance genes described to date, are presented in Table A (see Appendix).

The consequences of antimicrobial resistance depend on the role of the antimicrobial class in the treatment of human disease. The World Health Organisation (WHO) convened two expert meetings (WHO, 2005b, 2007) in order to classify antimicrobial drugs as “critically important”, “highly important”, and “important” based on two criteria, namely (i) sole therapies or one of few alternatives to treat serious human disease, and (ii) used to treat diseases caused by microorganisms that may be transmitted via non-human sources or diseases caused by microorganisms that may acquire resistance genes from non-human sources. A number of antimicrobial classes were categorised as critically important to human health. Participants concluded that from a public health perspective, the antimicrobial classes of greatest priority for risk management are: quinolones, 3rd/4th generation cephalosporins and macrolides (WHO, 2007).

Similarly, the World Animal Health Organisation (OIE) has developed and adopted a list ranking the importance of different antimicrobials for animal health (OIE, 2007b).

2.2. Definitions of resistance

2.2.1. Clinical resistance

Clinically-resistant infections are defined as those infections having a low probability of clinically responding to treatment, even if maximum doses of a given antimicrobial are administered (EUCAST, 2000; Acar and Röstel, 2003). The outcome of a treatment depends on many factors e.g. the pharmacokinetics of the drug, site of infection, status of the patient and properties of the causative agent. Clinical resistance cannot, therefore, be predicted by *in vitro* tests alone. The Minimum Inhibitory Concentration (MIC) of a drug for a bacterium isolated from clinical samples is used for guidance purposes, however.

A bacterial isolate is categorized as resistant when the obtained MIC of the drug is associated with a high likelihood of therapeutic failure of treatment with that drug. To facilitate the interpretation, threshold values or breakpoints are defined by national or international committees on the basis of, for example, pharmacokinetics, clinical trials and microbiology. Clinical breakpoints are intended for use in everyday clinical laboratory work to advise on therapy in the patient and may vary between countries and over time (Kahlmeter et al., 2003). The fact that the clinical break-points are defined differently by a number of National Committees hampers comparison of published data. Within EUCAST, activities to harmonise clinical breakpoints are ongoing (Kahlmeter et al., 2003; 2006).

2.2.2. Microbiological resistance

When a bacterium can tolerate higher concentrations of an antimicrobial than phenotypically related bacteria of the original or “wild type” strain (Acar and Röstel, 2003), it is defined as being resistant. Such isolates are phenotypically different from the wild type because of their acquisition of a resistance mechanism either by gene transfer or mutation (acquired resistance). Interpretation criteria for *in vitro* tests are based on the distribution of MICs among large collections of wild-type bacteria. An isolate is categorized as resistant when the MIC of a certain drug is higher than that which is expected for wild-type strains. An isolate classified as resistant by this criterion may well be classified as susceptible by clinical criteria.

The values used for categorisation are termed “epidemiological cut-off values”. As they are based on properties of a bacterial species, these interpretation criteria will not change over time or between countries (Kahlmeter et al., 2003). The use of epidemiological cut-off values provides an appropriate level of sensitivity when measuring resistance development in bacteria. These criteria have been harmonised between MS and are independent of the source of the bacterium investigated. EUCAST and EFSA have proposed the use of such criteria for monitoring of resistance in bacteria of concern both to human and veterinary medicine in the European Union (Kahlmeter et al., 2003; EFSA, 2006a).

In this Opinion, resistance is understood as microbiological resistance unless otherwise stated.

2.2.3. Inherent (intrinsic) resistance

Intrinsic resistance is a trait of a bacterial species. For example, the target of the antimicrobial agent may be absent in that species, the cell wall may have poor permeability for certain types of molecules or the bacterial species may inherently produce enzymes that destroy the

antimicrobial agent. These bacteria are clinically resistant, but should more accurately be referred to as “insensitive”.

2.2.4. *Acquired resistance*

A bacterial strain can acquire resistance either by mutation or by the uptake of exogenous genes by horizontal transfer from other bacterial strains. Genes encoding enzymes that can modify the structure of an antimicrobial are commonly transferable (penicillinases and cephalosporinases (*bla*-genes), acetyl transferases modifying e.g. aminoglycosides (*aac*-genes), as are genes leading to target modification (*erm*-genes), methicillin⁹-resistance (*mecA*-genes) and glycopeptide-resistance (*van*-genes). There are several mechanisms for horizontal gene transfer, and they often function in concert. Large plasmids with many different genes can be transferred from bacterium to bacterium by conjugation. Transposons can carry several resistance genes. They cannot replicate by themselves, but can move within the genome, e.g. from plasmid to plasmid or from chromosome to plasmid. Integrons can also encode several resistance genes. They cannot move by themselves, but encode mechanisms both to capture new genes and to excise and move cassettes with genes within and from the integron.

2.2.5. *Cross-resistance*

Antimicrobials are a diverse group of molecules, commonly ordered in classes with similar structure and mode of action (Table A, Appendix). Within a class, the target in the bacterial cell and the mode of action of the antimicrobial is the same or similar in each case. Therefore, some mechanisms of resistance will confer resistance to most or all members of a class, i.e. cross-resistance. Cross-resistance may also occur in relation to unrelated classes, if the target overlaps (as in the case of macrolides and lincosamides) or if the mechanism of resistance is of low specificity (e.g. affecting efflux pumps).

2.2.6. *Co-resistance*

Genes conferring antimicrobial resistance are frequently contained in larger genetic elements such as integrons, transposons or plasmids, and as such may be ‘linked’ to other, unrelated resistance genes. In such cases, multiple resistance genes may be transferred in a single event. When two or more different resistance genes are physically linked, this is termed “co-resistance”. Consequently, selection for one resistance will also select for the other resistance gene(s).

2.2.7. *Multiple resistance*

Multiple resistance (MR), sometimes referred to as “multi-resistance” is used here when a bacterial strain is resistant to several different antimicrobials or antimicrobial classes. There is no standard definition, which makes the term problematic and comparisons difficult.

⁹ Methicillin (International Nonproprietary Name) = Methicillin (United States Adopted Name)

3. Hazard identification

3.1. Direct and indirect hazards

The issue of antimicrobial resistance in food is addressed as existing either as a direct hazard or as an indirect hazard through resistance transfer¹⁰. The direct hazard is the presence on food of an antimicrobial-resistant pathogenic bacterium which can colonise or infect a human being after ingestion of the food, or as a hazard that arises if a person acquires the infection through handling contaminated food. The indirect hazard arises through resistance transfer and is defined as an antimicrobial-resistant bacterium that may transfer resistance genes to a bacterium pathogenic for humans, either directly, or via another commensal bacterium. In this case, the hazard is considered as being the resistance gene.

3.2. Resistance mechanisms and hazards

The resistance mechanisms involved may be classified in four large groups, as follows: (1) Enzymatic inactivation/degradation mechanisms such as β -lactamases degrading penicillins and cephalosporins and aminoglycoside modifying genes. (2) Alternative pathways, such as resistance to dihydrofolates antimicrobials e.g. sulphonamides and trimethoprim resistance. (3) Permeability changes, rendering the bacterium impermeable (altered porins) or a change in the rate of pumping out the antimicrobial (efflux). An example is tetracycline resistance. (4) Target alteration such as resistance to macrolides and (fluoro)quinolone antimicrobials.

3.3. Resistance transfer and hazard

Transfer of resistance genes between bacteria can occur at any point along the food chain, or within one body system (e.g. the intestine), or can occur between systems (e.g. from the intestinal tract to bacteria on the skin). These transfers can happen through three different mechanisms, (1) Conjugation, where a mobile genetic element (plasmid, transposon, gene cassette) can be transferred from one bacterium to another bacterium. (2) Transduction, where a bacteriophage takes up a resistance gene from one bacterium and transfers this to another bacterium. (3) Transformation, where naked DNA released from one bacterium is taken up by another bacterium.

3.3.1. *Transfer of antimicrobial resistance to bacteria by conjugation*

Conjugation is the mechanism by which genetic material transfers from one bacterium to another through a protein tunnel that temporarily connects the two bacteria. Such transfer may occur between bacteria of different species or even different genera. The elements transferred may be plasmid- or transposon-mediated. The transposable elements may be able to induce conjugation by themselves (self-transposable elements) or may need some functions coded by other genetic elements belonging to the bacterium itself, or to another mobile element. This is the most frequently reported mechanism of resistance transfer to date. The elements that are able to transfer often contain more than one gene for resistance and may encode linked resistance genes. One transfer may likewise deliver multiple resistances to the recipient bacterium.

¹⁰ A bacterium may also present both a direct and indirect hazard: e.g. a resistant pathogenic bacterium with a resistance gene(s) carried on a potentially transferable element.

3.3.2. *Transfer of antimicrobial resistance by transduction*

Transduction is the mechanism by which bacteriophages transfer genes from one bacterium to another. By taking up host DNA from one bacterium, and after lysis of the host cell (and release of the phages) the phage can introduce new genetic material into another (same) phage-susceptible bacterium.

3.3.3. *Transfer of antimicrobial resistance to bacteria by transformation*

While the processes of conjugation and transduction require viable donor cells, this is not the case for transformation. Successful transformation and expression of antibacterial resistance in bacterial cells is based on the following essential steps: 1) release of the DNA from the donor; 2) uptake of the DNA by competent bacteria in the vicinity; 3) stable incorporation of the DNA in the recipient cell and 4) expression of the incorporated DNA.

3.4. Food processing technologies and possible antimicrobial resistance development

Most food processing technologies aim to reduce the numbers of foodborne pathogens present, including AMR bacteria, as well as the overall bacterial load. Hence, food deterioration and the possibility of foodborne infections are reduced. This important beneficial effect has to be considered when evaluating any potential hazards arising from food processing with respect to antimicrobial resistance.

Emerging non-thermal processing/preservation technologies (e.g. high-pressure processing, ionizing radiation, pulsed electric field and ultraviolet radiation) are technologies designed to produce safe food, while maintaining its nutritional and sensory qualities. Experimental studies have shown that through damage to cell membranes, enzymes or DNA (Lado and Yousef 2002), such alternative preservation technologies could promote the generation or transfer of antimicrobial resistance (Zenz et al., 1998; Davison, 1999; IFT, 2002; Lado and Yousef 2002; Kharazmi et al., 2002; C  r  monie et al., 2004, 2006; Rodrigo et al., 2005, 2007; McMahon et al., 2007). Whilst these studies remain at the laboratory level, the relevance for industrial food processing remains to be defined.

3.5. Bacteria with multiple resistance as a hazard

For many bacteria, multiple resistance may create health problems, since the use of one antimicrobial will also select for resistance to other unrelated antimicrobials. Specific structures (integrons) that can collect and express antimicrobial resistance genes are present in bacteria. Furthermore, the genes encoding resistance in bacteria exhibiting multiple resistance may also be located on separate mobile elements.

Bacteria resistant to the latest categories of antimicrobials are also more likely to be multiply resistant (e.g. to 3rd and 4th generation cephalosporins), rendering the disease they cause more difficult to treat and prone to therapy failure.

3.6. Links between resistance and virulence as a hazard

Increased frequency of treatment failures and increased severity of infection due to infections with AMR bacteria are the principal human health concerns. They may be manifested by prolonged duration of illness, increased frequency of bloodstream infections, increased hospitalization, or increased mortality (WHO, 2005a).

Virulence of a bacterium is in general encoded by either a number of single genes or a cluster of genes, interplaying at different levels of the pathogenesis. Some genes, when deleted may be so essential in certain steps of the pathogenesis that virulence is abolished completely, while other genes are only of additional value to the virulence of the bacterium. Frequently, virulence genes are encoded on mobile genetic elements. Other elements contributing to pathogenicity are the so-called 'Pathogenicity Islands', 12 of which have been identified in *Salmonella enterica* (Hensel, 2004). Further virulence factors in some *Salmonella* serotypes and phage types are those which are involved in the iron sequestration system, thereby providing their host strains with the ability to survive in environments where iron is not readily accessible to the bacterium, such as blood. These virulence factors can have a substantive impact on the invasive ability of their host strains. Finally, genomic islands, such as SGI1 in *S. enterica*, may carry both virulence and antimicrobial resistance genes (Golding et al., 2007). As co-localisation of virulence genes and resistance genes on the same mobile genetic element has been reported (Carlson et al., 2007), the transfer of resistance, and simultaneously the transfer of the co-resident virulence genes, may give the bacterium, in addition to a newly acquired resistance, an enhanced virulence.

Genes that have functions both in virulence and antimicrobial resistance are also known. An example of this are some efflux pumps in *Campylobacter* (Lin et al., 2007; Quinn et al., 2007; Piddock et al., 2006).

MRSA frequently contain the genes associated with enterotoxins (Fey et al., 2003; Yarwood et al., 2002), which are the proteins which cause staphylococcal food poisoning. Different combinations of enterotoxins are associated with different MRSA clones (Ferry et al., 2006; Tristan et al., 2007). Enterotoxin genes are either located on mobile genetic elements, or within pathogenicity islands. Increased prevalence of MRSA amongst *S. aureus* strains could lead to a higher prevalence of toxinogenic *S. aureus*. It is not clear why there is an association with specific lineages of MRSA and enterotoxin genes. Food poisoning due to MRSA remains very rare (see Section 4.1.7.3).

3.7. The hazard of the bacterium as a carrier of resistance genes

The antimicrobial-resistant bacteria that pose a particular hazard are primarily those with resistance to the first-line drug of choice used in the treatment of a specific bacterial disease. Central to this is that upon ingestion of, or other contact with, these bacteria, the resistance gene(s) can be transferred directly or *via* an intermediary, to a human pathogenic bacterium. The likelihood of such transfer will be higher if the host is simultaneously exposed to an antimicrobial to which the bacteria are resistant (McConnell et al., 1991; Doucet-Populaire et al., 1991). Furthermore, such use will amplify resistance by selecting for any resulting transconjugants.

We can identify three different groups of resistant micro-organisms that may be of importance; Firstly, zoonotic agents and other food borne pathogens. They can directly pose a hazard, since in some cases, the conditions they cause need clinical treatment and, if resistant, they cannot be successfully treated with the antimicrobials against which the bacterium is resistant. Also, some of the bacteria remain for a certain period in the intestinal tract, where they may exchange or acquire resistance genes.

Secondly, commensals are also a potential AMR hazard. This is largely dependent on the capacity of the ingested food-derived commensals to come in contact with human commensals and pathogens. As the gastro-intestinal tract is the place with the highest abundance of host bacteria, the ability of the commensal to remain in this environment is of major importance for

the exchange of resistance genes. Other factors of influence are the mobile element on which the resistance genes are located, and the ability to form biofilms.

Thirdly, “industrial” or “technological” and /or other bacteria intentionally added to the food chain, may also be regarded as a potential hazard. These bacteria have a function in e.g. fermentation or preservation of the food product, or may be added specifically with a health claim, as in the case of probiotics. Bacteria added to the food chain should not carry potentially transferable resistance genes, (FAO/WHO, 2001; EFSA, 2005a; EFSA, 2007c) as it cannot be excluded that they may transfer their resistance genes directly or indirectly to pathogenic bacteria.

There is a paucity of information about the total presence, nature and evolution of antimicrobial resistance in the intestine. Likewise, there is only limited information about the rates of transfer of antimicrobial resistance in the gastrointestinal tract involving species other than *E. coli* and enterococci. Although only few data exist, mainly from *in vitro* models and from experiments on mice (e.g. Doucet-Populaire et al., 1991; McConnell et al., 1991), transfer of resistance from an ingested strain of *Escherichia coli* K12 to intestinal *E. coli* bacteria has been demonstrated in volunteers, albeit at low frequency (Anderson 1975). Also, *in vivo* transfer of *vanA* genes from *Enterococcus faecium* isolated from chicken to intestinal enterococci in human volunteers has been detected (Lester et al., 2006).

As for resistance genes in genetically modified food products, the reader is referred to Section 6.2.6 in this document, and to EFSA (2004b).

3.8. Transmission and exposure routes

Cross-contamination with AMR bacteria resulting from improper handling of food is a well known phenomenon and has been widely studied (Kusumaningrum et al., 2004; Mylius et al., 2007). *Campylobacter* spp. are more likely to be spread from primarily contaminated food like fresh chicken to other food prepared in the kitchen (e.g. ready-to-eat fresh salad) than are *Salmonella* spp. (Kusumaningrum et al., 2004). Fresh chicken with a high contamination level of antimicrobial-resistant *Campylobacter* will be a likely source of contamination for other foods if appropriate standards of food hygiene are not consistently applied at the distribution and retail phases of the food chain and, in particular, in the kitchen in the course of final food preparation and presentation.

In addition to the transmission routes mentioned above, other sources of food contamination with AMR bacteria are the smaller companion animals such as those kept as domestic pets in the private household.

Bacteria known to be spread by pets (such as dogs, cats, and exotic species including reptiles) include *Campylobacter*, *Salmonella* spp. (Marcus, 2008) and MRSA (Weese et al., 2006). As bacteria present in the intestinal tract of pets can also carry antimicrobial resistance of clinical relevance (Rossi et al., 2007), household pets could be a direct source of AMR bacteria in the kitchen.

Furthermore, cross-contamination in the kitchen can also result from a variety of sources including storage facilities such as refrigerators, and the use of work surfaces and towels that remain contaminated following the preparation of other foods (Kruse and Sorum, 1994).

4. Examples of hazards

4.1. Human pathogens

4.1.1. *Non-typhoid Salmonella*

4.1.1.1. Hazard identification and characterization

Salmonella is a zoonotic agent that readily infects humans. In general, treatment with antimicrobial drugs is not recommended for cases of salmonellosis in otherwise healthy individuals. Nevertheless, in the elderly, very young, or immunocompromised patients, treatment with an appropriate antimicrobial can be life-saving. Likewise, should a strain spread from the intestine to normally sterile body sites, then treatment with an appropriate drug is essential. In such cases, infection with an antimicrobial resistant *Salmonella* may pose an additional public health risk to that posed by infections that are susceptible.

