Foodborne antimicrobial resistance as a biological hazard

Draft Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-089)

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Panel Members


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 as a vector of an AMR bacterium demonstrated the complexity of the problem and the extensive data requirements for a formal risk ranking.

Resistant Salmonella and Campylobacter involved in human disease are mostly spread through foods. Contaminated poultry meat, eggs, pork and beef are prominent in this regard. Bovines are a major 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 community acquired MRSA. Food-associated MRSA, therefore, may be an emerging problem.
The principles that are applied to the prevention and control of the spread of pathogenic bacteria through the agency of food will also contribute to the prevention and control of AMR bacteria. However, antimicrobial resistance may increase the severity of the hazard, or may change the nature of the hazard and additional control measures for AMR 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 at the first instance towards fresh crop-based foods, raw poultry meat and raw pigmeat 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.

As the principal origin of human exposure to fluoroquinolone resistance via food appears to be poultry, whereas for cephalosprins it is poultry and pork, 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 who 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 ESFA

Antimicrobial resistant bacteria are biological hazards\(^1\) resulting in 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, 1969\(^2\). 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). The effect of this ban on the extent of bacterial antimicrobial resistance both within farm animals, and with regard to human health, however, is as yet unknown.

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 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.
- Development of antimicrobial resistant bacteria as a consequence of contact of pathogenic and commensal bacteria with residues of antimicrobial agents in food.

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\(^1\) Hazard- “a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect”

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.

**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 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.

3. To identify potential control options for reducing exposure.

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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 antimicrobials 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 thousand 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 a Codex Task Force (Codex Alimentarius Commission, 2007), which aims to assess the risks to human health associated with the presence in food and feed of antimicrobial-resistant organisms, antimicrobial resistance genes and residues of antimicrobials, and to develop risk management advice based on that assessment to reduce such a risk.

The two most important factors influencing the emergence and spread of AMR are the use of antimicrobial agents in different hosts and 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. 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, but 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 both sectors conditions exist that facilitate the spread of bacteria, such as high density and/or poor infection control.

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3 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 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 biocides 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. 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 food](image)

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 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

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4 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

5 Food from primary production includes fresh meat, fruit and vegetables. Water is also included in this category
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. Disinfectants and other biocides
are addressed only in the chapter dealing with the effect of food processing technologies on
antimicrobial resistance.

Antimicrobial susceptibility or resistance is generally defined on the basis of \textit{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, 2005, 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, 3\textsuperscript{rd}/4\textsuperscript{th} 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, 2007).
2.2. Definitions of resistance

2.2.1. Clinical resistance

Clinically-resistant infections are defined as those infections having a low probability of responding to treatment, even if maximum doses of a given antimicrobial are administered (EUCAST, 2000; Acar & 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 & 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 countries 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). Some of these criteria may need to be reviewed.

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 (penicillinas and cephalosporinas (*bla*-genes), acetyl transferases modifying e.g. aminoglycosides (*aac*-genes), as are genes leading to target modification (*erm*-genes), meticillin*-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.

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 transfer\(^7\). 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

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\( ^6 \) Meticillin (International Nonproprietary Name) = Methicillin (United States Adopted Name)

\( ^7 \) 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.
handling contaminated food. The indirect hazard arises through resistance transfer and is

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, viz. (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.

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), (Table 1), 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; Cérémonie 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.

Table 1. Mechanisms of action of food processing and preservation processes*

<table>
<thead>
<tr>
<th>Technology</th>
<th>Mechanism of inactivation</th>
<th>Possible link to AMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>Protein, DNA, RNA denaturation</td>
<td>Mutation</td>
</tr>
<tr>
<td>High Pressure</td>
<td>Changes in membrane structure and functionality</td>
<td>Transformation</td>
</tr>
<tr>
<td>Electric Pulsed Fields</td>
<td>Changes in membrane structure and functionality</td>
<td>Transformation</td>
</tr>
<tr>
<td>Ionizing Radiations</td>
<td>DNA, RNA denaturation</td>
<td>Mutation</td>
</tr>
<tr>
<td>UV light</td>
<td>DNA, RNA denaturation</td>
<td>Mutation</td>
</tr>
<tr>
<td>Natural Antimicrobials</td>
<td>Changes in membrane structure and functionality</td>
<td>Transformation</td>
</tr>
</tbody>
</table>

*This table has been adapted from Lado and Yousef (2002).

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, 2003).

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 SGII 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, 2005; EFSA, 2007c) as there is the possibility that during their residence on or in food, where they come in contact with other bacteria that may have resistance genes, the bacteria intentionally added may in turn acquire some or all of these resistances and later transfer these resistances to pathogenic bacteria.