Food is regarded as an important infection route for *Salmonella* including AMR *Salmonella*. There are numerous reports directly implicating foodborne AMR *Salmonella* in human disease (see 4.1.1.2 below), and a limited number of reports confirming transmission of AMR strains from the food animal, into foods, and subsequently to the human population. In 1984, a strain of *Salmonella* Newport with resistance to ampicillin and tetracyclines originating in cattle in the USA was traced through the food chain to humans (Holmerg et al., 1984); in 1998, an outbreak of multiresistant *S. Typhimurium* with additional resistance to quinolone antimicrobials, in which 15 persons were affected was traced through the food chain to pigs (Molbak et al., 1999). In the same year an outbreak of multiresistant *S. Typhimurium* DT 104 in the UK, involving over 200 persons, and in which the vehicle of infection was milk, was traced to the farm of origin (Walker et al., 2000). In all three examples, the causative organism was isolated from the food animal, from foods, and from patients.

To some extent antimicrobial resistance in *Salmonella* is serotype-dependent, with resistance and multiple resistance common in serotypes such as Typhimurium, Virchow, Derby and Newport (Threlfall et al., 2000a; Varma et al., 2006) and more recently Hadar (Threlfall et al., 2003) and Paratyphi B variant Java (Miko et al., 2003; Evans et al., 2005; Threlfall et al., 2005). In contrast, other serotypes important for public health, for example *S. Enteritidis* rarely display multiple resistance although resistance to antimicrobials such as nalidixic acid and ciprofloxacin is increasing in incidence, with over 20 % of isolates in infections within EU Member States from 2000-2005 exhibiting such resistance (Meakins et al., 2008).

Salmonella Typhimurium definitive phage type (DT) 104 is a multiresistant phage type with almost global epidemicity. Since first identified in the late 1980s in the UK (Threlfall, 2000) the organism has caused outbreaks in many countries throughout the world, with a variety of food associations (Molbak et al., 1999; Walker et al., 2000; Threlfall, 2000; Horby et al., 2003). Although declining in incidence in Europe, this *S. Typhimurium* strain remains a significant public health hazard world-wide.

The strain is typically penta-resistant (ampicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and tetracyclines (ACSSuT)), resistance encoded within a mobile genetic element designated *Salmonella* Genomic Island-1 (SGI-1). SGI-1 has also been identified in other Typhimurium phage types, as well as at least 10 other *Salmonella* serotypes including Agona, Albany, Newport and Paratyphi B variant Java.

A further multiresistant strain which has been associated with international food-borne outbreaks is *S. Typhimurium* DT 204b with resistance to up to 9 antimicrobial drugs. In 2000 the strain was responsible for at least one major international outbreak involving 10 countries epidemiologically-linked to contaminated salad vegetables (Crook et al., 2003).

Extended spectrum beta-lactamase (ESBL) resistance has recently arisen worldwide in *Salmonella*. Strains exhibiting such resistance have been detected in both humans and animals (Bertrand et al., 2006; EMEA, 2008). In Belgium, a cephalosporin-resistant *Salmonella* Virchow clone was found throughout the food chain. A similar spread was demonstrated for cephalosporin-resistant *S. Infantis*. In this case, ESBL resistance was located on a conjugative plasmid that had already spread to some other serotypes, including Paratyphi B variant Java and Typhimurium (Bertrand et al., 2006; Cloeckaert et al., 2007).

4.1.1.2. Exposure through foods

The occurrence of antimicrobial-resistant *Salmonella* spp. in pig meat in five Member States (MS) has been reported (EFSA, 2006b). The proportion of isolates reported (serotypes mostly unspecified, but including Derby, Enteritidis, Infantis, London, Saintpaul, Senftenberg, Typhimurium and Virchow) to be resistant to ampicillin in each of the five MS ranged from 21% to 35%, while resistance to sulphonamides (36 % to 52%) and tetracycline (38% to 59%) was also common. Resistance to ciprofloxacin was reported in 1% of isolates in Denmark, and resistance to enrofloxacin was reported in 0.6% of isolates by Italy. No data was reported for the other MS. In the UK, studies of shell eggs coordinated by the Food Standards Agency have demonstrated a substantive level of resistance in *S. Enteritidis* (most were resistant to nalidixic acid with reduced susceptibility to ciprofloxacin) isolated from eggs imported into the UK, but little if any resistance in isolates of *S. Enteritidis* from home-produced eggs (FSA, 2006). Similarly, studies of organisms from raw red meats in the UK has demonstrated contamination of beef, lamb and pork with a range of multiresistant *S. Typhimurium* phage types, with DT 104 and related strains predominating, whilst resistance was rare in other serotypes (Little et al., 2008).

4.1.1.3. Reports linking foodborne AMR *Salmonella* to human infections

The EFSA report on source attribution for human salmonellosis in meat summarises the findings from 5 attribution studies (EFSA, 2008b). This summary indicates that the proportion of foodborne cases varies, but is around 90 - 95% of cases. In the EU, amongst the foodborne cases, eggs, and egg products are the most frequently implicated sources. Meat is also an important source, with poultry and pork implicated more often than beef and lamb. The main food sources of *Salmonella* outbreaks in the UK are desserts (26%); poultry (25%); red meat (14%) and eggs (13%) (Adak, 2005). Results from a Dutch case-control study for *S. Enteritidis* and *S. Typhimurium* do not contradict the outbreak sources given above (Doorduyn et al., 2006). Doorduyn et al. (2006) found that for *S. Enteritidis*, consumption of raw eggs and products containing raw eggs were identified as risk factors (which may correspond to the 26% of outbreaks caused by the consumption of desserts in the UK study). Similarly for *S. Typhimurium*, occupational exposure to raw meat and the consumption of raw meat were identified as risk factors.

In a US FoodNet case-control study of sporadic multiple-resistant *Salmonella* Newport infections Varma et al. (2006) concluded that patients were more likely to have consumed uncooked ground beef or runny scrambled eggs or omelettes prepared in the home. Travel was not a risk factor for multiple-resistant *S. Newport*. Earlier studies (including outbreak

investigations) for the same hazard, incriminated consumption of ground beef, ground horse meat and cheeses made from nonpasteurised milk. In the published literature, contaminated milk (Olsen et al., 2004), lettuce (Horby et al., 2003), dried anchovy (Ling et al., 2002) and raw-milk cheese (Cody et al., 1999, Villar et al., 1999) among other foods have all been identified as food vehicles for outbreaks of multidrug-resistant *Salmonella* Typhimurium.

Microbial sub-typing can provide useful information during outbreak investigations but also at a population level. Isolates derived from humans, animals and food are obtained, analysed using a discriminatory method and are finally compared. This procedure has become popular as an attribution method for non-typhoidal *Salmonella* due to the fact that particular *Salmonella* serotypes are more likely to be observed in certain animals and/or foods. Using such information and routine surveillance data, attribution is routinely undertaken in both the Netherlands (Van Pelt et al., 1999) and Denmark (Hald et al., 2004) using statistical models. Using this approach, the main sources of *Salmonella* are identified to be eggs and pork in The Netherlands and table-eggs, pork and imported chicken in Denmark. In an extension of the study by Hald et al. attribution of antimicrobial-resistant *Salmonella* related cases has also been investigated (Hald et al., 2007). In this study they considered the attribution of resistant, multi-resistant and quinolone-resistant strains and concluded that (a) imported poultry and Danish eggs were important sources for quinolone-resistant *Salmonella*, (b) pork (Danish and imported) and imported beef for multidrug-resistant *Salmonella* infections and (c) Danish pork for resistant *Salmonella* infections. Also, (d) travel was associated with the acquisition by consumers, of multi-resistant and quinolone-resistant *Salmonella* strains.

4.1.2. Typhoidal *Salmonella*

4.1.2.1. Hazard identification and characterisation

Typhoid fever, sometimes known as enteric fever, is a disease caused by the bacterium *Salmonella enterica* serotype Typhi. Typhoid fever is a serious disease which can be life-threatening unless treated promptly with appropriate antimicrobials, which may be necessary before the results of laboratory sensitivity tests are available. The disease varies in severity, but nearly all patients experience fever and headache. Slightly less serious, but nevertheless very debilitating and possibly fatal, is enteric fever resulting from infections with *Salmonella* Paratyphi A. Again, appropriate antimicrobial treatment is essential and should be commenced as soon as the disease is diagnosed. The first-line antimicrobials of choice for infections with both *Salmonella* Typhi and Paratyphi A are fluoroquinolones such as ciprofloxacin, with third-generation cephalosporins and azithromycin as possible alternatives, particularly when the causative strains are resistant to the first-line antimicrobial. Both *Salmonella* Typhi and Paratyphi A are not indigenous to Member States, and the majority of infections are linked to travel to endemic areas such as the Indian sub-continent, Africa, or south and central America. Antimicrobial resistance to therapeutically important antimicrobials is of major concern, and strains with resistance or decreased susceptibility to antimicrobials such as ciprofloxacin are becoming widespread in developing countries, particularly the Indian sub-continent and consequently in travellers returning from these areas to Member States and elsewhere (Threlfall et al., 2008).

4.1.2.2. Exposure through foods

Salmonella Typhi and Paratyphi A do not have a food animal reservoir and infections are for the most part spread by eating food or drinking beverages that have been improperly handled by

an infected person, or by drinking water that has been contaminated by sewage containing the bacteria.

4.1.2.3. Reports linking foodborne AMR *Salmonella* Typhi to human infections

Substantive outbreaks of typhoid fever in developing countries caused by drug-resistant strains have been reported as a result of contamination of water supplies, with significant mortality (Mermin et al., 1999), which clearly demonstrates the importance of sanitation and an unpolluted water supply.

4.1.3. *Thermophilic Campylobacter*

4.1.3.1. Hazard identification and characterization

In recent years in the European Union, *Campylobacter* has been the most commonly reported diarrhoeal bacterial zoonotic pathogen (EFSA 2005b, 2006b). Most infections are caused by *Campylobacter jejuni* and *Campylobacter coli* of which *C. jejuni* accounts for the vast majority (>95%) of infections. Patients usually recover without antimicrobial therapy, but in some patients with severe, prolonged, or relapsing illness such therapy may be indicated. Macrolides are normally considered the drug of choice, but fluoroquinolones are also recommended (Blaser 1990; Goodman et al., 1990; Petrucci, et al., 1992; Salazar-Lindo, et al., 1986; Skirrow and Blaser, 2002). In a recent study of eleven randomised controlled trials of antibiotic treatment versus placebo in patients with *Campylobacter* infections, antibiotic treatment with erythromycin or fluoroquinolones significantly shortened the duration of intestinal symptoms (Ternhag et al., 2007). Increases in the occurrence of *Campylobacter* causing infections in man that are resistant to macrolides and fluoroquinolones have been reported in several countries (Endtz et al., 1991; Rautelin, et al., 1991; Reina et al., 1994; Sanchez et al., 1994; Gaudreau and Gilbert, 1998; Sjøgren et al., 1997; Hoge et al., 1998; Smith et al., 1999). As food animals are considered one of the most important sources of *Campylobacter* causing infections in man, the development of antimicrobial resistance in *Campylobacter* spp., due to the use of antimicrobial agents in food animals, is therefore a matter of concern should antimicrobial therapy be indicated.

Several studies have shown that infections with fluoroquinolone-resistant *Campylobacter* in humans are associated with adverse effects for human health, mainly measured by prolonged diarrhoea (Smith et al., 1999; Helms et al., 2005; Engberg et al., 2004; Nelson et al., 2004; *Campylobacter* sentinel, 2002). Although the results of these studies are not all statistically significant they point in the same direction and taken together they suggest that there is a longer duration of illness in patients infected with fluoroquinolone-resistant strains. In addition, it has been shown that there is an increased risk of death or invasive illness following an infection with a fluoroquinolone- or macrolide-resistant *Campylobacter* compared to susceptible strains (Helms et al., 2005). The effect of macrolide resistance on human health consequences have only been estimated in this one study and the results require to be verified in additional studies. In contrast a critical examination of available data by Wassenaar et al. (2007) has suggested that fluoroquinolone-resistant *Campylobacter* infections are not more severe than those caused by susceptible strains.

4.1.3.2. Exposure through foods

Thermophilic *Campylobacter*, especially *C. jejuni* and *C. coli* are normal inhabitants of the gastrointestinal tract of most warm-blooded animals including the major food-animals cattle, swine and poultry. Surveys of the faeces of healthy cattle and swine consistently show high isolation rates, often above 50%, but the actual carrier rate in food-animals is likely to be higher due to limitations in detection methods. Surveys of poultry, notably chicken, turkeys, ducks and geese, indicate large variations in the proportions of flocks that are infected. The large variation depends on the type of production system, the geographical location and on the time of year (season).

Because *Campylobacter* is often present in animal faeces, *Campylobacter* can be found where faeces contamination occurs. During evisceration, particularly of poultry, when the intestines and other internal organs are being removed, some degree of faecal contamination is inevitable no matter how stringent the hygiene measures that are applied. It is however important to note that, following evisceration, the *Campylobacter* present on carcasses do not multiply further. They may, however, be passed on to other products by cross-contamination. The rate by which *Campylobacter* dies in the food processing and food distribution system depends on many factors. The most important factors are temperature, oxygen tension and water activity (a_w) (Tomancova et al., 1991; Lee et al., 1998; Bhaduri and Cottrell, 2004). The potential transmission of *Campylobacter* along the food chain greatly depends on the contribution of each of the different processing steps from slaughter to meat packing.

Freezing or chilling of poultry meats has been shown to greatly reduce the number of live *Campylobacter* including AMR *Campylobacter* present on the product.

There are no significant biological reasons why resistant *Campylobacter* should not transmit equally well from animals to humans, as does sensitive *Campylobacter*. A temporal association between resistance emergence and its increase in animals and humans following the introduction of the antimicrobial in animal production has been shown by several studies (Endtz et al., 1991; Smith et al., 1999; Engberg et al., 2001). In addition, results of a further study have indicated that certain strains gain increased fitness, as defined by Luo et al. (2005), when acquiring fluoroquinolone resistance mutations (Luo et al., 2005).

Most studies conducted in several countries have identified poultry (especially consumption of undercooked chicken) as the main risk factor for sporadic campylobacteriosis (Wingstrand et al., 2006). Additional risk factors include foreign travel, drinking contaminated water or milk, barbecuing, swimming in contaminated water and contact with pet animals. Other epidemiological data also identify poultry as the main reservoir of human campylobacteriosis.

Development of AMR in *C. jejuni* and *C. coli* has important public health implications. Food is a recognized vehicle through which exposure can occur. Several studies have examined the occurrence of *Campylobacter* in various food categories (Meldrum and Wilson, 2007; Mena et al., 2008; Roasto et al., 2007). In a study reported from Korea, 770 retail raw meat samples were investigated for multi-drug resistant *Campylobacter* and these data demonstrated the widespread nature of the organism (Hong et al., 2007). Levesque et al., (2007) compared *Campylobacter jejuni* isolates from humans, with those recovered from various foods, including chicken, raw milk and the environment. Isolates from chickens were resistant to erythromycin, a feature that could lead to treatment failure in humans.

4.1.3.3. Reports linking foodborne AMR *Campylobacter* to human infections

A number of case-control studies have specifically addressed risk factors for fluoroquinolone-resistant *Campylobacter*. Examples include those by Smith et al. (1999); The *Campylobacter* Sentinel Surveillance Scheme Collaborators (2002); Engberg et al. (2004); Kassenborg et al. (2004); Nelson et al. (2004) and Johnson et al. (2008). All of these case-control studies identified foreign travel as a risk factor for acquisition of a fluoroquinolone-resistant *Campylobacter* infection. In most of the studies, it is not possible to conclusively say what the exposure food-stuff/route might have been when travellers visited these countries, although the *Campylobacter* sentinel study identified consumption of chicken and bottled water as risk factors for travel-related cases. Risk factors for non-travel related cases of fluoroquinolone-resistant *Campylobacter* were as follows: use of a fluoroquinolone before the collection of the stool specimen (Smith et al., 1999); consumption of cold meat (precooked) (The *Campylobacter* Sentinel Surveillance Scheme Collaborators 2002); consumption of fresh poultry other than chicken and turkey (Engberg et al., 2004); swimming (pool, ocean, lake or other places) (Engberg et al., 2004); consumption of chicken or turkey cooked at a commercial establishment (Kassenborg et al., 2004) and possession of non-prescribed antimicrobials (Johnson et al., 2008).

In Norway, the prevalence of fluoroquinolone resistance among *C. jejuni* isolates from imported and indigenous sporadic human cases of campylobacteriosis and from domestic broilers was assessed (Norström et al., 2005). Among the imported human isolates, 67.4% were resistant to ciprofloxacin compared with 6.5% of indigenous human isolates. The prevalence of resistance in indigenous human isolates was comparable with the prevalence of resistance in isolates from Norwegian broilers (1.2% fluoroquinolone resistant). No quinolone preparations are licensed for use in broilers in Norway

4.1.4. Verotoxigenic *Escherichia coli* (VTEC) of public health concern

4.1.4.1. Hazard identification and characterization

Verotoxigenic *Escherichia coli* (VTEC) has emerged as a public health threat since its identification in 1982 following an outbreak in the USA associated with the consumption of contaminated ground (minced) beef (Riley et al., 1983). *Escherichia coli* O157:H7 and O157:NM (non-motile) are major aetiological agents in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in humans (Mead and Griffin, 1998). Currently more than 200 *E. coli* serotypes are recognized, and these have been isolated from animals, food and other sources. Of these, only 60 serotypes have been linked with human disease. In the US, the Centres for Disease Control & Prevention (CDC) estimate that *E. coli* O157:H7 causes approximately 73,400 illnesses and 60 deaths each year (Mahon et al., 1997; Mead et al., 1999).

Bovines are a major VTEC reservoir and resistant strains may colonise the human population via the food chain. Earlier reports signalled an increase in antimicrobial resistance among O157 and non-O157 serotypes (Aarestrup and Wagner, 1999; Farina et al., 1996; Kim et al., 1994; Threlfall et al., 2000b; Schroeder et al., 2002; White, 2002) whilst others suggested that the occurrence of antimicrobial resistance among three VTEC serotypes was low (Walsh et al., 2006).

The use of antimicrobials for the treatment of human infections with VTEC is controversial. In general, antimicrobials are not recommended as their usage may exacerbate symptoms, particularly haemolytic uraemic syndrome. Antimicrobials may be of use in the early stage of

infection and in some countries, fosfomycin has been used for treatment, with some success (Igarashi, 2002).

4.1.4.2. Exposure through foods

Food products derived from bovines can represent a source from which these pathogens can enter the food chain. Schroeder et al. (2002) described the characterization of 27 *E. coli* cultured directly from food (including beef, pork and unspecified sources), of which 17 were defined as VTEC based on the presence of *stx1* and/or *stx2* markers. Among these isolates 26% were resistant to sulfamethoxazole and a similar percentage was resistant to tetracycline. Apart from these two drug classes, the majority of isolates were susceptible to the complete panel of antimicrobials tested. Interestingly when VTEC and non-VTEC isolates were compared, resistance among the latter was higher, although both were susceptible to third generation cephalosporins (ceftriaxone and ceftiofur). Whereas in a study by Klein and Bülte (2003) in Germany no multiresistant VTEC isolates could be identified, another study recently showed more than 50% of porcine VTEC isolates and 25% of bovine isolates as multiresistant in Germany (von Müffling et al., 2007). In both studies resistance to tetracyclines was most common.

Herd-level surveillance among dairy animal populations is a convenient way to assess the risk of pathogen transmission through milk (Murphy et al., 2005). Using this approach, over a two-year period, 16 VTEC strains were recovered from animals supplying raw milk for the manufacture of cheese. With the exception of one isolate, resistant to streptomycin, all were susceptible to the panel of drugs tested. In a more recent study (Murphy et al., 2007), baseline data were obtained on the prevalence and characteristics of VTEC microorganisms in lactating animals (bovines, ovines and caprines) supplying milk to the farmhouse cheese sector and for the manufacture of raw milk ice cream. Milk samples were analysed for the presence of serotypes O111, O157 and O26 and the susceptibility patterns determined. No O111 serotype was recovered. All of the O157 isolates were susceptible to the panel of 15 antimicrobials tested and among the O26 isolates, three (of 17) were defined as multi-drug resistant, a further three resistant to ampicillin, three more were resistant to tetracycline and only one isolate was resistant to streptomycin. The genetic basis of resistance was not examined in these studies.

There is a paucity of information regarding the mechanisms of antimicrobial resistance among VTEC strains. In a study of 274 VTEC strains (recovered from poultry, bovines, swine and humans) class 1 integrons were detected in 16% of the study population (Singh et al., 2005). These structures facilitate the emergence and dissemination of antimicrobial resistance among strains independent of origin. Similarly, in a study of 105 epidemiologically-unrelated VTEC strains belonging to serogroup O111 from humans and cattle isolated in Germany between 1983 and 2003, resistance was detected in 76 % of isolates mediated by a range of resistance genes including those coding for resistance to ampicillin, chloramphenicol, aminoglycosides, tetracyclines and trimethoprim (Guerra et al., 2006). It is acknowledged that the emergence of resistance among these strains may further limit therapeutic options.

4.1.4.3. Reports linking foodborne AMR VTEC to human infections

Comparisons between non-VTEC and VTEC isolates from food animals and both symptomatic and asymptomatic humans, showed that the former appear to display a broader resistance profile (Bettelheim et al., 2003). Nevertheless, whilst resistance among VTEC strains is still relatively low, (Walsh et al., 2006) surveillance will be important to recognize any future changes in these early trends. One report described antimicrobial resistance in VTEC strains (4

of 59 isolates examined or 6.8%), cultured from patients presenting with diarrhoea and urinary tract infection (UTI) to common antimicrobials including ampicillin and tetracycline (Banerjee et al., 1999). Murphy et al., (2007) similarly reported the identification of multiple-resistant strains recovered from bovine milk used in the production of farmhouse cheese (Murphy, 2007); however, it is not known if any of these isolates caused human infection. In VTEC strains isolated in Bosnia and Germany, all were resistant to sulphonamide, many of the porcine isolates were resistant to oxy- and chlortetracycline, and bovine isolates were resistant to sulphonamide/trimethoprim and ampicillin (von Muffling et al., 2007).

4.1.5. *Shigella*

4.1.5.1. Hazard identification and characterization

The normal presentation of bacillary dysentery caused by strains of *Shigella* of subgroups A, B, C (*Shigella dysenteriae*, *Sh. flexneri*, *Sh. boydii*) and D (*Sh. sonnei*) is that of mild to moderate gastroenteritis. The disease is self-limiting and the primary therapy is oral rehydration. However, symptoms can be severe in the very young, the very old, the malnourished, and patients with other underlying diseases. In such cases, administration of an effective antimicrobial should commence as soon as clinical diagnosis is made. Ampicillin was the antimicrobial of choice until the mid-1980s. Following the widespread emergence of ampicillin-resistant strains this antimicrobial was compromised and was replaced by co-trimoxazole (trimethoprim plus sulphamethoxazole) and nalidixic acid as the first-line drugs. More recently, the American Public Health Association have recommended that, should antimicrobial therapy be indicated for cases of acute shigellosis, then oral trimethoprim-sulphamethoxazole, ciprofloxacin or ofloxacin should be used for adults and oral trimethoprim-sulphamethoxazole, nalidixic acid or parenteral ceftriaxone for children (Chin, 2000).

Within the European Union infections with *Shigella dysenteriae*, *Sh. flexneri*, *Sh. boydii* are normally associated with travel to countries outside Europe, particularly the Indian sub-continent, Africa and south and central America. Infections with *Sh. sonnei* are indigenous in many European countries (Cheasty et al., 2004).

4.1.5.2. Exposure through foods

Although being a foodborne pathogen, shigellae are not considered to have a food animal reservoir and infections are normally a result of person-to person transmission. However, contamination of foods or water by human faecal material has led to human cases. Foods implicated in human cases of shigellosis include fresh fruit and vegetables, raw oysters, deli meats and unpasteurized milk

4.1.5.3. Reports linking foodborne AMR *Shigella* to human infections

Certain food products which have been subjected to contamination by human sewage and that have been responsible for national or international outbreaks have been linked to AMR *Shigella*. Seafood has been particularly implicated and of note have been oysters (Terajima et al., 2004) and ready-to-eat shrimps (Duran and Marshall, 2005). In 2007 imported baby corn originating from Thailand was linked to drug-resistant shigellosis cases in Australia, and to an outbreak involving more than 100 persons in Denmark (Stafford et al., 2007). In all the above outbreaks the causative organism was *Sh. sonnei*.

4.1.6. *Vibrio*

4.1.6.1. Hazard identification and characterization

As with *Shigella*, infections with *Vibrio* spp. are normally the result of contamination of food and water with human faecal material. The standard treatment in such cases is oral rehydration, but should antimicrobials be required, then tetracyclines have for many years been the first-line choice. More recently, because of the emergence of strains with resistance to tetracyclines, fluoroquinolones have been used when treatment has been indicated.

Recently the efficacy of fluoroquinolones has been jeopardised following the emergence of resistant strains in the Indian sub-continent and Europe. There is no evidence to suggest that the emergence of resistance to critical antimicrobials in *V. cholerae* is linked to the use of antimicrobials in food production animals.

4.1.6.2. Exposure through foods

Vibrio cholerae serogroups O1, O139 and non-O1/non-O139 are not considered to have a traditional food animal reservoir. Infections result from the ingestion of faecally contaminated water or food in the case of cholera, or the consumption of seafood such as shrimp (Duran and Marshall 2005; Boinapally and Jiang 2007), and cockles or oysters in the case of *V. parahaemolyticus*. This is due to the fact that the principal reservoir for these bacteria is the aquatic environment. When such strains have been antimicrobial-resistant, then infections or substantive outbreaks with resistant strains have resulted (Weber et al., 1994). Though *V. vulnificus* has been obtained from a number of sources in inshore marine areas, the significant food contamination is of shellfish and particularly of oysters. The vibrio is very heat sensitive and has not been reported on processed foods (ICMSF, 2005; Bang and Drake, 2002; Kim et al., 1997)

4.1.6.3. Reports linking foodborne AMR *Vibrio* spp. to human infections

A variety of food products have been involved in foodborne AMR *Vibrio* outbreaks, most often seafood and seafood products. The causative agent was *V. parahaemolyticus*, and up to 10% of the isolates were multi-resistant (Wong et al., 2000). A case control study identified *V. cholerae* as a source of foodborne infection, with 36% of the isolates being multi-resistant. In particular, raw seafood, unboiled water or cooked crabs were identified as associated with illness (Weber et al., 1994). Virtually all reported *V. vulnificus* food borne cases have resulted from consumption of raw oysters by susceptible individuals with no information about AMR (ICMSF, 2005).

4.1.7. **Meticillin¹¹-resistant *Staphylococcus aureus* (MRSA)**

Staphylococcus aureus is a bacterium found on the skin or in the nostrils of humans. The bacterium is carried temporarily and rarely poses a problem for people in full health, although it is a major cause of nosocomial infections, often causing postsurgical wound infections. Some strains are capable of producing an enterotoxin that can cause foodborne intoxication.

Meticillin-resistant *Staphylococcus aureus* (MRSA) has a generally decreased sensitivity to β -lactam antimicrobials, and is thus a serious clinical problem in hospital environments. The resistance is based on a specific penicillin-binding protein, PBP2' (or PBP2a), coded by *mecA*

¹¹ Meticilin (International Nonproprietary Name) = Methicillin (United States Adopted Name)

(Hartman and Tomasz, 1984). The *mecA* gene is typically part of an integron associated with the *S. aureus* chromosome (Katayama et al, 2000).

From being considered as almost exclusively a nosocomial pathogen, MRSA has during the last two decades emerged in the community. Furthermore, it has recently also caused infections in, and colonized, domestic pets and food production animals. MRSA has been detected in cattle, chickens, horses, pigs, dogs, rabbits, seals, birds and cats. The colonization in animals has in several cases been implicated in infections in humans and infection with MRSA may today be considered as a zoonosis. It is, important to distinguish between the epidemiology of MRSA in relation to production animals, where a new clone appears to be emerging, and pet animals infected with classical human variants of MRSA (Manian, 2003; Weese et al., 2006).

4.1.7.1. Hazard identification and characterisation

Meticillin-resistant *Staphylococcus aureus* (MRSA) has been identified amongst pig farmers, and abattoir workers, indicating, in this instance, not a food hazard but an occupational hazard. Multilocus sequence typing indicates that there is a common type. However, MRSA which has also been identified on meat, (Kitai et al., 2005; van Loo et al., 2007b; Normanno et al., 2007, VWA, 2008), may be a hazard as a consequence of handling contaminated meat. Juhasz-Kaszanyitzky et al. (2007) reported subclinical MRSA mastitis in dairy cattle. Milk derived from cows with subclinical MRSA mastitis may be considered as a source of MRSA in milk.

One specific clone (ST398) has been found in several countries including Austria, Belgium, Canada, Denmark, France, Germany, The Netherlands and Singapore where it has been isolated from both production animals and humans (van Loo et al., 2007a, b; Tan et al., 1994; Wijaya et al., 2006). Surveys in Sweden and Switzerland were negative. With our current knowledge it seems quite evident that ST398 is a MRSA clone transmitted from production animals to humans. Its origin is currently unknown. Further studies are underway in several countries, but it seems likely that MRSA ST398 is widespread in the food animal population, most likely in all Member States with intensive animal production. For example, a study in Germany found a prevalence of MRSA of 12.5% of 678 isolates from pigs in 17.9% of the 62 farms investigated, all of the isolates being typed as ST398 (Blaha, 2008). A recent study from the Netherlands found MRSA in small quantities in turkey (31% of samples), chicken (27%), veal (17%), pork (10%), beef (10%) and lamb (6%) meats at retail. Most (84%) of the MRSA found was to be ST398 (VWA, 2008). The reason for the colonization of MRSA ST398 in pigs and other production animals and the epidemiology of this clone are currently not known; it possibly first emerged in 2003, as it was not detected in 2002 in the human monitoring done in Holland, or in monitoring from 1992-2003 of human isolates in Germany.

Case-control studies in both Denmark and The Netherlands have shown that the people at risk of being colonised with ST398 are those persons working or living on farms and mainly those in direct contact with animals (van Loo et al., 2007). This is also underlined by the above mentioned German study (Blaha, 2008) who found that people working with live animals on farms showed a higher rate of nasal contamination (41.8%) than those working in slaughterhouses or in diagnostic laboratories (13.4% of a total of 86 persons investigated). All positive isolates were ST398.

A baseline study on the prevalence of MRSA in holdings of breeder pigs is now being carried out across Member States¹² (EFSA, 2007b), and an assessment by EFSA of the public health significance of MRSA in animals and foods (EFSA-Q-2008-300) is being prepared.

¹² OJ, L 14, 17.1.2008, p10-25

4.1.7.2. Exposure through foods

In Korean studies, MRSA has been frequently isolated from food producing animals, albeit at a relatively low frequency. While the study of Lee (2003) indicated the few animal isolates were closely related to human isolates, the studies of Kwon et al. (2005, 2006) suggested that isolates from bovine milk had a different subtype of the resistance cassette and might not be related to community-acquired human cases nor to the ST398 clone recently identified in Europe. Two Japanese poultry-associated MRSA strains shared the characteristics of community-acquired human MRSA strains (Kitai et al., 2004).

In an Italian study, MRSA were found at a frequency of 3.75% in *S. aureus* isolates derived from different foods of animal origin. Four isolates were from bovine milk and two from dairy products (pecorino and mozzarella cheese). The strains were able to produce enterotoxins, but no association with cases of food poisoning nor to relatedness to human MRSA isolates was made (Normanno et al., 2007).

4.1.7.3. Reports linking foodborne MRSA to human infections

The first food-associated MRSA outbreak resulting in several fatalities was described by Kluytmans et al. in 1995. The origin was most likely a colonized health care worker (HCW) who was responsible for preparing the food for haematology patients. Identical strains were isolated from the HCW, a food item (peeled banana) and from the first patient involved in the outbreak. The HCW had no direct contact with patients. Airborne transmission was thought to play an important role in the subsequent spread of the outbreak. Jones et al. (2002), reported a small outbreak involving a family which had consumed a contaminated meal purchased from a delicatessen. The origin of this outbreak was most likely also human. Further outbreaks have apparently not been reported. However, animal products remain a potential source of MRSA. Food-associated MRSA, therefore, may now be an emerging problem.

4.1.8. *Listeria monocytogenes*

4.1.8.1. Hazard identification and characterisation

Foods associated with transmission of *Listeria monocytogenes* to humans become contaminated either at source (e.g. from the general environment), from sites within food production environments, or by cross-contamination during subsequent stages in the food chain. Penicillin, ampicillin, amoxicillin with or without gentamicin or trimethoprim-sulfamethoxazole (TMP-SMX) are recommended for the treatment of listeriosis. Although plasmids conferring resistance to tetracyclines, chloramphenicol, macrolides, streptomycin and trimethoprim have been described, all strains are susceptible to these antimicrobial agents or combinations of such. All strains of *L. monocytogenes* are intrinsically highly resistant to cephalosporins (Johnson et al., 1996; Threlfall et al., 1998; Hansen et al., 2005); this may present therapeutic problems if this class of antimicrobial agent is used for blind therapy (therapy in the absence of confirmation of the causative agent of infection).

There is relatively little evidence for emergence of antimicrobial resistance in the bacterium; indeed the resistance pattern has remained virtually unchanged for the past 40 years (Johnson et al., 1996; Threlfall et al., 1998; Charpentier and Courvalin, 1999; Hansen et al., 2005).

4.1.8.2. Exposure through foods

Transmission of *L. monocytogenes* occurs through several routes; however the majority of cases are considered to occur as a result of eating contaminated food (McLauchlin 1996).

4.1.8.3. Reports linking foodborne AMR *L. monocytogenes* to human infection.

Epidemiological analysis of both sporadic cases and outbreaks of human listeriosis have shown that the foods associated with transmission are predominantly ready-to-eat foods, with those extended (usually refrigerated) shelf-life foods capable of supporting the growth of *L. monocytogenes* (McLauchlin, 1996) being of particular importance. Antimicrobial resistance is not a therapeutic problem for the treatment of listeriosis; this situation may be reversed if resistance develops or horizontal transfer of key resistance genes occurs. It should be emphasised that there are no reports linking foodborne *L. monocytogenes* with antimicrobial drug resistance to cases of human infection.

4.2. Commensals

Commensal bacteria are those bacteria that live in or upon the host without causing disease. Mostly, this co-existence is of mutual benefit. However, many commensals can cause disease if they enter body sites that are normally sterile or when the host's immune defence is impaired (Sharp, 1999). As discussed in Section 3.7, commensal bacteria that contaminate food can harbour transferable resistance genes. During the passage through the intestine, these bacteria may transfer their resistance genes to host-adapted bacteria or to pathogens. Exchange of resistance genes between bacteria from different sources can also occur in the kitchen environment (Kruse and Sørum, 1994; Walsh et al., 2008). The most studied species are commensal *Escherichia coli* and *Enterococcus* spp.