There is a paucity of information about the total presence, nature and evolution of antimicrobial resistance in the intestine. In particular, as the two most studied bacteria of the gastrointestinal tract, namely E. coli and enterococci, represent only 1% of the intestinal flora, and that the majority of the bacteria in the intestine cannot be cultivated in vitro, there is a strong bias in the direction of what is happening in these minority populations. Likewise, there is only limited information about the rates of transfer of antimicrobial resistance in the gastrointestinal tract. Although only few data exist, mainly from in vitro models and from experiments on mice (e.g. Doucet-Populaire et al., 1991; McConnell, 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 faecius 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 4.4 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 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., 2000; Varma et al., 2006). In contrast, other serotypes important for public health, for example *S. Enteriditis* rarely display multiple resistance although resistance to antimicrobials such as nalidixic acid coupled with decreased susceptibility to 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. Although declining in incidence in Europe, this S. Typhimurium strain remains a significant public health hazard world-wide. In addition to the outbreaks listed above the strain has been epidemiologically-linked to at least one major international outbreak involving 10 countries linked to contaminated salad vegetables (Horby et al., 2003).

The strain is typically penta-resistant (ampicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and tetracyclines (ACSSuT)) 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.

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 has been reported (EFSA, 2006b). The proportion of isolates reported (serotypes mostly unspecified, but including Derby, Enteritidis, Infantis, London, Saintpaul, Senftenberg, Typhomurium and Virchow) to be resistant to ampicillin in each Member State 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. In the UK, studies of shell eggs coordinated by the Food Standards Agency have demonstrated a substantive level of resistance in S. Enteritidis 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 & 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, 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

Substantial 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; Petruccelli, et al., 1992; Salazar-Lindo, et al., 1986; Skirrow and Blaser, 2000). However, 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 a matter of concern.

Several studies have shown that infections with quinolone-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 excess risk of death or invasive illness following an infection with a quinolone- or macrolide-resistant _Campylobacter_ compared to susceptible strains (Helms et al., 2005). The effect of macrolide resistance on human health consequences have however, only been estimated in this one study and the results require to be verified in additional studies.
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 et al., 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. In addition, it is thought that the aeration resulting from preparation of ground meat products helps reduce the number of viable cells of Campylobacter, which are sensitive to high oxygen tension.

There are no significant biological reasons why resistant Campylobacter should not transmit equally well from animals to humans, as does sensitive Campylobacter. Hypothetically there may be a small fitness loss when a strain of Campylobacter mutates and becomes resistant to quinolones: however, this loss of fitness is soon regained by the strain’s ability to adapt. Thus, when resistance emerges in Campylobacter in animals, resistant Campylobacter can be transmitted to humans. 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 that certain strains gain increased fitness 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 & 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.

An extensive retail study carried out on the island of Ireland (Whyte et al., 2004) examined 2,391 food samples over a 20-month period. This study investigated food sold at retail level in three major population centres. Five hundred and forty three Campylobacter were recovered using enrichment methods, the majority of which were identified as C. jejuni (83.4%). When the resistance profiles of these isolates were determined (McGill et al., 2006) using a panel of eight drugs, more than half of the collection was resistance to ceftiofur (58%).

4.1.3.3. Reports linking foodborne AMR Campylobacter to human infections

A number of case-control studies have specifically addressed risk factors for quinolone-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. (2007). All of these case-control studies identified foreign travel as a risk factor for acquisition of a quinolone-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 quinolone-resistant Campylobacter were as follows: use of a quinolone 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. 2007).

4.1.4. Verotoxigenic Escherichia coli (VTEC)

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 & 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) estimates 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, 1999; Farina et al., 1996; Kim et al., 1994; Threlfall et al., 2000; 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 outside the EU, fosfomycin has been used for treatment, with some success (Igarashi, 2002). This antimicrobial is not used within the EU.

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 cefotiofur). 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 multi-resistant in Germany (v. 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 serotypes were 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. 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., 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 chlorotetracycline, 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 subcontinent, Africa and south and central America. Infections with *Sh. sonnei* are indigenous in many European countries (Cheasty et al., 2005).

4.1.5.2. Exposure through foods

Shigella are not considered to have a food animal reservoir and infections are normally a result of person-to person transmission, or contamination of foods or water by human faecal material. 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.

4.1.6.2. Exposure through foods

*Vibrio* spp., both serotypes O:1 and non-O:1, are not considered to have a traditional food animal reservoir. Infections are the result of the ingestion of contaminated water, or the consumption of seafood such as shrimp (Duran & Marshall 2005; Boinapally & Jiang 2007), cockles or oysters. When such strains have been antimicrobial-resistant, then infections or substantive outbreaks with resistant strains have resulted (Weber et al 1994).

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).

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

*Staphylococcus aureus* is a normally harmless commensal 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, despite the limited therapeutic uses of meticillin itself. The resistance is based on a specific penicillin-binding protein, PBP2’ (or PBP2a), coded by *mecA* (Hartman and Tomasz, 1984). The *mecA* gene is typically part of an integron associated with the *S. aureus* chromosome (Katayama et al, 2000).