4.2.1. *Escherichia coli*

Commensal *E. coli* from the intestines of animals and humans contaminate foods of animal origin, vegetables and water and may carry transferable resistance genes (Sunde and Norström, 2006). One of the best documented specific examples of spread of resistance genes from animal to human bacteria is transposon-encoded streptothricin resistance (Tschäpe, 1994). Following the introduction of nourseothricin for use as a growth promoter in pig production, resistance emerged in commensal *E. coli* of pigs and farmers, and later in urinary isolates of *Salmonella*, and in *E. coli* and *Shigella* causing disease in humans (Hummel et al., 1986; Tschäpe, 1994). Another example is the spread of apramycin resistance, an antimicrobial used exclusively in animals. Apramycin resistance was first described in *E. coli* and *S. Typhimurium* from animals (Chaslus-Dancla et al., 1986; Wray et al., 1986) and has since been demonstrated in various enterobacteria from animal, human and environmental sources (Chaslus-Dancla et al., 1989; Hunter et al., 1994; Hunter et al., 1993; Threlfall et al., 1986).

4.2.1.1. Hazard identification and characterisation

Infections with multi-resistant Gram-negative bacteria are currently among the major challenges in health-care settings in Europe and elsewhere. Gram-negative pathogens can be recipients of resistance genes transferred from commensals such as *E. coli* and may cause serious infections in, e.g. the blood-stream, abdomen, lungs and urinary tract and can lead to septicaemia (Livermore et al., 2007). Meanwhile, multi-resistant *E. coli* are also increasingly associated with urinary tract infections in the community (Calbo et al., 2006; Woodford et al., 2007).

4.2.1.2. Exposure through foods

Phenotypic data on resistance to antimicrobials in *E. coli* isolated from food have been compiled from the summary report on zoonoses in the EU (EFSA, 2006b) and from national reports on monitoring of resistance. Comparability is hampered by differences in inclusion criteria, testing methodology and choice of interpretation criteria as the epidemiological cut-off values were not uniformly used for compilation of some of the reports quoted (see 2.2.2). Notwithstanding this, resistance to ampicillin, streptomycin, tetracyclines and trimethoprim is common in *E. coli* from beef, poultry meat and pork. These antimicrobials are also those frequently employed in animal husbandry for therapy and prophylaxis. A high proportion of isolates resistant to fluoroquinolones is reported from poultry in some countries. Resistance to 3rd generation cephalosporins is still not common according to these reports, but The Netherlands has reported a rapid increase in resistant isolates from broilers and broiler products and Canada has reported very high figures, also from broilers (see Appendix, Table B).

4.2.1.3. Reports linking foodborne AMR *E. coli* to human infections

Most food-derived *E. coli* will be transient and non-pathogenic to the host. Resistance genes may be transferred to host-adapted species or to zoonotic pathogens such as *Salmonella*, during passage through the intestine or in food. Indeed, non-pathogenic multidrug-resistant *E. coli* in the intestine are an important reservoir of resistance genes (Osterblad et al., 2000) and these *E. coli* isolates of animal origin may colonize the human intestine at least temporarily (Linton et al., 1977; Marshall et al., 1990; Orskov and Orskov, 1992). Transfer of resistance genes from *E. coli* to *Salmonella* has been demonstrated experimentally in poultry intestinal tract (Gast and Stephens, 1986; Poppe et al., 2005). Further, there are some reports indicating acquisition of resistance plasmids by *E. coli* and *Salmonella* in the human gut (Su et al., 2003; Yan et al., 2005). Exchange of resistance genes between bacterial clones has also been demonstrated experimentally in water, soil, on kitchen towels, on cutting boards, and on the surface of food (Kruse and Sørum, 1994; Walsh et al., 2008).

Spread of resistance genes between bacteria colonising animals and man has been shown. For example, some studies have shown that the same R plasmids can be transferred between bacterial strains from bovines and humans (Oppegaard et al., 2001). Some categories of food may often be contaminated with *E. coli*, including resistant isolates (Sunde and Nordström, 2006), and these bacteria reside long enough in the intestines of humans to be able to transfer resistance genes to the residential flora. It is therefore highly probable that food is a vehicle for spread of resistance genes between different ecosystems.

4.2.2. *Enterococcus*

Enterococci (former D streptococci or faecal streptococci) are natural commensals of the human and animal gut. In addition, they occur in foods as contaminants but are also present as acidifying microorganisms in many traditional and artisanal fermented products (Franz et al., 2003) (See also 4.3).

Enterococci are intrinsically resistant to cephalosporins, low concentrations of aminoglycosides, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole. In addition, many strains harbour transmissible genetic elements for acquired resistance for various antimicrobials (tetracycline, erythromycin, chloramphenicol) (Cetinkaya et al., 2000). The acquired resistance to glycopeptide antimicrobials (vancomycin, teicoplanin) has received most attention, because of the rapid increase in the occurrence of vancomycin resistant *Ent. faecalis* and *Ent. faecium* strains (VRE) both among clinical isolates and food and faecal strains

(Bates et al., 1994; Bates, 1997) since the first report of their isolation (Leclercq et al., 1988; Uttley et al., 1989). While vancomycin resistance compromises the treatment of nosocomial infections, there is an additional concern of the eventual transfer of the resistance to methicillin-resistant staphylococci (MRSA), which would lead to extremely serious clinical consequences. This transfer has been reported to occur under laboratory conditions (Noble et al., 1992), and more recently clinical isolates of MRSA have also been found to harbour vancomycin resistant genes, originating from enterococci (CDC, 2002).

4.2.2.1. Hazard identification and characterization

Since the 1970s, enterococci have become important agents in hospital-acquired infections, causing mainly urinary tract and wound infections and endocarditis. The virulence factors associated with enterococci include adhesins, invasions and hemolysin (Eaton and Gasson, 2001; Franz et al., 2003). The most common species detected in nosocomial infections is *Enterococcus faecalis*. Occasionally also infections caused by *Ent. faecium* are encountered, while clinical cases associated with other species (*Ent. gallinarum*, *Ent. casseliflavus*, *Ent. durans*, *Ent. avium*, *Ent. raffinosus*) are rarely reported (Cetinkaya et al., 2000).

4.2.2.2. Exposure through foods

VREs can be isolated from animal faeces and from foods of animal origin (Wegener et al., 1997; Bates, 1997; Klein, 2003; Eisner et al., 2005; Kaszanyitzky et al., 2007), and also from healthy, non-hospitalized humans (Balzereit-Scheuerlein and Stephan, 2001). The former use of a glycopeptide antimicrobial, avoparcin, as an antimicrobial growth promoter has contributed to the formation of a reservoir of VRE in food-producing animals. Because of these concerns, the growth promoter use of avoparcin has been restricted or banned in many third countries, and in the EU since 1997.

Molecular studies indicate a high degree of clonality among the vancomycin resistance determinants of animal origin. For example, an *Ent. faecium*-strain carrying a specific variant of resistance transposon Tn1546 has been simultaneously detected in swine isolates from Denmark, Spain, Portugal and Switzerland (Novais et al., 2005). In the studies of Garcia-Migura et al. (2007a, b) the Tn1546 isolated from 19 unrelated farms show a very low diversity of Tn types, while otherwise the genotypic diversity between the different farm isolates was high. These findings suggest that horizontal transfer may have a more important role in the persistence of vancomycin resistance than the clonal spread.

4.2.2.3. Reports linking foodborne AMR enterococci to human infections

There is little evidence of human infections being directly linked to the consumption of VRE-contaminated foods. Apparently few, if any systematic studies on the prevalence of the known enterococcal virulence factors among the food-associated VRE-strains have been done, while generally the occurrence of virulence determinants in food isolates and in human commensals appears to be low compared to clinical isolates (Eaton and Gasson, 2001; Franz et al., 2001; Lempiäinen et al., 2005).

The question as to how frequently animal and food strains of enterococci can permanently colonise humans, is to some extent an open one. Molecular studies indicate a certain overlap between human isolates and strains from pigs and, to some extent, with isolates from poultry and cattle (Bruinsma et al., 2002). In a Danish study the presence of a vancomycin-resistant *Ent. faecium*, apparently related to the swine-associated clone mentioned above (Novais et al.,

2005), has been detected in a healthy volunteer after seven years since the ban on avoparcin usage (Hammerum et al., 2004).

The *in vivo* transfer of *vanA* genes from chicken isolates to human strains in human volunteers has been detected (Lester et al., 2006), this being perhaps the strongest direct evidence of the potential of animal strains to spread resistance to human strains with a resulting clinical significance.

While the direct clinical infection in humans by VRE from food sources apparently is rare although not totally excluded as a possibility, the reservoir of VRE in food-producing animals presents a definite risk of resistance genes being transferred to virulent human strains through food and other routes.

4.3. Bacteria deliberately added to the food chain or being an integral part of the food

Fermentation is an ancient practice to improve the hygienic, nutritional and sensory quality of perishable foodstuffs. Fermented foods all over the world are being prepared using microorganisms that are either added as starter cultures, or, in more traditional or artisanal production conditions, by back-slopping or relying on spontaneous fermentation in conditions favouring the desired microbial community. The use of bacteria intended to promote the well being of the host (probiotics) both in food and feed or the use of microbial preparation, as protective cultures represent new ways by which deliberately added microorganisms enter the food chain.

Microorganisms present in fermented food (see review of Wigley, 2000) are mainly lactic acid bacteria (LAB). Typical food-associated LAB include members of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus*. *Streptococcus thermophilus* is a commonly used species. In addition, enterococci occur in many traditional products, while some staphylococcal and micrococcal species (*S. carnosus*, *S. xylosus*, *M. varians*) are used in certain meat fermentations as colour and flavour producers. Propionic acid bacteria are typically added to Emmenthal cheese. In probiotic food preparations LAB, often of intestinal origin (mainly lactobacilli but also, particularly in feed applications, enterococci), or bifidobacteria, are frequently used (Ouwehand et al., 2002).

4.3.1. Hazard identification and characterisation

While occasional opportunistic infections caused by lactobacilli, usually associated with a severe underlying disease, are reported (Gasser 1994; Salminen et al., 2006), no reports of clinical cases associated with industrial starters have been found. A few cases of human infections associated with a probiotic *Lactobacillus rhamnosus* strain have been described (Rautio et al., 1999; de Groote et al., 2005). Considering the extensive use of this bacterium in these products, these cases remain isolated incidents, and there have been no indications of therapy failures due to any antimicrobial resistance in the strain.

There are relatively few studies on the prevalence of antimicrobial resistance markers in currently used starter cultures, or in bacteria isolated from fermented foods. In an EU Sixth Framework Program project ACE-ART, however, approximately 1400 isolates of lactic acid bacteria and bifidobacteria of human, animal, food and feed origin were screened for typical and atypical antibiotic resistances. Some of the results have already been published (Florez et al., 2006, 2007; Korhonen et al., 2007; Mättö, et al., 2007; Tosi et al., 2007; Egervarn et al., 2008). The general finding was that conspicuous transferable resistances were rare, and the most common resistance occasionally detected was against tetracycline.

4.3.2. *Exposure through foods*

While there has been no indication of widespread prevalence of antimicrobial resistance in strains used as industrial starter cultures, there are studies indicating that antimicrobial-resistant microorganisms can at least occasionally be isolated from fermented foods (Teuber et al., 1999) or among probiotic strains (Masco et al., 2006).

In their review, Teuber et al. (1999) list a number of acquired antimicrobial resistances detected in lactic acid bacteria isolated from foods. They most often occur among enterococci, which, in addition to vancomycin resistance, can also harbour other resistance determinants, the most common being genes associated with resistances to tetracycline, erythromycin and chloramphenicol. These resistance determinants are often carried on conjugative plasmids, which makes their transfer possible both among the enterococcal strains and even between different bacterial genera.

As in the case of enterococci, lactococci and lactobacilli harbouring multiresistance plasmids have been isolated from dairy products (Gfeller et al., 2003; Teuber et al., 1999). A high incidence of tetracycline and erythromycin resistance determinants *erm*(B) and *tet*(S) were detected among lactobacilli isolated from hand made artisanal cheeses in Turkey (Cataloluk and Gogebakan, 2004). Tetracycline resistance determinants *tet*(M) and *tet*(S) have been found to be relatively common in lactic acid bacteria associated with raw meat, while in the process of preparation of fermented dry sausages *tet*(M) became dominant among the tetracycline resistant isolates (Gevers et al., 2003).

In a recent study by Klare et al. (2007) 473 lactic acid bacteria strains representing nutritional or probiotic strains or human and animal isolates were screened for antimicrobial resistance. Six probiotic or nutritional cultures were found to be multi-resistant, possessing *tet*(W), *tet*(M) or *erm*(B) determinants. In a study carried out in the USA (Wang et al., 2006) high incidences of antimicrobial resistance were observed, particularly *tet*(S)/(M) and *erm*(B) markers in the lactococcal and *St. thermophilus* isolates from retail dairy products, the frequency of resistant strains ranging from 10^2 - 10^7 CFU g⁻¹ food.

Antimicrobial resistance determinants including among others *tet*(W), have also been detected in bifidobacteria, including seven *Bifidobacterium animalis* subsp. *lactis* and *B. bifidum* strains used as probiotics (Masco et al., 2006).

4.3.3. *Antimicrobial-resistant starter and probiotic bacteria and human infections*

While food-associated fermentative bacteria, whether antimicrobial-resistant or not (with the possible exception of enterococci) do not present a clinical problem, they might act as a reservoir of transmissible antimicrobial resistance determinants. Should strains with transferable antimicrobial resistance genes become widespread in the food chain, the eventual transfer of resistances to food associated or intestinal pathogens might become a possibility, although the probability of such an event leading to actual clinical consequences is difficult to evaluate. Nevertheless, avoiding the use of strains harbouring transmissible antimicrobial resistance determinants in food or feed fermentation, or as probiotics is a prudent precaution (von Wright, 2005), as reflected in the acceptance criteria of bacterial strains intended for use as animal feed additives (SCAN, 2003; EFSA, 2005a).

5. Categorisation of food with respect to risk of AMR

Major food categories to be considered include food of (1) animal origin including fish, (2) plant origin, and (3) water used directly in the food chain. Mixed products are also considered, as most ready-to-eat (RTE) foods and convenience products consist of foods of different origin (e.g. pizzas, etc.). The latter category is included as one of the four main categories.

A further subdivision of food categories is concerned with the manufacturing process that has been applied, i.e. if the products are offered at retail level and at the point of consumption as fresh or raw, minimally processed or processed (i.e. heat treated or fermented) foods.

Several categorisation schemes for food exist (as for example, reviewed by Ireland and Moller, 2000), or are under development (e.g. EFSA¹³ and ISO¹⁴). These have been developed for specific purposes. For the estimation of food consumption, data categories are usually defined according to the nutritional value, taking into account the regional diversity of food. Other listings concern the estimation of risk exposure to contaminants and residues; these follow lists used for consumption data in order to estimate the amount of intake of specific food categories. The categorisation used for the outbreak reporting in EFSA's Zoonoses Report (EFSA 2006b, 2007d, e) refers to the origin of the food, i.e. animal species. These categories are useful for the purpose of attribution of pathogens to products of animal origin. For the description of the role of food in the dissemination of antimicrobial resistance these latter categories can be used in part. The number of categories should not be too high; Additional information has also to be considered, such as information about the manufacturing process used, as elucidated below. A combination of these categorisation systems has been used here and is presented in Table 1.

Antimicrobial resistance is dependent on live microorganisms and the transfer of resistance genes (see Chapter 3). Therefore any processing step that reduces or increases the bacterial load has an influence on the risk of exposure to antimicrobial resistant bacteria. For the purpose of this Opinion, food categories are defined according to their impact on bacterial survival and growth. For different microorganisms different influences might apply, but in general the effects on the bacterial microflora can be described as shown below.

The categorisation follows a recommendation drafted by the former Federal Institute for Consumer Protection and Veterinary Medicine (BgVV, 2000), (now the Federal Institute for Risk Assessment, BfR). This general approach has been adapted for the present purpose and simplified.

The categorisation takes into account the treatment by the manufacturer (e.g. heat treatment, other stabilizing procedures, use of preservation agents, fermentation), the possibility of recontamination after this treatment and the type of packaging used. The recommended shelf-life or best-before date are considered as well. The intended use at consumer level plays an important role. For instance, consumption without further heat treatment in the kitchen, and the consumption of specialised products by defined groups of consumers (e.g. diet or infant food). The food matrix and characteristics also have a major influence on the bacterial microflora. This concerns both intrinsic and extrinsic factors, including pH-value, NaCl-content, a_w -value, redox potential, temperature, storage conditions, etc.).

Some categories are only of importance for bacteria capable of multiplying in the food matrix (e.g. *Salmonella*, *Listeria*). For bacteria that are unable to multiply (e.g. *Campylobacter*) in such matrices, the nature of the food is not of importance. Bacteria introduced from the pre-harvest

¹³ EFSA Article 36 funded research project CFP/EFSA/DATEX/2007/02

¹⁴ ISO 16140 in revision by TC34 SC9 WG3

and harvest phases of the food chain pose a different risk to that posed by bacteria intentionally introduced during processing.

Table 1 presents a categorisation of food for the purpose of undertaking an initial consideration of issues relating to AMR. Notional sub-categories of the main categories are listed so as to introduce technological factors as well as production factors for consideration. Formal sub-categorisation of food groups would require to be based on such factors as the log reduction of the bacterial load of concern brought about by the particular process used, and other considerations. For the purposes of this Opinion any assessment based upon the sub-categories as presented in Table 1 is qualified due to a lack of precision regarding the impact of the specific process used on the particular food product's microflora.

Any assignment of foods to the different AMR categories listed in Table 1 above is highly subjective. Some foods may belong to two or more categories depending on the consumption habits in different Member States. A simplified list is used here only to illustrate the different categories and to provide a basis for a quantitative comparison of the relative contribution each makes to the transmission of AMR bacteria to humans. Cross-contamination with these bacteria at different stages of the food production chain (especially at retail level and in the home) would affect the level of risk posed by such food irrespective of its category and would in effect lead to a higher cumulative exposure than would be expected from the original food category alone.

5.1. Source attribution

In order to reduce the public health burden from foodborne infections (including that which results from antimicrobial-resistant bacteria), it is important to have an insight into the source(s) from which such bacteria gain entry to the food of concern. However this is not an easy exercise because (1) many bacteria have multiple hosts, (2) there can be a vast range of foodstuffs derived from one type of food-producing animal, and (3) humans may have exposures to more than one possible source. Techniques developed for source attribution are based on (a) outbreaks; (b) analytical epidemiology of sporadic cases (e.g. case-control studies); (c) microbial sub-typing; (d) comparative exposure assessment and (e) expert opinion (Batz et al., 2005). EFSA reports on methods for source attribution for human illness from foodborne microbiological hazards (EFSA, 2008c), and source attribution for human salmonellosis from meat (EFSA, 2008b), considers each of these in detail. Outbreak investigations, case-control studies and microbial sub-typing studies have most commonly been applied to the area of antimicrobial-resistant bacteria. For foodborne attribution, most attention has been centred on *Campylobacter* and *Salmonella* (see sections 4.1.1 and 4.1.3). A source attribution analysis, including antimicrobial-resistant *Salmonella*, is conducted annually in Denmark (Hald et al., 2007).

Table 1. **An example of categorisation of food including production and processing factors to facilitate the assessment of exposure of the consumer to AMR factors.**

Category concerning AMR (AMR Category)	
Category and Subcategory of food	
1.	Milk and dairy products (cows, goats sheep, buffalo, horse)
1.1.	Milk
1.2.	Dairy products (other than cheeses)
1.3.	Cheese
2.	Eggs and egg products
3.	Red meats
3.1.	Bovine meat and products thereof
3.2.	Pig meat and products thereof
3.3.	Sheep meat and products thereof
3.4.	Other or mixed red meat and products thereof
4.	Poultry meats
4.1.	Broiler meat (<i>Gallus gallus</i>) and products thereof
4.2.	Turkey meat and products thereof
4.3.	Other or unspecified poultry meat and products thereof
5.	Aquaculture and marine
5.1.	Fish and fish products
5.2.	Crustaceans, shellfish, molluscs and products thereof
6.	Vegetables, cereals, fruits
6.1.	Vegetables and juices and other products thereof
6.2.	Cereal products including rice and seeds/pulses (nuts, almonds)
6.3.	Fruit, berries and juices and other products thereof
7.	Herbs and spices
8.	Mixed or buffet meals*
9.	Other foods*
10.	Tap water including well-water

* Assessed by considering the food component subjected to the least amount of heat treatment or exposure to comparable treatment, e.g. raw meat, raw vegetable, smoked fish.

6. On assessing the risk of the acquisition of antimicrobial resistant bacteria or bacteria-borne antimicrobial resistance genes *via* the food chain

In accordance with the Terms of Reference, a reliable approach for assessing the risk to humans of acquiring antimicrobial resistant bacteria or bacteria-borne antimicrobial resistance genes from food is required. This is a complex task, as there are many possible routes of acquisition, in addition to food, such as direct contact with livestock and companion animals, exposure to the environment, human-to-human transmission, etc. Here we focus only on the food routes.

Human exposure to antimicrobial resistant bacteria is difficult to measure (qualitatively or quantitatively). Data on the quantitative numbers of antimicrobial resistant bacteria in different food products and data on the human consumption habits are in some cases available but may not be in the required format for input into a risk assessment. It is unknown whether antimicrobial resistant bacteria survive or multiply during stages of the food-chain (e.g. processing, cooking etc.) to a greater extent than susceptible bacteria. Likewise, in terms of the dose-response characteristics, there are very limited data on whether resistant bacteria are more pathogenic or cause more severe illness than the antimicrobial-susceptible equivalent. In addition, it is very difficult and the data very sparse, to properly take into account the significance of transferable resistance genes and their 'indirect' impact on human health. Few if any risk assessments in this area have considered this consequence, presumably due to the significant data requirements and, overall, the scientific uncertainty associated with within-host resistance gene transfer.

It is recognised that there are many different types of consumers and that their consumption habits (e.g. type and quantity of food consumed) and susceptibility to antimicrobial resistant infections may differ. For example, the 'consumer' can be classified as an infant, youth, adult or an elderly person and within each of these categories, could be further classified as healthy, clinically ill or immuno-compromised. In the ideal world, a risk assessment would also take into account a secondary classification to reflect cultural and religious status and optional dietary preferences (vegetarians, vegans). Likewise, taking into account the risk of exposure, farming families and workers, along with workers in the meat trade deserve consideration as distinct entities as, either usually or occasionally, they may consume raw dairy and meat products. Other groups engaged in the wholesale or retail food trade may be similarly exposed. Many types of consumers are identified here and we acknowledge the potential impact of each type on their risk of exposure and any consequences, due to the large scope of the given Terms of Reference, consideration of consumer type is not taken any further here.

6.1. Issues to be considered in relation to risk assessment applied to the area of antimicrobial resistance

Risk assessment is a scientific tool that can be used to evaluate the level of exposure and the subsequent risk to human health due to a specific microorganism or particular type of resistance. Both qualitative and quantitative risk assessment approaches have been utilised to estimate the risk to human health from antimicrobial resistant bacteria (Snary et al., 2004). Written guidelines and accepted procedures are available (e.g. Codex Alimentarius Commission, 1999) for microbial food safety risk assessments and for antimicrobial resistance. The OIE guidelines, which are specific to antimicrobial resistance risk assessment, can also be used (OIE, 2007a). Risk assessments in the area of antimicrobial resistance, and especially those investigating the impact of the use of antimicrobial agents in food animal production on human health, are challenging. This is because the data needs are greater than for non-resistance risk assessments and for many aspects there are significant data gaps.

6.1.1. Data requirements for an antimicrobial resistance risk assessment

The data requirements will be determined by the risk analysis question posed and the level of detail (or resolution) used to address this question. There is no standard list; however, previous data gaps/deficiencies were identified by Snary et al., (2004) and are summarised in Table 2. Here some of the data requirements (and gaps/deficiencies) and hence the challenges encountered in the area of antimicrobial resistance risk assessment, are discussed, but are limited to risk assessments that focus on transmission of antimicrobial-resistant bacteria through the food chain.

If the focus of the risk assessment is on the use of a certain antimicrobial in food-animal production it is often the case that data on the usage of antimicrobial agents for food animal species are in many Member States totally lacking. If such data are available, it is very rarely, if ever, known down to the individual animal, herd or flock level. The exact association between usage of a given antimicrobial agent and the emergence and spread of resistance is seldom known and even though several studies have shown that usage is an important factor for the emergence of resistance, the association is not linear, but is also determined by the way the antimicrobial agent is used. Thus, on the one hand it has been indicated that increased dosages might help to avoid the selection for quinolone resistance in *Salmonella* (Wiuuff et al., 2003), while on the other hand, continuous feeding of tylosin supplemented feed to chickens might select for macrolide resistant *C. jejuni*, whereas single treatments are less likely to do so (Lin et al., 2007).

Table 2. **Key data limitations/issues affecting microbial risk assessments (MRAs) applied to the area of antimicrobial resistance (Snary et al., 2004)**

Data limitation/issue	Effect on MRA
Definition of resistance – Harmonization of MIC/disc-diffusion breakpoints required.	Data from different sources are not comparable. May limit the amount of data available for the MRA.
Microbiological methods, e.g. – Selective plating v testing of one isolate from non-selective plate – Enrichment v non-enrichment – Molecular versus phenotypic methods	- The amount of data available for the MRA may be limited if the methods are not comparable. - Cannot compare selective plating against the testing of one isolate without knowledge of the ratio of resistant to susceptible bacteria. - If enriched the number of organisms is increased and therefore cannot directly be used in the MRA.
Multiple levels of the sampling framework	Large variability of sampling methods between studies. Therefore data from different sources may not be comparable; could limit the amount of data available for the MRA.
Small sample sizes	If the sample size is small at any level of the sampling framework, the uncertainty about the associated parameter will be large. This may contribute to a large uncertainty associated with the final risk estimate.
Little data available on indicator organisms (resistant or susceptible) compared to pathogenic bacteria.	Surrogate organisms etc. may be used to overcome the data gap, thus increasing the level of uncertainty in the output of the model. This uncertainty may not be quantified.
Sensitivity and specificity of the tests used.	MRA may overestimate or underestimate the risk.
Causality unclear	Large assumptions made on the causality of antimicrobial resistance. This leads to a higher level of uncertainty in the model results, but which may be difficult to quantify.
Lack of quantitative microbiological data	Microbial load of resistant bacteria in/on different sources is unknown, therefore either not modelled or key assumptions made.
Little information on the use of antimicrobial agents for – veterinary use (at animal and farm level) – human use	Causality is difficult to consider. May lead to a large degree of uncertainty in the results of the model.

6.1.2. Requirements for a risk assessment

It is very difficult to provide a general definition for a risk assessment. This is because the most important attribute is that it is 'fit for purpose', i.e. it answers the risk question posed within the constraints of the resources available (e.g. time, data and expertise).

As described in the Terms of Reference, the aim here is (1) to identify in terms of qualitative risk, the extent to which food serves as a source for the acquisition, by humans of antimicrobial resistant bacteria or bacteria-borne antimicrobial resistance genes; (2) to rank the identified risks and (3) to identify potential control options for reducing exposure. Therefore, in the ideal world, for the Terms of Reference given, all factors that increase/decrease the risk of human exposure (both in terms of prevalence and microbial load) to a particular antimicrobial-resistant bacteria from a particular source would be considered as part of the risk assessment and the required data would be available. In particular, the food pathways would take the form of a farm-to-consumption risk assessment and would be able to take into account the variability of production systems and consumer preferences between the different EU Member States. In addition, as discussed earlier, detailed risk assessments would be produced for the other sources of antimicrobial-resistant bacteria and would need to take into account the different exposures and consequences that sub-populations of consumers may have. In addition to exposure assessment, a full risk assessment would also need to take into account the dose-response relationship of developing infection and clinical illness. Specific for AMR risk assessment, the consequences of resistance on outcome of infection and illness (e.g. treatment failure), would need to be included. Finally, the problem of within-host (human) resistance gene transfer would need to be taken into account to ensure that the risks of 'direct' and 'indirect' transmission are comparable. This would be difficult if using the probability of exposure as the end-point of the risk assessment were used.

Developing a risk assessment along the lines described above would allow the risks to be estimated for each possible source and hence ranked. In addition, due to the detail included in the risk pathways, many control measures (individually or simultaneously) could be investigated and their effect on the risk and ranking assessed. However, to do this in full, would be extremely challenging and would probably take multiple person-years. This is especially due to the number of antimicrobial classes that would need to be considered (see Appendix: Table A), the bacteria of interest (*Salmonella*, *Campylobacter*, VTEC, *Staphylococcus aureus*, etc) and the number of potential sources, where the food routes alone account for 20 different categories (Table 1) without, however, taking into account the precise degree of processing (e.g. raw, minimally processed and processed).

For the above reasons the assessment presented here for illustrative purposes only, is an assessment of the risk of exposure to AMR bacteria where food is the vehicle of interest. While the assessment is designed to be qualitative, this is nevertheless a large and complex risk question. Consequently, a simplified approach to assessing the extent to which food serves as a source is preferred here. This approach allows the food types to be compared and hence ranked for a particular antimicrobial-resistant bacterium. There is no intention to propose a method whereby specific bacterial species/antimicrobial resistance combinations could be prioritised. Due to the simplicity of the approach, the template is not amenable, as more complex risk assessments would be, in terms of assessing the effect of control options. Neither is this approach put forward as a means of addressing such issues as emerging risks.

6.2. Construction of an exposure assessment template: Food as a source of antimicrobial resistant bacteria. An example.

6.2.1. Exposure pathway

Rather than adopting a farm-to-fork approach, in this example, the risk pathway commences at the point of retail sale (Figure 2). This removes the need to include the earlier production stages of farm, transport and lairage, abattoir and further processing. In addition, it is closer to the point of consumption of a food-stuff and, advantageously, many Member States collect information on the prevalence of bacteria and also antimicrobial resistance at the point of retail. Even though the pathway does not implicitly take into account undercooking of food products or cross-contamination the prevalence of the antimicrobial-resistant bacteria at the point of sale is used as a proxy to indicate the degree of contamination that is entering the home/restaurant. Contamination that may occur following purchase is not considered in this example. The probability of bacteria being present in food at retail and the probability that bacteria present in food at retail are resistant to an antimicrobial class of interest can be combined, multiplicatively, to provide an overall probability of the food at retail being contaminated with antimicrobial-resistant bacteria. This is combined with the probability that the food is purchased and consumed; such data should be available from consumption studies (e.g. Harrington et al., 2001). Each of the data requirements for the proposed risk pathway are considered below. The preferred end-point of the risk pathway would be the probability of a human being exposed to the antimicrobial-resistant bacteria of interest due to the consumption of the food of interest. Because data on cross-contamination and the effect of food preparation practices on the viability of AMR bacteria are scarce, as well as dose-response and consequence data, the exposure assessment, in this example, stops at the point of purchase of food at retail. Existing data on cross-contamination involving non-AMR bacteria could be substituted, however, as an extension to the present study.

The hazard characterization phase of the complete risk assessment process (e.g. dose-response; severity of illness; treatment failure, etc.) is not considered here. Given the end-point of exposure, this template can also be used for indicator bacteria that carry resistance genes. However it is essential that the interpretation of the final results takes into account the differences in the hazards. This applies, in particular, when comparing the more 'direct' transmission of resistant foodborne pathogens (e.g. macrolide-resistant *Campylobacter*; fluoroquinolone-resistant *Salmonella*) to the more 'indirect' risk from indicator bacteria (e.g. ESBL-resistant *E. coli* and vancomycin-resistant *Ent. faecium*) and also the indirect risk posed by pathogens carrying readily transferable resistance genes (e.g. *Salmonella* carrying plasmid borne ESBL resistance) where gene transfer within the human gut needs to be considered as part of the discussion. The same would apply to bacteria that are intentionally added to foods, such as fermentation bacteria. A full assessment of the effect of exposure to horizontally spread genes would be extremely complex and require data that are not readily available.

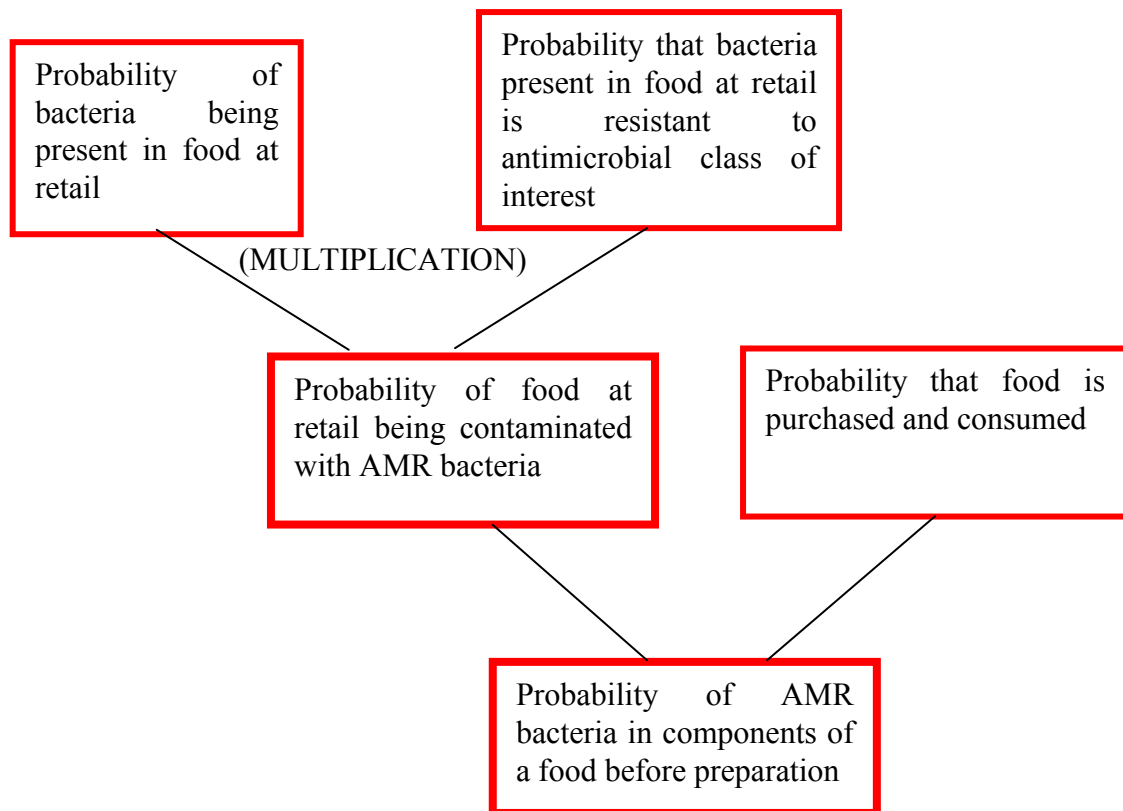


Figure 2. Example of a risk pathway for assessing the contribution of different foods to the occurrence of AMR bacteria in meal components.

Finally, it should be noted that although the adoption of a simplified approach such as this makes the risk ranking much more feasible, it loses resolution in terms of being able to explicitly consider the impact of control measures on the risk ranking. Consequently, unless information is gained on the effect of interventions on any of the 3 model parameters, namely the probability that (i) the bacteria in question are present in food at retail; (ii) such bacteria are resistant to the antimicrobial class of interest; (iii) the food is purchased and consumed, it will not be possible to assess the impact of control measures on the risk ranking. Therefore the identified potential control options for reducing exposure may not be included within the risk assessment framework, but by using other relevant evidence (e.g. epidemiological studies) can be used to support the recommendation of such control options.

6.2.2. Data requirements and availability

For each of the data requirements in the risk pathway, an estimate by category is provided, either using available data or expert opinion. In order to allow transparency and consistency between food-types and other sources, the categories are defined by broad ranges of probabilities. The number of categories is limited to three or four, because the presently available data do not justify more precise categories.