Infection in humans with MRSA is a major problem in hospitals. It is estimated that compared to infections with meticillin susceptible *S. aureus*, there is a 2-fold increase in mortality. It should, however, be remembered that these estimates are based on hospitalized patients and nosocomially acquired infections, and not on community associated MRSA cases.

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 has indicates that there is a common type. However,
MRSA which has also been identified on meat, although in low numbers and in only a few
cases (Kitai et al., 2005; van Loo et al., 2007b; Normanno et al. 2007), may be a hazard as a
consequence of handling contaminated meat.

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 pig, cattle and perhaps
poultry populations, 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).
ST398 is mainly found to colonise animals, but have also in a few cases been found to cause
infections. A limited number of reports have been recorded, this is probably due to difficulties
in isolating this bacterium from animals because it is necessary to use selective enrichment. It
must be expected that several new reports will be published in the near future. 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 States⁹ (EFSA, 2007b)

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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 MRSAs shared the characteristics of community-acquired human MRSA-strains (Kitai et al. 2004).

In an Italian study, MRSAs 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 community-acquired 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. Due to the extreme seriousness of the systemic infection, antimicrobial treatment is generally recommended for listeriosis cases. The standard treatment for cases of listeriosis is with a penicillin and an aminoglycoside: ampicillin plus gentamycin is the most frequently used combination and all strains are susceptible to these antimicrobial agents. All strains of *L. monocytogenes* are intrinsically highly resistant to cephalosporins and fluoroquinolones (Johnson et al., 1996; Threlfall et al., 1998; Hansen et al., 2005); this may present therapeutic problems when either of these antimicrobial agents are 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.

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 & 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 & Norström, 2006). One of the best documented specific examples of spread of resistance genes from animal to human bacteria is transposon-encoded streptomycin resistance (Tschäpe, 1994). Following the introduction of norlethamicin 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 sepsicaemia (Livermore et al., 2006). 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 quinolones 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 & 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 & 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 4.3.1).

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 *E. faecalis* and *E. faecium* strains (VRE) both among clinical isolates and food and faecal strains
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 E. faecium are encountered, while clinical cases associated with other species (E. gallinarum, E. casseliflavus, E. durans, E. avium, E. raffinosis) 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 E. 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 VR 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; Lempiainen 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 E. 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

Fermented foods all over the world are being prepared using micro-organisms 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 (probiotics) both in food and feed represents a new way by which deliberately added micro-organisms enter the food chain.

There are relatively few studies on the prevalence of antimicrobial resistance markers in starter cultures, or in bacteria isolated from fermented foods. However, 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 even among probiotic strains (Masco et al., 2006).

4.3.1. Hazard identification and characterisation

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 enterococci) or bifidobacteria, are frequently used (Ouwehand et al., 2002).

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 el 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.
4.3.2. Exposure through foods

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 multi-resistance plasmids have been isolated from dairy products (Gfeller et al., 2003; Teuber et al. 1999). A high incidence of tetracycline and erythromycin resistance determinants \textit{erm}(B) and \textit{tet}(S) were detected among lactobacilli isolated from hand made artisanal cheeses in Turkey (Cataloluk and Gogebakan, 2004). Tetracycline resistance determinants \textit{tet}(M) and \textit{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 \textit{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 \textit{tet}(W), \textit{tet}(M) or \textit{erm}(B) determinants. In a study carried out in the USA (Wang et al. 2006) high incidences of antimicrobial resistance were observed, particularly \textit{tet}(S)/(M) and \textit{erm}(B) markers in the lactococcal and \textit{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 \textit{tet}(W), have been detected also in bifidobacteria, including seven \textit{Bifidobacterium animalis} subsp. \textit{lactis} and \textit{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 (EFSA, 2005).

4.4. Risk assessment of the presence of antimicrobial resistance genes in genetically modified organisms

In genetically modified organisms the presence of antimicrobial resistance genes is considered in the risk assessment process carried out by EFSA in line with the provisions of Regulation
(EC) 1829/2003\(^{10}\) and Directive 2001/18/EC (Article 4.2)\(^{11}\). Under Regulation (EC) 1829/2003, the assessment of the environmental risk shall comply with the requirements referred to in Directive 2001/18/EC. According to Directive 2001/18/EC, Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antimicrobials in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antimicrobial resistance markers in GMOs which may have adverse effects on human health and the environment. The risk assessment process is described in EFSA’s guidance documents for genetically modified plants - *Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed* (EFSA, 2004c) - and for genetically modified microorganisms (GMMs) - *Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Microorganisms and their Derived Products Intended for Food and Feed Use* (EFSA, 2006e). According to these guidance documents, for any gene sequence including the genes conferring resistance to antimicrobial agents, information is assessed including data on the presence of genes, their location within the genome and their potential for transfer to other organisms. In addition, the guidance document on GMMs recommends that alternative technologies that do not rely on antimicrobial resistance marker genes should be used for selection purposes in GMMs.

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 are 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. 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 Zoonoses Report of the EFSA for the Community refers to the origin of the food, i.e. animal species and which part of the animal. 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 2.