6.2.2.1. Probability of bacteria being present in food at retail

At a national level, data are likely to be available for the probability of the bacteria of interest being present in food at retail (i.e. prevalence). However, in practice, data quality and quantity will vary between Member States (MS). For example, some MS may have carried out large, structured, retail surveys and others carried out smaller studies where the sampling was done by convenience rather than randomised. In addition, the microbiological methods may differ between studies and between food types. As for all the probabilities being considered, significant variation between MS and even regions within MS may be expected.

6.2.2.2. Probability that bacteria present in food at retail are resistant to antimicrobial class of interest

Antimicrobial resistance testing can also vary between countries (and will have the same study design issues as identified above). Many studies pick a single colony of the bacteria and test this for antimicrobial resistance using a non-selective culture plate; other studies put the culture substrate onto primary isolation media containing an antimicrobial. Depending on the ratio of resistant to susceptible bacteria in the sample, this may affect the estimated probability of the antimicrobial-bacteria being present in the food and may make it difficult to compare studies and hence the different food sources.

6.2.2.3. Probability of AMR bacteria in food at retail

This probability is simply a multiplication of the two probabilities discussed above. Three categories are distinguished (high, >1%; medium, 0.01 - 1 %; low, <0.01%)

6.2.2.4. Probability that food is purchased and prepared for consumption

The probability of the food of interest being purchased and consumed can be obtained from national consumption studies. Such studies vary in the level of detail of the different food stuffs consumed and also the desired output from such studies, as many are designed for nutritional rather than food-safety purposes. The food categories suggested in Section 5 are extensive in number, but are still a simplification of the diverse range of foods that Europeans eat. For each food stuff, where appropriate, different levels of processing are taken into account as this will affect the risk of the consumer being exposed to the hazard of interest. However, it may not be possible to break down the consumption data to, for example, the level of raw, minimally processed and processed. Consequently, if not available, this must be done using expert opinion and hence bringing further uncertainty into the assessment. Four categories are detected, which characterise the proportion of consumers who would eat a meal including the food of concern on a random day.

6.2.3. *Presenting the risk estimate*

In this preliminary risk assessment, the risk of preparing a meal with food components that are contaminated with AMR bacteria is simply presented by cross-tabulation. This eliminates the need to apply seemingly simple, but arbitrary combinatorial rules.

6.2.4. *Case-study 1: Fluoroquinolone-resistant Campylobacter jejuni in the UK*

The framework described above is now adopted for a risk assessment for fluoroquinolone-resistant *C. jejuni* in the UK. For each food-stuff, information is obtained for the 3 parameters

listed above, i.e. probability of bacteria being present in food at retail; probability that bacteria present in food at retail is resistant to antimicrobial class of interest and the probability food purchased and consumed. Table C in the Appendix provides the information that was collected and the outcome of the assessment. It is important to note that the data collection was not exhaustive and that the data included is what was readily available to the working group at the time. Consequently, it has been necessary to make many assumptions when constructing Table C. It is therefore essential to note that this is not a formal risk assessment but, rather, an example of how to complete the template and the type of information that would be informative. Taking this approach, broiler meat that is bought raw is expected to have the highest probability of carrying fluoroquinolone-resistant *C. jejuni*. Other products with relatively high risk are raw beef and pork. Of course, in the majority of cases, these products will be cooked but, as described before, using this output provides a measure of the degree of contamination that is entering the home/restaurant. Note that raw vegetables, fruit etc., and also certain beef products are typically consumed without further cooking; in these cases the relative risk of consumer exposure would actually be higher than suggested in this Table.

The completion of the template for just one combination of bacteria, antimicrobial and country demonstrates the size of the task requested by the TOR. In particular, the data collection phase would be significant. As seen in Table C, data for the more common food sources of the bacteria will be plentiful, but not for those that are deemed to be more unusual. The same applies for the antimicrobial resistance data, where it is frequently assumed that the probability of *Campylobacter* being fluoroquinolone-resistant in minimally processed and processed foods is the same as in fresh/raw food stuffs. Such a probability is based upon the implicit assumption that any decay of fluoroquinolone-resistant *C. jejuni* during processing happens at the same rate as for other *Campylobacter* spp. Care must also be taken when comparing antimicrobial resistance data as different break-points may have been used, e.g. the majority of data referred to use a break-point of 16 mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin; however the study on raw/fresh broilers by Wilson (2003) used a breakpoint of 30 mg/litre for nalidixic acid. Small sample sizes are also an issue. In quantitative risk assessment, the uncertainty can be described, often using a *Beta* distribution, but this is not possible using a qualitative risk assessment approach. In Table C, when sample sizes have been low the working group has erred on the side of caution and/or made assumptions.

Detailed consumption data can also be difficult to obtain. Using the Irish data (Harrington et al., 2001), although there was good information on each type of food, there was no information on the level of processing for each food-type at the point of retail, i.e. whether the food consumed was purchased at retail as fresh/raw, minimally processed or processed. Consequently many assumptions had to be made, thereby adding to the uncertainty associated with the individual risk estimates. For some of the food types, there was no information available, or if there was information, the sample size was very small, e.g. in the case of minimally processed fruit, berries and juices. These limitations should be considered when comparing this source of fluoroquinolone-resistant *C. jejuni* to other food-types.

Table 3. **Examples of the risk of purchasing food contaminated with fluoroquinolone-resistant *Campylobacter***
(Prevalence data from UK (see Table C in the appendix), “purchasing” data from

Prevalence of FQ-resistant <i>Campylobacter</i>	Frequency of consumption of purchased food items			
	Daily (>50%)	Weekly (5-50%)	Monthly (0.5-5%)	Rarely (<0.5%)
High (>1%)	Raw broiler meat			
Medium (0.01-1%)	Raw beef Raw pork	Raw sheep Raw turkey	Offal Private water supplies	Raw milk Raw dairy
Low (<0.01%)	Processed milk Processed dairy Processed cheese Eggs Fish Vegetables Soft fruit Juices Cereals Community water supplies	Processed beef Processed pork Processed sheep Processed poultry Raw shellfish	Game	Raw cheese

6.2.5. *Future development of risk assessment approaches*

As has been indicated before, full risk assessments of AMR bacteria in foods are demanding in terms of data availability and resources. This is not a characteristic of the risk assessment as such, but rather of the risk management questions. Comparing the risks of different pathogens in different foods is a demanding task that cannot be done by any approach without adequate resources. Likewise, the impact of potential control options at some stage in the production chain on the risk of consumer exposure and/or health is a complex issue.

A possible strategy is to build up AMR risk assessments on the basis of available risk assessments for sensitive bacteria.

For the purpose of risk ranking, an exposure assessment model as presented by Evers et al. (2008) could be adopted. This model, which is also discussed in the EFSA Opinion on *Salmonella* in meat (EFSA, 2008b), estimates exposure per person per day, averaged over a specified population (e.g. all inhabitants of one country). Exposure is estimated separately for all relevant sources (different food products, but also animal contact, environment, etc.). For food sources, the average exposure is estimated by multiplication of (averages of) the daily intake of the food product, the fraction of contaminated products at retail, the concentration of pathogens in contaminated products at retail and the fraction of pathogens that is eventually ingested by consumers. For foods that are consumed raw, this fraction is 1; for foods that are cooked before consumption the fraction is between 0 and 1 as this fraction can result from undercooking and/or cross-contamination. Similar factors are taken into account for environmental (e.g. water) exposure. For animal contact, in the course of food preparation, calculations involve factors such as the frequency of human-animal contact and the (probability of) ingestion of faeces per contact.

Results of all exposures can be cumulated to calculate the total exposure or can be ranked to identify the most significant sources of exposure. There are currently many data gaps, and uncertainty analysis is an essential component of the calculations. Uncertainty is explicitly included in the exposure estimates. For AMR risk assessment, additional data are necessary on the prevalence of resistant bacteria in all exposure routes to be considered. Such data are available from the literature, from special studies or by using proxies (e.g. the prevalence of AMR bacteria in raw milk is similar to that in cattle faeces).

The exposure assessment model can be regarded as an extension to the cross-tabulation approach presented in this document. The exposure model takes the same variables into account but is based on quantitative rather than categorical estimates. Furthermore, it also includes the effects of preparation and cross-contamination.

Risk assessment models that need to evaluate the effect of interventions on AMR risk can also be built on existing (farm-to-fork) models. For example, EFSA has currently outsourced a QMRA on *Salmonella* in pigs¹⁵. This model can in future be simply extended to include the spread of resistant salmonellae in the food chain by multiplying the prevalence of *Salmonella* at the farm gate by the percentage of bacteria examined that are resistant. The underlying assumption would be that the behaviour of resistant bacteria in the food chain is similar to that of sensitive bacteria of the same genus, species or serotype. In the absence of selective pressure, this appears to be a realistic assumption. A more complex question would be to evaluate, *inter alia*, the impact of a reduced use of antibacterial agents in primary production. This would be subject to the development of new modules on the relationship between usage of and resistance to antimicrobials.

6.2.6. Antimicrobial resistance genes in genetically modified organisms

For GM plants, which can be present in food:

While this opinion primarily deals with AMR bacteria in foods, concerns have been raised about the possible link between the presence of antibiotic resistance marker genes in GM plants and the increase of resistance against relevant antibiotics in human, animals and in organisms in the wider environment as a result of horizontal gene transfer. The GMO Panel in its opinions concludes that this route of antibiotic resistance development is extremely unlikely. The conclusion is based on the fact that the successful transfer of a functional antibiotic resistance gene (or any other gene) from a GM plant into a bacterium would require a series of biologically complex and unlikely events to occur (EFSA 2004b and EFSA 2007¹⁶).

The use of antibiotic resistance marker genes in GM plants has been the subject of several reviews (Gay and Gillespie, 2005, Goldstein et al., 2005, Miki and McHugh, 2004, Nap et al., 1992, Nielsen et al., 1998, Ramessar et al., 2007) and expert consultations: Working Party of the British Society for Antimicrobial Chemotherapy (Bennett et al., 2004), FAO/WHO Consultation on Foods Derived from Biotechnology (FAO/WHO, 2000), Scientific Steering Committee of the European Commission (SSC, 1999) Zentrale Kommission für die Biologische Sicherheit, DE (ZKBS, 1999), The Advisory Committee on Novel Foods and Processes, UK (ACNFP, 1996). It has been concluded in these reports that the frequencies of gene transfer from plants to bacteria are likely to be extremely low and that the presence of

¹⁵ EFSA Article 36 Cooperation, call for proposal, CFP/EFSA/BIOHAZ/2007/01: Quantitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs. www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178622332966.htm

¹⁶ www.efsa.europa.eu/EFSA/Statement/gmo_statement_nptII_0.pdf

antibiotic resistance marker genes in GM plants do not pose a relevant risk to human or animal health or to the environment.

7. Prevention and control options

Proposals for the control of the transmission of AMR bacteria and resistance genes to humans through the agency of food require to take account of the factors that give rise to AMR and to the ways in which food becomes exposed to contamination with these agents (Figure 1). Regarding the former, since the use of antimicrobials in human and veterinary medicine is the major initiating factor for such resistance, the control and conditions of use of antimicrobials in both fields have been extensively addressed elsewhere and are the subject of various reports (WHO, 2000, 2001; Codex, 2005). The induction of AMR through the therapeutic use of antimicrobials in human and veterinary medicine is an inevitable consequence of the ethical need, on health and welfare grounds, to use such treatments in a clinical situation. Of particular concern is the impact of their continued use in the form of mass medication for the treatment of infectious diseases in food animals kept in intensive production systems. This issue has been comprehensively addressed elsewhere (WHO, 1997, 2000).

The dissemination of bacteria that have acquired AMR attributable to human medical treatment, both in a hospital setting and in the home, occurs in both the general environment and in the home. When this occurs in the environment of the kitchen, then food becomes exposed as the result of direct or indirect contamination, e.g. through handling. Consequently, the prevention and control of food contamination with antimicrobial-resistant bacteria, both pathogenic and otherwise, from this and other sources, relies on the consistent and effective application of good food hygiene practices. Of equal if not greater importance is the prevention and control of the spread of AMR bacteria originating from primary food animal production and to a lesser extent from contamination in the course of harvesting, processing and distribution. Here again the sustained application of good hygienic practices (Council Directive 93/43/EEC) throughout the food chain provides a varying degree of assurance against the introduction of AMR bacteria onto or into food, as has been adequately demonstrated in the case of known pathogens and the control of spoilage bacteria.

Recently, other concerns have arisen in relation to bacterial contaminants of processed foods in which AMR has resulted from transformation induced by the effects of modern processing or preservation methods, and the possibility that the increasing use of biocides in industry and in the home may itself induce AMR in a wide range of bacteria. The dissemination of such AMR bacteria, were they to arise, would likely be addressed through the application of good hygienic practices or, where necessary, by modification or suspension of the food processing involved and by the selective use, or the withdrawal from use, of biocides shown to induce AMR. In industry the dissemination of those AMR microorganisms can be mitigated by using proper and convenient validated physical treatments (for example, sterilization or pasteurization treatments).

Further control options that address the induction of AMR in bacteria and that are aimed at limiting the spread of such bacteria are discussed below.

7.1. Controlling spread of infections and of resistant bacteria

7.1.1. Preventing infectious diseases in animals and plants

Reduction of animal diseases can be expected to reduce the need to use antimicrobials in food production. Measures to reduce disease in animals include specific measures such as vaccination and the prevention of spread through biosecurity and general operational hygiene management measures.

The use of antimicrobials for treatments against plant pathogenic bacteria is generally not permitted in European countries, although the situation varies between MS (McManus et al., 2002). However, in other parts of the world where antimicrobials are registered for use against the fire blight of Rosaceae, widespread resistance to streptomycin has been reported for *Erwinia amylovora*, the causal agent of fire blight (McManus et al., 2002).

Measures which may help in reducing the inoculum and the spread of bacterial disease in plants are the use of healthy pathogen-free plant propagation material and resistant plant varieties, the application of protective treatments with copper compounds and the adoption of correct cultural hygiene practices.

7.1.2. Control and prevention of *Salmonella* and other zoonotic bacteria in animals

The use of programmes aimed at the prevention and control of *Salmonella* and other zoonotic bacteria in primary animal production, can lead to a reduction in the level of contamination of related food products at retail, and thereby also reduce the risk of human exposure to AMR salmonellae from those food products. The occurrence of *Salmonella* and AMR salmonellae in other food commodities is also likely to be reduced as the risk of cross-contamination is reduced.

In all cases, antimicrobials have no part to play in control programs for *Salmonella* in food production (e.g. EFSA, 2004a, 2006c).

7.1.3. Improved hygiene

Improved hygiene at all steps of the food chain, including primary production, is effective in reducing the number of foodborne pathogens in food. This will also reduce the numbers of foodborne pathogens that are resistant to antimicrobials.

7.1.4. Processing

Most food processing technologies aim to reduce the level of contamination of foods with bacterial pathogens as well as the overall bacterial load. As a consequence, the presence of foodborne AMR bacteria is also reduced. In addition, bacteria added intentionally to the food chain may also contribute to the control of foodborne pathogens, they should however be free of potentially transferable antimicrobial resistance before their application in feed or food processing.

7.1.5. Recirculation

Animal manures and municipal sludges, particularly in the latter case those sludges that contain effluents from hospitals, contain many types and numbers of pathogenic and other bacteria,

including AMR bacteria in both these categories. Hospital effluent has been demonstrated to contain a high proportion of AMR *E. coli* (Chitnis et al., 2000; Reinhaller et al., 2003) and vancomycin resistant enterococci on occasion (Iversen et al., 2002). This is not to discount the contribution of AMR bacteria contributed by the effluent from the general population.

The WHO has indicated that in countries that do not experience epidemics of enteric disease, it is acceptable to discharge the effluent from health-care establishments to municipal sewers without pretreatment, provided specific requirements are met (WHO, 1999). If these requirements cannot be met, the wastewater should be managed and treated accordingly.

Proper management of both these materials, if their application on land used for food production or their discharge into waterways and estuaries serving aquaculture is intended, can effectively address the risk they pose for food animals and fresh produce produced on these lands and in these waterways. Any consequential transmission onto food of AMR bacteria and genes derived from animal manures and municipal sludges can be controlled concurrently by employing processes used for the treatment of manures and municipal sludge that effectively mitigate any food-related risks to human health posed by such use.

7.2. Appropriate usage of antimicrobials

Reduction of the use of antimicrobials in general, or of specific antimicrobial classes, reduces the selective pressure and in many cases will thereby reduce the prevalence of resistant bacteria. A reduction of use can be obtained in different ways as detailed in, e.g. the SSC report (1999), the OIE guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine (OIE, 2007a), WHO Global principles for the containment of antimicrobial resistance in animals intended for food (WHO, 2000) and the WHO global strategy on containment of antimicrobial resistance (WHO, 2001). For example, a drastic reduction in their use may be encouraged through regulatory interventions, such as withdrawal of authorization or other restrictions. However, antimicrobials are needed to treat animal diseases and therefore, these measures can only be applied where such use is unnecessary or where there are clear alternatives available.

A sharing of responsibility on the part of all stakeholders, i.e. advisers, producers, processors, distributors and those involved in final stages of food preparation, that is based on a knowledge and appreciation both of the consequences of AMR for the human patient and the means of retaining an effective arsenal of antimicrobial agents, is recognised as a key factor when addressing AMR as a global issue of grave concern. Measuring the effectiveness of interventions and identifying priority areas

Monitoring of the occurrence of antimicrobial resistance, as well as the extent of use of antimicrobials and their use in human medicine and food animal production can provide relevant background information when considering trends in antimicrobial resistance and its prevalence in relevant bacteria, including pathogens and non-pathogens, in foods. This information can provide a further means to identify problems, to measure the effect of interventions and to support policies on the use of antimicrobials.

8. Issues of immediate concern

Fluoroquinolone antimicrobials are an important addition to the list of antimicrobials available for the treatment of infectious diseases. The appearance and spread of strains of enterobacterial pathogens such as *Salmonella* Typhi, Typhimurium and Enteritidis with reduced sensitivity to

ciprofloxacin coupled with high-level resistance to nalidixic acid has been described in sections 4.1.1. and 4.1.2. above. A new development has been the emergence of strains with plasmid-mediated fluoroquinolone resistance in several countries world-wide, in both humans and food-production animals. Such plasmid-mediated resistance has resulted in an increased MIC to ciprofloxacin, with adverse effects for treatment (Hopkins et al., 2008). Strains with such resistance are rapidly increasing in incidence, and may pose a significant threat to public health.

The potential role of food and environmental sources in the epidemiology of transferable resistance genes has gained increased attention in relation to the rapid and recent emergence of resistance to 3rd generation cephalosporins, in particular of the CTX-M-type (Canton and Coque, 2006; Livermore et al., 2007). The genes coding for these enzymes may be located on highly transferable plasmids and are found in bacteria causing infections in hospitals, but also in infections acquired in the community, in *Salmonella* from cases of human infection and food animals and commensal *E. coli* isolated from animals (Livermore et al., 2007). The genes encoding this type of resistance may be physically linked in mobile genetic structures with fluoroquinolone resistance, and also with genes encoding resistance to other resistance genes (Canton and Coque, 2006). The role of food, water and the environment in the spread of apparently epidemic plasmids encoding multiple resistance is not clear, but deserves immediate attention.

Concerns regarding the increasing rate of isolation of MRSA from food producing animals and whether or not there is a link to public health through food now need to be addressed.

In view of experimental findings, the possibility that new food processing and preservation treatments may induce AMR in commensal and other bacteria, as a result of transformation, merits attention.

In relation to food as a vehicle for their transmission to humans, other aspects of AMR and the emergence of new patterns of resistance in bacteria that to-date have attracted little attention are now likely to arise as a focus of concern due to changing patterns in medical and veterinary use of antimicrobials, in food consumption and in international trade in food.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The principles that are applied to the prevention and control of the spread of pathogenic bacteria *via* food will also contribute to the prevention and control of the spread of antimicrobial-resistant pathogenic bacteria. As antimicrobial resistance in foodborne pathogens and commensals represents a specific public health hazard, additional control measures for antimicrobial-resistant bacteria may therefore be necessary.
- The present extent of exposure to AMR bacteria *via* food is difficult to determine and the role of food in transfer of resistance genes has not been fully explored to-date. Any further expansion of the occurrence of resistance among bacteria in foods, including fresh crop-based foods, is likely to have an influence on human exposure.
- Foodborne bacteria, including known pathogens and commensal bacteria, display an increasing, extensive and diverse range of resistance to antimicrobial agents of human and veterinary importance.

- Antimicrobial resistance in food exists both as a direct hazard and as an indirect hazard through resistance transfer.
 - The direct hazard is the presence on food of an AMR pathogenic bacterium which can colonise or infect a human being after ingestion of the food, or as a hazard that arises if a person acquires the infection through handling contaminated food.
 - The indirect hazard arises through resistance transfer and is defined as an antimicrobial-resistant bacterium that may transfer resistance genes to another bacterium that is either a commensal or a bacterium pathogenic for humans.
- In all cases where antimicrobial treatment in humans is indicated, resistance to the antimicrobials of choice is of clinical importance.
- Resistant *Salmonella* and *Campylobacter* involved in human disease are mostly spread through foods. With regards to *Salmonella*, contaminated poultry meat, eggs, pork and beef are prominent in this regard. For *Campylobacter*, contaminated poultry meat is prominent.
- Cattle are a major VTEC reservoir and resistant strains derived from bovines can colonise the human population *via* contaminated foods of bovine origin more commonly than from other foods.
- Food is also an important source for human infections with antimicrobial resistant *Shigella* spp. and *Vibrio* spp.
- Animal-derived products remain a potential source of MRSA. Food-associated MRSA, therefore, may be an emerging problem.
- Bacteria intentionally added to the food chain have on occasion exhibited AMR. As such they are to be regarded as an indirect foodborne hazard as in this case the hazard is considered as being the resistance gene.
- The public health consequences of exposure to antimicrobial-resistant commensal bacteria through food are less well defined.
- Recently identified links between AMR and virulence in foodborne pathogens are a cause for concern. Any enhancement of virulence in known pathogenic bacteria, and potentially in other bacteria as yet unidentified as pathogenic, as a consequence of acquiring resistance attributable to antimicrobial usage or gene transfer can adversely affect the outcome of treatment.
- By way of example, a qualitative ranking of food as a vector of an AMR bacterium demonstrated the complexity of the problem and the extensive data requirements for a formal ranking of risk.
- Any control measure requires to be viewed in the context of the critical and irreplaceable role specific antimicrobial therapeutic agents or groups of agents play in the treatment and management of life-threatening infectious diseases in humans.
- There are few examples of control programmes that directly control AMR as the hazard using measures that specifically address food, the final product, as the vehicle of concern. In terms of impact, controls operated at the pre-harvest phase, for example those aimed at the control and limitation of antimicrobial usage, are potentially the most effective and as such are capable of playing a major role in determining the AMR-status of food as presented for sale.

RECOMMENDATIONS

The development and application of new approaches to the recognition and control of food as a vehicle for AMR bacteria and related genes based on epidemiological and source attribution studies directed towards fresh crop-based foods, raw poultry meat raw pigmeat and raw beef are recommended.

The use of epidemiological cut-off values provides an appropriate level of sensitivity when measuring resistance development in bacteria. These criteria have been harmonised for use in both in human and veterinary medicine in the European Union. It is now important that these matters be addressed globally.

Specific measures to counter the current and developing resistance of known pathogenic bacteria to fluoroquinolones as well as to 3rd and 4th generation cephalosporins found in a variety of foods and in animals in primary production now require to be defined and put in place as a matter of priority.

As a major source of human exposure to fluoroquinolone resistance *via* food appears to be poultry, whereas for cephalosporin resistance it is poultry, pork and beef that are important, these food production systems require particular attention to prevent spread of such resistance from these sources.

If a full risk assessment for a specific food-bacterium combination, in respect of AMR, should be undertaken, methodologies currently available for the risk assessment of foods require to be modified for uniform adaptation at both MS and EU level for the risk assessment of those combinations (including foods originating from food animals, fish, fresh produce (e.g. lettuce etc.) and water, as a vehicle for the transmission of AMR bacteria and related genes).

Further research on the role of commensals and of bacteria intentionally added as an aid to food processing in the transmission of AMR *via* food to the human flora, aimed at identifying ways in which such transmission from these agents can be prevented, is recommended.

The role of food, water and the environment in the spread of apparently epidemic plasmids encoding multiple resistance is not clear, but deserves immediate attention.

Overall, control of all the routes by which AMR bacteria and their related genes can arise in the human patient, of which food is but one such route, requires a response from all stakeholders to acknowledge their responsibilities for preventing both the development and spread of AMR, each in their own area of activity including medicine, veterinary medicine, primary food animal production, food processing and food preparation, as well as in the regulation of food safety.

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APPENDICES

Table A: **Classes of antimicrobials, examples of substances used in human and veterinary medicine and examples of resistance genes.**

Note: There is generally complete or partial cross resistance within each class or subclass unless otherwise indicated

Class	Examples of substances used in:		Examples of resistance genes	Comments
	<u>Human medicine</u>	<u>Veterinary medicine;</u> <u>food production animals in EU</u>		
Aminoglycosides	amikacin, gentamicin, netilmicin, tobramycin	apramycin, gentamicin, streptomycin	<i>aac</i> , <i>aad</i> (<i>ant</i>), <i>aph</i> , <i>armA</i> , <i>rpsL</i> (<i>strA</i>) <i>rpsD</i> , <i>rpsE</i> , <i>strB</i>	No general cross-resistance within class, but some types of resistance will involve cross-resistance between some aminoglycosides
	kanamycin, spectinomycin, streptomycin	neomycin, spectinomycin		
Amphenicols	chloramphenicol, tiamphenicol	chloramphenicol, florfenicol, tiamphenicol	<i>cat</i> , <i>cfr</i> , <i>cml</i> , <i>flo</i>	cfr confers cross-resistance to amphenicols, lincosamindes, pleuromutilins, streptogramins, linezolid
Beta-lactam antibiotics				Cross resistance within subclasses but also, depending on mechanism, between subclasses
<i>Penicillins</i>	benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	<i>bla_Z</i> (<i>bla_{PC}</i>), <i>bla_{TEM}</i> , <i>bla_{SHV}</i> , <i>bla_{OXA}</i>	
<i>Beta-lactamase resistant penicillins</i>	cloxacillin, dicloxacillin (meticillin)	cloxacillin, dicloxacillin	<i>bla_{OXA}</i> , <i>mecA</i>	
<i>Cephalosporins, first generation</i>	cephalexin, cefazolin, cephalotin	cefazolin, cephalexin	<i>bla_{TEM}</i> , <i>Bla_{SHV}</i> , <i>bla_{CTX}</i> , <i>bla_{CMY}</i> , some <i>bla_{OXA}</i>	
<i>Cephalosporins, second generation</i>	cefuroxime, loracarbef	-		
<i>Cephalosporins, third generation</i>	ceftazidime, ceftriaxone	ceftiofur		
<i>Cephalosporins, fourth generation</i>	cefepime, cefpirome	cefepime, cefquinome		
<i>Cephameycins</i>	cefoxitin		<i>bla_{CMY}</i> , <i>bla_{AAC}</i>	
<i>Carbapenems</i>	ertapenem, imipenem, meropenem	-	<i>bla_{IMP}</i> , <i>bla_{VIM}</i> , <i>bla_{KPC}</i> some <i>bla_{OXA}</i>	

Class	Examples of substances used in:		Examples of resistance genes	Comments
	<u>Human medicine</u>	<u>Veterinary medicine;</u> <u>food production animals in EU</u>		
Cyclic polypeptides	bacitracin	(bacitracin)	<i>bcrABD</i>	Formerly used as feed additive in EU
Glycopeptides	teicoplanin, vancomycin	(avoparcin)	<i>van (A-E)</i>	Formerly avoparcin was used as feed additive in EU
Ionophores	-	monensin, salinomycin		Used as coccidiostats
Lincosamides	clindamycin, lincomycin	clindamycin, lincomycin	<i>cfr, erm</i>	Cross-resistance also to macrolides and streptogramin B for certain resistance genotypes
Lipopeptides	daptomycin	-		
Macrolides & ketolides	erythromycin, spiramycin, azithromycin, clarithromycin	spiramycin, tylosin, tulathromycin	<i>erm, ere, mef, msr</i>	Cross-resistance also to lincosamides and streptograminB for certain resistance genotypes
Nitrofurans	furazolidone, nitrofurantoin	-		Used formerly as veterinary medicine
Nitroimidazoles	metronidazole, tinidazole	-		Dimetridazole and ronidazole used formerly as veterinary medicine
Orthosomycins	-	avilamycin	<i>emtA</i>	Used formerly as feed additive
Oxazolidones	linezolid	-	<i>cfr</i>	
Pleuromutilins	-	tiamulin, valnemulin	<i>cfr</i>	
Polymixins	colistin, polymixin B	colistin, polymixin B		
Quinolones	nalidixic acid, ciprofloxacin, norfloxacin, moxifloxacin	danofloxacin, enrofloxacin,	<i>aac(6')-Ib-cr, gyrA, parC, qepA, qnr,</i>	Incomplete cross-resistance. <i>aac(6')-Ib-cr</i> also confers resistance to kanamycin
Quinoxalines	-	carbadox, olaquinox	<i>oqxAB</i>	Olaquinox used formerly as feed additive in the EU
Streptogramins	pristinamycin, quinpristin/dalfopristin	(virginiamycin)	<i>cfr, erm, vga, vgb</i>	Formerly virginiamycin was used as feed additive in EU Cross-resistance between streptogramin B, lincosamides and macrolides for certain resistance genotypes
Sulphonamides & trimethoprim	sulfadiazine, sulfamethoxazole, trimethoprim	sulfadiazine, sulfadoxine, sulfamethoxazole, trimethoprim	<i>dfr, sul</i>	
Tetracyclines	chlortetracycline, doxycycline, oxytetracycline	chlortetracycline, doxycycline, oxytetracycline	<i>tet</i>	

Class	Examples of substances used in:		Examples of resistance genes	Comments
	<u>Human medicine</u>	<u>Veterinary medicine;</u> food production animals in EU		
Miscellaneous	-	flavophospholipol (bambermycin)		Used formerly as feed additive
	fosfomycin	-	<i>fosAB</i>	
	fusidic acid	fusidic acid	<i>fusB</i>	
	mupirocin	-	<i>mupA</i>	Used in human medicine topically for MRSA decontamination
	rifampicin	(rifampicin)	<i>rpoB</i>	Use in vet. med limited to foals

Table B. **Occurrence of resistance to antimicrobials in *Escherichia coli* from food products (percent resistant isolates)^a** (Sources: The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005 and national reports)

Country	Year	Food type	Number of isolates	Reported resistance (%)											Data source
				Ampicillin	3rd gen cephalosporin	Chloramphenicol	Ciprofloxacin- /Enrofloxacin	Nalidixic acid	Gentamicin	Neomycin-/kanamycin	Streptomycin	Sulphonamides	Tetracycline	Trimethoprim	
Austria	2003	beef	40	2	-	2	2*	5	0	0	12	-	12	2	Remost 2004
Belgium	2005	beef	238	13	2	4	1	2	-	-	12	19	16	11	EFSA 2006
Denmark	2004	beef	196	8	0	1	0*	0	0	2	9	7	9	4	DANMAP 2005
The Netherlands	2004	beef	34	12	3	3	9*	3	0	0	-	15	12	12	MARAN 2005
Norway	2005	beef	90	3	0	0	0	0	0	0	10	3	2	3	EFSA 2006
Austria	2003	poultry	34	26	-	0	30*	32	4	0	35	-	38	18	Remost 2004
Belgium	2005	broiler	148	37	3	7	3	28	-	-	24	37	39	25	EFSA 2006
Denmark	2004	broiler	216	15	0	<1	0*	6	0	0	1	15	9	3	DANMAP 2004
The Netherlands	2004	poultry	115	54	11	8	30*	33	4	12	-	50	47	43	MARAN 2005
Austria	2003	pork	56	16	-	12	4*	4	0	2	54	-	55	12	Remost 2004
Belgium	2005	pork	86	10	-	7	1	2	-	-	19	15	21	15	EFSA 2006
Denmark	2004	pork	178	15	0	2	0*	2	2	3	13	18	26	10	DANMAP 2004
The Netherlands	2004	pork, organic	155	16	1	6	0*	0	4	38	-	27	38	17	MARAN 2005
Germany	2005	mixed meat	50	2	0	0	0	-	0	0	10	14	12	4	EFSA 2006
Portugal	2005	cheese	33	30	0	3	6	-	36	42	91	-	58	-	EFSA 2006
<i>Non-EU countries</i>															
Canada QC	2003	broiler	112	50	33	18	0*	1	18	11	48	43	57	-	CIPARS 2003
Canada; ON	2003	broiler	136	35	18	5	2*	2	7	9	32	24	51	-	CIPARS 2003
Canada QC	2003	pork	61	20	2	10	0*	0	2	3	28	31	48	-	CIPARS 2003
Canada ON	2003	pork	91	20	2	8	0*	0	1	6	17	30	55	-	CIPARS 2003
Canada QC**	2003	beef	84	7	0	1	1*	1	1	2	2	7	19	-	CIPARS 2003
Canada ON**	2003	beef	100	8	2	2	0*	0	0	2	6	14	23	-	CIPARS 2003

* cut-off of >0.06 i.e. the same as for DANMAP has been used to define resistance for the compilation of this table; ** ON = Ontario, QC = Quebec

References for Table B:

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Table C: Qualitative risk assessment for fluoroquinolone-resistant *Campylobacter jejuni* in the UK: example of use of template.

Please note - Data used is that which was readily available to working group or from informal expert opinion.
THIS IS NOT A FORMAL RISK ASSESSMENT.

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
1. Milk and dairy products (cows, goats sheep, buffalo, horse)	1.1 Milk	A. Fresh or raw	19/1097 (1.7%) unpasteurised cows' milk samples positive. (de Louvois & Rampling, 1998) 0/100 of unpasteurised goats' milk samples positive (Little & de Louvois, 1999) 0/26 of unpasteurised ewe's milk samples positive (Little & de Louvois, 1999).	Data from abattoir study used as an indicator of probability of antimicrobial resistance at retail (Teale, 2002). ¹⁸ 0/99 <i>C. jejuni</i> cattle isolates were resistant to nalidixic acid or ciprofloxacin. 0/65 <i>C. jejuni</i> sheep isolates were resistant to nalidixic acid or ciprofloxacin. Also - referred to data for bovine/sheep meat	Assumption. Prohibited from sale in Scotland.
		B. Minimally processed	Assumption. Lower probability than for raw milk.	Assumption. Same as raw milk.	Assumption.
		C. Processed	Pasteurisation very effective at killing <i>Campylobacter</i> . However outbreaks have occurred where pasteurisation has failed or from bird-pecked milk.	Assumption. Same as raw milk.	99.3% of participants in Irish survey consumed milk (Harrington et al., 2001). Assumed that majority is pasteurised.
	1.2 Dairy products	A. Fresh or raw	Assumption. Same as raw milk.	Assumption. Same as raw milk.	Assumption. Raw cows' cream prohibited from sale of in Scotland.
		C. Processed	Assumption. Same as processed milk.	Assumption. Same as raw milk.	74.6% of participants in Irish survey consumed dairy products (Harrington et al., 2001). Assumed that majority is purchased processed / pasteurised.
	1.3 Cheese	A. Fresh or raw	Assumption. Risk for cheese made from unpasteurised milk is assumed to be very low. This takes into account the risk for unpasteurised milk and also maturation time.	Assumption. Same as raw milk.	Assumption.

¹⁷ UK data (MAFF 2000) readily available to the working group gave % of all household purchasing each type of food in survey week, which was unsuitable for the risk assessment template; therefore Irish consumption data were used.