Antimicrobial resistance is dependent on live microorganisms and/or 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). 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, aw-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 and harvest phases of the food chain pose a different risk to that posed by bacteria intentionally introduced during processing.

Table 2 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 2 is qualified due to a lack of precision regarding the impact of the specific process used on the particular food product’s microflora.
Table 2. An example of categorisation of food including production and processing factors to facilitate the assessment of exposure of the consumer to AMR factors.

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

* 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.

Any assignment of foods to the different AMR categories listed in Table 2 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). An EFSA report on source attribution for human salmonellosis from meat (EFSA, 2008), 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). These annual calculations clearly identify imported chicken products as the main source of human infection with quinolone-resistant Salmonella in Denmark.

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 (Vose et al., 2001b). 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. The few risk assessments already undertaken, namely those that have considered the risk to human health from multiple bacteria (Hurd et al., 2004); multiple antimicrobial classes (Institute of Medicine, 1989); resistance gene transfer or across different food products and other sources (Wooldridge, 1999; Hill et al., 2005) are referred to here. Risk assessments tend only to focus on the transmission through the food chain: this does not take into account the possible direct spread to people working in the food industry, or environmental spread.

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 3. 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.
### Table 3: Key data limitations/issues affecting microbial risk assessments (MRAs) applied to the area of antimicrobial resistance (Snary et al., 2004)

<table>
<thead>
<tr>
<th>Data limitation/issue</th>
<th>Effect on MRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition of resistance</td>
<td>• Data from different sources are not comparable. May limit the amount of data available for the MRA.</td>
</tr>
<tr>
<td>– Harmonization of MIC/disc-diffusion breakpoints required.</td>
<td></td>
</tr>
<tr>
<td>Microbiological methods</td>
<td>• The amount of data available for the MRA may be limited if the methods are not comparable.</td>
</tr>
<tr>
<td>– Selective plating v testing of one isolate from non-selective plate</td>
<td>• Cannot compare selective plating against the testing of one isolate without knowledge of the ratio of resistant to susceptible bacteria.</td>
</tr>
<tr>
<td>– Enrichment v non-enrichment etc.</td>
<td>• If enriched the number of organisms is increased and therefore cannot directly be used in the MRA.</td>
</tr>
<tr>
<td>Multiple levels of the sampling framework</td>
<td>• 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.</td>
</tr>
<tr>
<td>Small sample sizes</td>
<td>• 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.</td>
</tr>
<tr>
<td>Little data available on indicator organisms (resistant or susceptible) compared to pathogenic bacteria.</td>
<td>• 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.</td>
</tr>
<tr>
<td>Sensitivity and specificity of the tests used.</td>
<td>• MRA may overestimate or underestimate the risk.</td>
</tr>
<tr>
<td>Causality unclear</td>
<td>• 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.</td>
</tr>
<tr>
<td>Lack of quantitative microbiological data</td>
<td>• Microbial load of resistant bacteria in/on different sources is unknown, therefore either not modelled or key assumptions made.</td>
</tr>
<tr>
<td>Little information on the use of antimicrobial agents for</td>
<td>• Causality is difficult to consider. May lead to a large degree of uncertainty in the results of the model.</td>
</tr>
<tr>
<td>– veterinary use (at animal and farm level)</td>
<td></td>
</tr>
<tr>
<td>– human use</td>
<td></td>
</tr>
</tbody>
</table>
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* (Wiuff 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).

### 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 2) 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.


6.2.1. 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. 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 endpoint 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: **Recommended 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 Chapter 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: Quinolone-resistant Campylobacter jejuni in the UK

The framework described above is now adopted for a risk assessment for quinolone-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 quinolone-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 quinolone-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 quinolone-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 16mg/litre for nalidixic acid and 1 mg/litre for
ciprofloxacin; however the study on raw/fresh broilers by Wilson (2003) used a breakpoint of
30µg 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 quinolone-resistant *C. jejuni* to other food-types.
Table 4. Risk of purchasing food contaminated with fluoroquinolone-resistant Campylobacter

(Prevalence data from UK, “purchasing” data from Ireland)

<table>
<thead>
<tr>
<th>Prevalence of FQ-resistant Campylobacter</th>
<th>Frequency of consumption of purchased food items</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily (&gt;50%)</td>
</tr>
<tr>
<td>High (&gt;1%)</td>
<td>Raw broiler meat</td>
</tr>
<tr>
<td>Medium (0.01-1%)</td>
<td>Raw beef</td>
</tr>
<tr>
<td></td>
<td>Raw pork</td>
</tr>
<tr>
<td>Low (&lt;0.01%)</td>
<td>Processed milk</td>
</tr>
<tr>
<td></td>
<td>Processed dairy</td>
</tr>
<tr>
<td></td>
<td>Processed cheese</td>
</tr>
<tr>
<td></td>
<td>Eggs</td>
</tr>
<tr>
<td></td>
<td>Fish</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
</tr>
<tr>
<td></td>
<td>Soft fruit</td>
</tr>
<tr>
<td></td>
<td>Juices</td>
</tr>
<tr>
<td></td>
<td>Cereals</td>
</tr>
<tr>
<td></td>
<td>Community water supplies</td>
</tr>
<tr>
<td></td>
<td>Private water supplies</td>
</tr>
</tbody>
</table>

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.).
food sources, the average exposure is estimated by multiplication of (averages of) the daily intake of the food product, 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 pigs. 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.

**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 either for the treatment of infectious diseases in food animals kept in intensive production systems,
or for the less defendable reason of growth promotion in these animals. These issues have 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. Control and prevention of infectious diseases in animals

Reduction of animal illness can be expected to reduce the need to use antimicrobials. Measures to reduce disease include specific measures such as vaccination and the prevention of spread though biosecurity and general operational hygiene management measures.

The use of programmes aimed at the prevention and control of salmonella 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).
7.1.2. 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.

7.1.3. 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.

7.1.4. 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; Reinthaler 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 on the prudent use of antimicrobials (OIE, 2007), 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 section 4.1.1.above. A new development has been the emergence of strains with plasmid-mediated quinolone resistance in several countries world-wide, in both humans and food-production animals. Such plasmid-mediated resistance has resulted in the MIC to ciprofloxacin, in strains with decreased susceptibility, to be raised to therapeutic levels, 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 & 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 through the agency of food will also contribute to the prevention and control of antimicrobial-resistant bacteria. However, antimicrobial resistance may increase the severity of the hazard, or may change the nature of the hazard, compared to the non-resistant bacteria. 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.

Resistant *Salmonella* and *Campylobacter* involved in human disease are mostly spread through foods. Contaminated poultry meat, eggs, pork and beef are prominent in this regard. In all cases the antimicrobials concerned can be of clinical importance in human medicine.

Bovines 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.

The emergence of antimicrobial resistance in VTEC may facilitate the ability of AMR strains to preferentially colonise the bovine gastrointestinal tract, when selective pressures are applied.

Animal-derived products remain a potential source of community-acquired 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.

Control of the land application of animal manures and of municipal sludges, in particular, those containing hospital effluent, on land used for food animal production and in particular, on land used for the production of fresh produce that is likely to be consumed in the raw state, is considered as an essential measure for the control of AMR transfer from the pre-harvest phase of the food chain to the final food product.

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 in the first instance towards fresh crop-based foods, raw poultry meat and raw pigmeat are recommended.

The use of epidemiological cut-off values provides an appropriate level of sensitivity when measuring resistance development in bacteria. While these criteria have been harmonised for use in both in human and veterinary medicine in the European Union these criteria may now need to be reviewed.

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 the principal origin of human exposure to fluoroquinolone resistance via food appears to be poultry, whereas for cephalosprins it is poultry and pork, 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 who 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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**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