¹⁸ Breakpoint of 16 mg/ml for nalidixic acid; 1 mg/ml for ciprofloxacin

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		B. Minimally processed (smoked)	Assumption.	Assumption. Same as raw milk.	Assumption.
		C. Processed	Assumption.	Assumption. Same as raw milk.	78.0% of participants in Irish survey consumed cheese (Harrington et al., 2001). Assumed that majority is purchased processed / pasteurised.
2. Egg and egg products		A. Fresh or raw	0/226 eggs produced from hens <u>faecally shedding</u> <i>C. jejuni</i> were positive; 2 of the shell surfaces were <i>C. jejuni</i> positive (Doyle, 1984). 2/187 (1.1%) eggs produced from hens <u>faecally shedding</u> <i>C. jejuni</i> were positive due to penetration of the egg shell. 0/142 eggs derived from birds of unknown status were <i>C. jejuni</i> positive (Shanker et al., 1986)	Assumption. Same fresh or raw broiler meat. However, use of quinolones may <u>significantly</u> differ between layers and broilers - high level of uncertainty	92.2% of participants in Irish survey consumed egg & egg products (Harrington et al., 2001). Assumed that majority purchased as raw.
		B. Minimally processed (as in desserts)	Assumption. Lower probability than for fresh or raw eggs.	Assumption. Same as fresh or raw eggs.	Assumption.
		C. Processed	Assumption. Lower probability than for fresh or raw eggs.	Assumption. Same as fresh or raw eggs.	Assumption.
3. Red meats	3.1 Bovine	A. Fresh or raw	71/1514 (4.7%) of meat cuts and 6/49 (12.2%) offal portions were positive for Campylobacter. 43/49 (87.7%) isolates were <i>C. jejuni</i> (Little et al., 2008) 47/96 (49%) of ox liver samples were positive for <i>C. jejuni</i> . (Kramer et al, 2000)	13.9% of <i>C. jejuni</i> resistant to nalidixic acid; 11.6% resistant to ciprofloxacin (43 isolates tested) (Little et al., 2008) ¹⁹ 15.1% of 53 <i>C. jejuni</i> isolates isolated from ox liver were resistant to nalidixic acid; 3.8% were resistant to ciprofloxacin ²⁰ . (Kramer et al., 2000)	87.8% of participants in Irish survey consumed bovine meat and products (Harrington et al. 2001). Assumed that majority is purchased as raw and as meat cuts (i.e. not offal).

¹⁹ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

²⁰ Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		B. Minimally processed (cured and/or smoked)	Assumption.	Assumption. Same as fresh or raw bovine meat.	Assumption
		C. Processed	0/3085 ready-to-eat burgers (of which 90% were beef) purchased from burger outlets were positive for <i>Campylobacter</i> (Little et al., 2001). 1/546 (0.18%) ready to eat beef (cold) positive for <i>Campylobacter</i> (Elson et al., 2004) 0/371 VP-MAP ready to eat beef positive for <i>Campylobacter</i> (Sagoo et al., 2007)	Assumption. Same as fresh or raw bovine meat.	Assumption. Significant proportion is purchased as processed.
	3.2 Pig	A. Fresh or raw	66/1309 (5%) of meat cuts and 24/131 (18.3%) offal portions were positive for <i>Campylobacter</i> . 36/68 (52.9%) isolates were <i>C. jejuni</i> (Little et al., 2008) 34/99 (34.3%) of pigs liver samples were positive for <i>C. jejuni</i> . (Kramer et al., 2000)	27.8% of <i>C. jejuni</i> resistant to nalidixic acid; 19.4% resistant to ciprofloxacin (36 isolates tested) (Little et al., 2008) ²¹ 33.3% of 36 <i>C. jejuni</i> isolates isolated from pigs liver were resistant to nalidixic acid; 5.6% were resistant to ciprofloxacin ²² . (Kramer et al., 2000)	93.7% of participants in Irish survey consumed pig meat and products (Harrington et al. 2001). Assumed that significant proportion is purchased as raw (includes sausages) and not as offal.
		B. Minimally processed (cured and/or smoked)	Assumption. Lower probability than for fresh or raw pig meat.	Assumption. Same as fresh or raw pig meat.	93.7% of participants in Irish survey consumed pig meat and products (Harrington et al. 2001). Assumed that significant proportion is purchased as minimally processed (includes bacon).

²¹ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

²² Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		C. Processed	0/1423 ready to eat ham (cold) positive for <i>Campylobacter</i> . 0/243 ready to eat pork (cold) positive for <i>Campylobacter</i> . (Elson et al., 2004) 0/1351 VP-MAP ready to eat ham positive for <i>Campylobacter</i> . 0/206 VP-MAP ready to eat pork positive for <i>Campylobacter</i> (Sagoo et al., 2007)	Assumption. Same as fresh or raw pig meat.	93.7% of participants in Irish survey consumed pig meat and products (Harrington et al. 2001). Assumed that significant proportion is purchased as processed (includes ham).
	3.3 Sheep	A. Fresh or raw	55/744 (7.4%) of meat cuts and 59/161 (36.6%) offal portions were positive for <i>Campylobacter</i> . 64/90 (71.1%) isolates were <i>C. jejuni</i> (Little et al., submitted) 72/96 (75%) of lambs liver were positive for <i>C. jejuni</i> . (Kramer et al., 2000).	12.5% of <i>C. jejuni</i> resistant to nalidixic acid; 10.9% resistant to ciprofloxacin (64 isolates tested) (Little et al., 2008) ²³ 7% of 100 <i>C. jejuni</i> isolates isolated from lambs liver were resistant to nalidixic acid; 4% were resistant to ciprofloxacin ²⁴ . (Kramer et al. 2000).	34.5% of participants in Irish survey consumed sheep meat and products (Harrington et al. 2001). Assumed that majority is purchased as raw and not as offal.
		B. Minimally processed (cured and/or smoked)	Assumption. Lower probability than for fresh or raw sheep meat.	Assumption. Same as fresh or raw sheep meat.	Assumption.
		C. Processed	0/19 ready to eat lamb (cold) positive for <i>Campylobacter</i> (Elson et al., 2004) Note - low sample size. Assumption. Lower probability than for fresh or raw sheep meat.	Assumption. Same as fresh or raw sheep meat.	Assumption.
	3.4 Other	A. Fresh or raw	GAME & OTHER MEATS ²⁵ : 5/47 (10.6%) of meat cuts; 0/1 (0%) offal portions and 0/3 (0%) whole animals were positive for <i>Campylobacter</i> . 3/4 (75%) were <i>C. jejuni</i> (Little et al., 2008) Note - low sample size.	GAME & OTHER MEATS: 0% of <i>C. jejuni</i> resistant to nalidixic acid; 0% resistant to ciprofloxacin (3 isolates tested) (Little et al., 2008) ²⁶ Note - low sample size. Assumption.	4.1% of participants in Irish survey consumed 'other' red meat and products (Harrington et al. 2001). Assumed that majority is purchased as raw and as meat cuts.

²³ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

²⁴ Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

²⁵ hare, rabbit, venison, goat, mutton

²⁶ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		B. Minimally processed (cured and/or smoked)	Assumption. Lower probability than for fresh or raw 'other' meat.	Assumption. Same as fresh or raw 'other' red meat.	Assumption.
		C. Processed	Assumption. Lower probability than for fresh or raw 'other' meat.	Assumption. Same as fresh or raw 'other' red meat.	Assumption
4. Poultry meats	4.1 Broiler	A. Fresh or raw	In large UK study, 50% of chickens sampled (5394) were positive for <i>Campylobacter</i> spp. [56% fresh chicken; 31% frozen chicken]. Of 1636 isolates tested, 74% were <i>C. jejuni</i>. (Food Standards Agency, 2003). 187/301 (62.1%) whole birds and 896/1477 (60.7%) portions were positive for <i>Campylobacter</i>. 163/239 (68.2%) were <i>C. jejuni</i> (Little et al., submitted). In Northern Ireland study 141/412 (34%) of local whole chickens were <i>C. jejuni</i> positive. From imported (frozen) chicken breasts 33/150 (22%) were <i>C. jejuni</i> positive. (Wilson, 2003). 156/198 (77.3%) of chilled chicken breast or thigh portions were positive for <i>C. jejuni</i>. (Kramer et al., 2000).	In large UK study, resistance to ciprofloxacin was detected in 13% of <i>C. jejuni</i> isolates. (Food Standards Agency, 2003). 11% of <i>C. jejuni</i> resistant to nalidixic acid; 9.4% resistant to ciprofloxacin (64 isolates tested) (Little et al., submitted)²⁷ 187/595 (31.4%) and 12/595 (2.0%) <i>Campylobacter</i> spp. isolates (from raw whole chickens) resistant to nalidixic acid and ciprofloxacin, respectively²⁸ (Anon., 2005). In Northern Ireland study, 9/141 (6.4%) of isolates from local whole chickens were resistant to nalidixic acid and 12/141 (8.5%) resistant to ciprofloxacin. From imported (frozen) chicken breasts, 6/33 (18.1%) were resistant to nalidixic acid and 6/33 (18.1%) resistant to ciprofloxacin. ²⁹ (Wilson, 2003). 15% of 194 <i>C. jejuni</i> isolates were resistant to nalidixic acid; 10.8% were resistant to ciprofloxacin³⁰. (Kramer et al., 2000).	84.3 % of participants in Irish survey consumed broiler meat and products (Harrington et al., 2001). Assumed that majority is purchased as raw.
		B. Minimally processed (cured and/or smoked)	Assumption.	Assumption. Same as fresh or raw broiler meat.	Assumption

²⁷ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

²⁸ Anon. 2005 used the HPA breakpoints, i.e 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

²⁹ Wilson, 2003 used the following concentrations of antimicrobials in the discs susceptibility testing: 30µg for nalidixic acid; 1µg for ciprofloxacin.

³⁰ Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		C. Processed	0/121 ready to eat chicken (cold) positive for <i>Campylobacter</i> (Elson et al., 2004) 0/495 VP-MAP ready to eat chicken positive for <i>Campylobacter</i> (Sagoo et al., 2007) 0/449 chicken sandwich samples were positive for <i>Campylobacter</i> (Little et al., 2002).	Assumption. Same as fresh or raw broiler meat.	Assumption
	4.2 Turkey	A. Fresh or raw	1/2 (50%) of whole birds and 71/212 (33.5%) portions were positive for <i>Campylobacter</i> . 7/15 (46.7%) were <i>C. jejuni</i> . (Little et al., submitted)	16.7% of <i>C. jejuni</i> resistant to nalidixic acid; 16.7% resistant to ciprofloxacin (6 isolates tested) (Little et al., submitted) ³¹ Note - low sample size.	24.1 % of participants in Irish survey consumed turkey meat and products (Harrington et al., 2001). Assumed that a significant proportion is purchased as raw.
		B. Minimally processed (cured and/or smoked)	Assumption. Lower probability than fresh or raw turkey meat	Assumption. Same as fresh or raw turkey meat.	24.1 % of participants in Irish survey consumed turkey meat and products (Harrington et al., 2001). Assumed that a lesser proportion is purchased as minimally processed.
		C. Processed	0/411 ready to eat turkey (cold) positive for <i>Campylobacter</i> (Elson et al., 2004) 1/523 VP-MAP ready to eat turkey positive for <i>Campylobacter</i> (Sagoo et al., 2007)	Assumption. Same as fresh or raw turkey meat.	24.1 % of participants in Irish survey consumed turkey meat and products (Harrington et al., 2001). Assumed that a significant proportion is purchased as processed.
	4.3 Other	A. Fresh or raw	DUCK: 2/7 (28.6%) of whole birds and 37/70 (52.9%) portions were positive for <i>Campylobacter</i> . 8/19 (42.1%) isolates were <i>C. jejuni</i> . (Little et al., submitted) OTHER ³² : 11/23 (47.8%) of whole birds and 1/12 (8.3%) portions were positive for <i>Campylobacter</i> . 2/4 (50.0%) were <i>C. jejuni</i> . (Little et al., submitted) Note - low sample size	DUCK. 0% of <i>C. jejuni</i> resistant to nalidixic acid; 0% resistant to ciprofloxacin (9 isolates tested) (Little et al., submitted) OTHER: 0% of <i>C. jejuni</i> resistant to nalidixic acid; 0% resistant to ciprofloxacin (3 isolates tested) (Little et al., submitted) ³³ Note - low sample sizes. Assumption. Same as fresh chicken & turkey.	2.3% of participants in Irish survey consumed other poultry meat and products (Harrington et al. 2001). Assumed that majority is purchased as raw.

³¹ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

³² grouse, guinea fowl, ostrich, partridge, pheasant, poussin, quail, wood pigeon

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		B. Minimally processed (cured and/or smoked)	Assumption.	Assumption. Same as fresh or raw 'other' poultry meat.	Assumption
		C. Processed	Assumption.	Assumption. Same as fresh or raw 'other' poultry meat.	Assumption
5. Aquaculture and marine	5.1 Fish	A. Fresh or raw	Assumption	Assumption.	65.1% of participants in Irish survey consumed fish and fish products (Harrington et al. 2001). Assumed that significant proportion is purchased as raw.
		B. Minimally processed (pickled and/or smoked)	Assumption	Assumption.	Assumption
		C. Processed	Assumption	Assumption.	65.1% of participants in Irish survey consumed fish and fish products (Harrington et al. 2001). Assumed that significant proportion is purchased as processed.
	5.2 Crustaceans, shellfish, molluscs	A. Fresh or raw	In Irish study, 3/117 (2.5%) of seafood sampled (oysters and mussels) were <i>C. jejuni</i> positive (Whyte et al., 2004). 47% of 331 mixed bivalves (cockles, mussels, scallops) shortly after harvesting were positive for <i>Campylobacter</i> spp. Only 2% of these were <i>C. jejuni</i> . (Wilson & Moore, 1996).	Assumption.	8.8% of participants in Irish survey consumed crustaceans, shellfish and molluscs (Harrington et al., 2001). Assumed that small proportion is purchased as processed.
		B. Minimally processed	Assumption	Assumption.	Assumption.

³³ Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
		C. Processed	3/49 (6%) of depurated oysters were positive for <i>Campylobacter</i> spp. None were <i>C. jejuni</i> . (Wilson & Moore, 1996). Note - low sample size for <i>C. jejuni</i> .	Assumption.	8.8% of participants in Irish survey consumed crustaceans, shellfish and molluscs (Harrington et al., 2001). Assumed that majority is purchased as processed.
6. Vegetables, cereals, fruits	6.1. Vegetables and juices	A. Fresh or raw	0/2870 open ready to eat salad samples positive for <i>Campylobacter</i> (Sagoo, et al., 2003a) 0/3827 ready to eat salad vegetable samples positive for <i>Campylobacter</i> (Sagoo et al., 2003b) 0/151 unprepared non-UK whole lettuce positive for <i>Campylobacter</i> (Little et al., 1999) 0/2883 ready-to-eat organic vegetables positive for <i>Campylobacter</i> (Sagoo et al., 2001)	Assumption. Animal/human wastes spread onto land	99.9% of participants in Irish survey consumed vegetables & juices and products (Harrington et al., 2001). Assumed that majority purchased as raw.
		B. Minimally processed (pickled vegetables)	Assumption. Lower probability than for fresh or raw vegetables.	Assumption. Same as fresh/raw	Assumption.
		C. Processed	Assumption. Lower probability than for minimally processed vegetables.	Assumption. Same as fresh/raw	99.9% of participants in Irish survey consumed vegetables & juices and products (Harrington et al., 2001). Assumed that significant proportion is purchased as processed.
	6.2 Cereal products	A. Fresh or raw	Assumption.	Assumption. Animal/human wastes spread onto land	100% of participants in Irish survey consumed cereal products (Harrington et al., 2001). Assumed that large proportion is purchased as fresh/raw.
		B. Minimally processed	Assumption. Lower probability than for fresh/raw cereals	Assumption. Same as fresh/raw	Assumption
		C. Processed	Assumption. Lower probability than for fresh/raw cereals	Assumption. Same as fresh/raw	100% of participants in Irish survey consumed cereal products (Harrington et al., 2001). Assumed that majority are purchased as processed.

Food category	Food sub-category	Processing factors	Probability of bacteria being present in food at retail	Probability that bacteria present in food at retail is resistant to antimicrobial class of interest	Probability food purchased and consumed ¹⁷
	6.3 Fruit, berries and juices	A. Fresh or raw	0/143 strawberries were positive for <i>Campylobacter</i> spp.; 0/162 melons were positive for <i>Campylobacter</i> spp. (Williamson et al., 2003)	Assumption.	91.4% of participants in Irish survey consumed fruits, berries, juices and products (Harrington et al., 2001). Assumed that majority are purchased raw.
		B. Minimally processed	Assumption	Assumption.	Assumption
		C. Processed	Assumption	Assumption.	Assumption
7. Herbs and spices		A. Fresh or raw	Assumption	Assumption. Animal/human wastes spread onto land	58.1% of participants in Irish survey consumed herbs and spices (Harrington et al., 2001). Assumed that lesser proportion are purchased as fresh herbs/spices.
		B. Minimally processed	Assumption	Assumption. Same as fresh/raw herbs and spices	Assumption
		C. Processed	Assumption	Assumption. Same as fresh/raw herbs and spices	58.1% of participants in Irish survey consumed herbs and spices (Harrington et al., 2001). Assumed that majority are purchased as processed (i.e. dried).
8. Mixed or buffet meals*					
9. Other foods*					
10. Tap water, including well-water		A. Not chlorinated	Assumption	Assumption. Private water supplies may be contaminated by run-off from nearby land	Assumption
		B. Chlorinated	Assumption	Assumption. Same as unchlorinated water.	94.2% of participants in Irish survey consumed tap water (Harrington et al., 2001). Assumed that majority is chlorinated.

* As assessed in respect of the food component subjected to the least amount of heat treatment or exposure to comparable treatment, e.g. raw meat, raw vegetable, smoked fish.

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