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples of substances used in:</th>
<th>Veterinary medicine: food production animals in EU</th>
<th>Examples of resistance genes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td></td>
<td>No general cross-resistance within class, but some types of resistance will involve cross-resistance between some aminoglycosides</td>
</tr>
<tr>
<td></td>
<td>amikacin, gentamicin, netilmicin, tobramycin</td>
<td>apramycin, gentamicin, streptomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>kanamycin, spectinomycin</td>
<td>neomycin, spectinomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amphenicol</strong></td>
<td>chloramphenicol, tiamphenicol</td>
<td>chloramphenicol, florfenicol, tiamphenicol</td>
<td></td>
<td>Crf confers cross-resistance to amphenicols, lincosamindes, Pleuromutilins, streptogramins, linezolid</td>
</tr>
<tr>
<td><strong>Beta-lactam antibiotics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Penicillins</strong></td>
<td>Benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)</td>
<td>benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)</td>
<td></td>
<td>Cross resistance within subclasses but also, depending on mechanism, between subclasses</td>
</tr>
<tr>
<td><strong>Penicillins - antistaphylococcal</strong></td>
<td>cloxacillin, dixcloxacillin (methicillin)</td>
<td>cloxacillin, dixcloxacillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cephalosporins, first generation</strong></td>
<td>cephalaxin, cefazolin, cephalotin</td>
<td>cefazolin, cephalaxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cephalosporins, second generation</strong></td>
<td>cefuroxime, loracarbef</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cephalosporins, third generation</strong></td>
<td>ceftazidime, ceftriaxone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cephalosporins, fourth generation</strong></td>
<td>cefepime, cefpirome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cephamycins</strong></td>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carbapenems</strong></td>
<td>ertapenem, imipenem, meropenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclic polypeptides</strong></td>
<td>Bacitracin</td>
<td>(bacitracin)</td>
<td></td>
<td>Formerly used as feed additive in EU</td>
</tr>
<tr>
<td>Class</td>
<td>Examples of substances used in:</td>
<td>Veterinary medicine: food production animals in EU</td>
<td>Examples of resistance genes</td>
<td>Comments</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------------</td>
<td>--------------------------------------------------</td>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>teicoplanin, vancomycin</td>
<td>- (avoparcin)</td>
<td>van (A-E)</td>
<td>Formerly avoparcin was used as feed additive in EU</td>
</tr>
<tr>
<td>Ionophores</td>
<td>-</td>
<td>monensin, salinomycin</td>
<td></td>
<td>Used as coccidiostats</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>clindamycin, lincomycin</td>
<td>clindamycin, lincomycin</td>
<td>cfr, erm</td>
<td>Cross-resistance also to macrolides and streptograminB for certain resistance genotypes</td>
</tr>
<tr>
<td>Lipopeptides</td>
<td>Daptomycin</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrolides &amp; ketolides</td>
<td>erythromycin, spiramycin, azithromycin, clarithromycin</td>
<td>spiramycin, tylosin, tulathromycin</td>
<td>erm, ere, mef, msr</td>
<td>Cross-resistance also to lincosamides and streptograminB for certain resistance genotypes</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>furazolidone, nitrofurantoin</td>
<td>-</td>
<td></td>
<td>Used formerly as veterinary medicine</td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>metronidazole, tinidazole</td>
<td>-</td>
<td></td>
<td>Dimetridazole and ronidazole used formerly as veterinary medicine</td>
</tr>
<tr>
<td>Orthosomycins</td>
<td>-</td>
<td>avilamycin</td>
<td>emtA</td>
<td>Used formerly as feed additive</td>
</tr>
<tr>
<td>Oxazolidones</td>
<td>linezolid</td>
<td>-</td>
<td>cfr</td>
<td></td>
</tr>
<tr>
<td>Pleuromutilins</td>
<td>-</td>
<td>tiamulin, valnemulin</td>
<td>cfr</td>
<td></td>
</tr>
<tr>
<td>Polymixins</td>
<td>colistin, polymixin B</td>
<td>colistin, polymixin B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td>nalidixic acid, ciprofloxacin norfloxacin, moxifloxacin</td>
<td>danofloxacin, enrofloxacin, aac(6’)-Ib-cr, gyrA.parC, qepA, qnr,</td>
<td></td>
<td>Incomplete cross-resistance</td>
</tr>
<tr>
<td>Quinoxalines</td>
<td>-</td>
<td>carbadox, olaquindox</td>
<td>opxAB</td>
<td>Olaquindox used formerly as feed additive in the EU</td>
</tr>
<tr>
<td>Streptogramins</td>
<td>pristinamycin, quinpristin/dalfopristin</td>
<td>- (virginiamycin)</td>
<td>cfr, erm, vga, vgb</td>
<td>Formerly virginiamycin was used as feed additive in EU</td>
</tr>
<tr>
<td>Sulphonamides &amp; trimethoprim</td>
<td>sulfadiazine, sulfamethoxazole, trimethoprim</td>
<td>sulfadiazine, sulfadoxine, sulfamethoxazole, trimethoprim</td>
<td>dfr, sul</td>
<td>Cross-resistance between streptograminB, lincosamides and macrolides for certain resistance genotypes</td>
</tr>
</tbody>
</table>
### Examples of substances used in:

<table>
<thead>
<tr>
<th>Class</th>
<th>Human medicine</th>
<th>Veterinary medicine: food production animals in EU</th>
<th>Examples of resistance genes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>chlortetracycline, doxycycline, oxytetracycline</td>
<td>chlortetracycline, doxycycline, oxytetracycline</td>
<td>Tet</td>
<td></td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>-</td>
<td>flavophospholipol (bambermycin)</td>
<td>foxAB</td>
<td>Used formerly as feed additive</td>
</tr>
<tr>
<td>fosfomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fusidic acid</td>
<td></td>
<td></td>
<td>fusidic acid</td>
<td></td>
</tr>
<tr>
<td>Mupirocin</td>
<td></td>
<td></td>
<td>mupA</td>
<td>Used in human medicine topically for MRSA decontamination</td>
</tr>
<tr>
<td>Rifampicin</td>
<td></td>
<td>(rifampicin)</td>
<td>rpoB</td>
<td>Use in vet. med limited to foals</td>
</tr>
</tbody>
</table>
### Table B. Occurrence of resistance to antimicrobials in *Escherichia coli* from food products (percent resistant isolates)* (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)

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Food type</th>
<th>Number of isolates</th>
<th>Reported resistance (%)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ampicillin</td>
<td>3rd gen cephalosporin</td>
</tr>
<tr>
<td>Austria</td>
<td>2003</td>
<td>beef</td>
<td>40</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Belgium</td>
<td>2005</td>
<td>beef</td>
<td>238</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Denmark</td>
<td>2004</td>
<td>beef</td>
<td>196</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2005</td>
<td>beef</td>
<td>34</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Norway</td>
<td>2005</td>
<td>beef</td>
<td>90</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Austria</td>
<td>2003</td>
<td>poultry</td>
<td>34</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Belgium</td>
<td>2005</td>
<td>broiler</td>
<td>148</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>Denmark</td>
<td>2004</td>
<td>broiler</td>
<td>216</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2005</td>
<td>poultry</td>
<td>115</td>
<td>54</td>
<td>11</td>
</tr>
<tr>
<td>Austria</td>
<td>2003</td>
<td>pork</td>
<td>56</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Belgium</td>
<td>2005</td>
<td>pork</td>
<td>86</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Denmark</td>
<td>2004</td>
<td>pork</td>
<td>178</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2005</td>
<td>pork, organic</td>
<td>155</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Germany</td>
<td>2005</td>
<td>mixed meat</td>
<td>50</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Portugal</td>
<td>2005</td>
<td>cheese</td>
<td>33</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

**Non-EU countries**

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Food type</th>
<th>Number of isolates</th>
<th>Reported resistance (%)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ampicillin</td>
<td>3rd gen cephalosporin</td>
</tr>
<tr>
<td>Canada QC</td>
<td>2003</td>
<td>broiler</td>
<td>112</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>Canada; ON</td>
<td>2003</td>
<td>broiler</td>
<td>136</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>Canada QC</td>
<td>2003</td>
<td>pork</td>
<td>61</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Canada ON</td>
<td>2003</td>
<td>pork</td>
<td>91</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Canada QC**</td>
<td>2003</td>
<td>beef</td>
<td>84</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Canada ON**</td>
<td>2003</td>
<td>beef</td>
<td>100</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

* 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
Table C: Qualitative risk assessment for quinolone-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.

<table>
<thead>
<tr>
<th>Food category</th>
<th>Food subcategory</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
</table>
| 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). Breakpoint of 16 mg/ml for nalidixic acid; 1 mg/ml for ciprofloxacin. 0/99 C. jejuni cattle isolates were resistant to nalidixic acid or ciprofloxacin. 0/65 C. jejuni sheep isolates were resistant to nalidixic acid or ciprofloxacin. Also - referred to data for bovine/sheep meat | Assumption. Prohibited from sale in Scotland. | Assumption.

|  | C. Processed | Pasteurisation very effective at killing Campylobacter. 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. |

13 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.

14 Breakpoint of 16 mg/ml for nalidixic acid; 1 mg/ml for ciprofloxacin
<table>
<thead>
<tr>
<th>Food category</th>
<th>Food sub-category</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Assumption. Same as processed milk.</td>
<td>Assumption. Same as raw milk.</td>
<td>74.6% of participants in Irish survey consumed dairy products (Harrington et al. 2001). Assumed that majority is purchased processed / pasteurised.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>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.</td>
<td>Assumption. Same as raw milk.</td>
<td>Assumption.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption. Same as raw milk.</td>
<td>78.0% of participants in Irish survey consumed cheese (Harrington et al. 2001). Assumed that majority is purchased processed / pasteurised.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption. Same as raw milk.</td>
<td>92.2% of participants in Irish survey consumed egg &amp; egg products (Harrington et al. 2001). Assumed that majority purchased as raw.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. Fresh or raw</td>
<td>Assumption.</td>
<td>2/187 (1.1%) eggs produced from hens faecally shedding C. jejuni were positive due to penetration of the egg shell. 0/142 eggs derived from birds of unknown status were C. jejuni positive (Shanker et al., 1986)</td>
<td>92.2% of participants in Irish survey consumed egg &amp; egg products (Harrington et al. 2001). Assumed that majority purchased as raw.</td>
</tr>
<tr>
<td>Food category</td>
<td>Food sub-category</td>
<td>Processing factors</td>
<td>Probability of bacteria being present in food at retail</td>
<td>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</td>
<td>Probability food purchased and consumed&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Probability of AMR bacteria being in food purchased that will be consumed</td>
</tr>
<tr>
<td>---------------</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Minimally processed (as in desserts)</td>
<td>Assumption. Lower probability than for fresh or raw eggs.</td>
<td>Assumption. Same as fresh or raw eggs.</td>
<td>Assumption.</td>
<td>Assumption.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption. Lower probability than for fresh or raw eggs.</td>
<td>Assumption. Same as fresh or raw eggs.</td>
<td>Assumption.</td>
<td>Assumption.</td>
</tr>
<tr>
<td>3. Red meats</td>
<td>3.1 Bovine</td>
<td>A. Fresh or raw</td>
<td>71/1514 (4.7%) of meat cuts and 6/49 (12.2%) offal portions were positive for Campylobacter. 43/49 (87.7%) isolates were <em>C. jejuni</em> (Little et al., submitted) 47/96 (49%) of ox liver samples were positive for <em>C. jejuni</em>. (Kramer et al., 2000)</td>
<td>13.9% of <em>C. jejuni</em> resistant to nalidixic acid; 11.6% resistant to ciprofloxacin (43 isolates tested) (Little et al., submitted)&lt;sup&gt;15&lt;/sup&gt; 15.1% of 53 <em>C. jejuni</em> isolates isolated from ox liver were resistant to nalidixic acid; 3.8% were resistant to ciprofloxacin&lt;sup&gt;16&lt;/sup&gt;. (Kramer et al., 2000)</td>
<td>87.8% of participants in Irish survey consumed bovine meat and products (Harrington <em>et al.</em> 2001). Assumed that majority is purchased as raw and as meat cuts (i.e. not offal).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption.</td>
<td>Assumption. Same as fresh or raw bovine meat.</td>
<td>Assumption</td>
<td>Assumption</td>
</tr>
<tr>
<td>Food category</td>
<td>Food sub-category</td>
<td>Processing factors</td>
<td>Probability of bacteria being present in food at retail</td>
<td>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</td>
<td>Probability food purchased and consumed&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Probability of AMR bacteria being in food purchased that will be consumed</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>--------------------</td>
<td>----------------------------------------</td>
<td>-------------------------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>0/3085 ready-to-eat burgers (of which 90% were beef) purchased from burger outlets were positive for Campylobacter (Little et al., 2001). 1/546 (0.18%) ready to eat beef (cold) positive for Campylobacter (Elson et al., 2004) 0/371 VP-MAP ready to eat beef positive for Campylobacter (Sagoo et al., 2007)</td>
<td>Assumption. Same as fresh or raw bovine meat.</td>
<td>Assumption. Significant proportion is purchased as processed.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 Pig</td>
<td>A. Fresh or raw 66/1309 (5%) of meat cuts and 24/131 (18.3%) offal portions were positive for Campylobacter. 36/68 (52.9%) isolates were C. jejuni (Little et al., submitted) 34/99 (34.3%) of pigs liver samples were positive for C. jejuni. (Kramer et al., 2000) 27.8% of C. jejuni resistant to nalidixic acid; 19.4% resistant to ciprofloxacin (36 isolates tested) (Little et al., submitted)&lt;sup&gt;17&lt;/sup&gt; 33.3% of 36 C. jejuni isolates isolated from pigs liver were resistant to nalidixic acid; 5.6% were resistant to ciprofloxacin&lt;sup&gt;18&lt;/sup&gt;. (Kramer et al., 2000)</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption. Lower probability than for fresh or raw pig meat.</td>
<td>Assumption. Same as fresh or raw pig meat.</td>
<td>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).</td>
<td></td>
</tr>
</tbody>
</table>

<sup>17</sup> Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin

<sup>18</sup> Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.
<table>
<thead>
<tr>
<th>Food category</th>
<th>Food sub-category</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed&lt;sup&gt;13&lt;/sup&gt;</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>0/1423 ready to eat ham (cold) positive for <em>Campylobacter</em>. 0/243 ready to eat pork (cold) positive for <em>Campylobacter</em>. (Elson et al., 2004) 0/1351 VP-MAP ready to eat ham positive for <em>Campylobacter</em>. 0/206 VP-MAP ready to eat pork positive for Campylobacter (Sagoo et al., 2007)</td>
<td>Assumption. Same as fresh or raw pig meat.</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>3.3 Sheep</td>
<td>A. Fresh or raw</td>
<td>55/744 (7.4%) of meat cuts and 59/161 (36.6%) offal portions were positive for <em>Campylobacter</em>. 64/90 (71.1%) isolates were <em>C. jejuni</em> (Little et al., submitted) 72/96 (75%) of lambs liver were positive for <em>C. jejuni</em>. (Kramer et al., 2000).</td>
<td>12.5% of <em>C. jejuni</em> resistant to nalidixic acid; 10.9% resistant to ciprofloxacin (64 isolates tested) (Little et al., submitted)&lt;sup&gt;19&lt;/sup&gt; 7% of 100 <em>C. jejuni</em> isolates isolated from lambs liver were resistant to nalidixic acid; 4% were resistant to ciprofloxacin&lt;sup&gt;20&lt;/sup&gt;. (Kramer et al. 2000).</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption. Lower probability than for fresh or raw sheep meat.</td>
<td>Assumption. Same as fresh or raw sheep meat.</td>
<td>Assumption.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<sup>20</sup> Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.
<table>
<thead>
<tr>
<th>Food category</th>
<th>Food sub-category</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3.4 Other</td>
<td>A. Fresh or raw GAME &amp; OTHER MEATS: 5/47 (10.6%) of meat cuts; 0/1 (0%) offal portions and 0/3 (0%) whole animals were positive for <em>Campylobacter</em>. 3/4 (75%) were C. jejuni (Little <em>et al.</em>, submitted) Note - low sample size.</td>
<td>GAME &amp; OTHER MEATS: 0% of C. jejuni resistant to nalidixic acid; 0% resistant to ciprofloxacin (3 isolates tested) (Little <em>et al.</em>, submitted)(^{22}) Note - low sample size. Assumption.</td>
<td>4.1% of participants in Irish survey consumed ‘other’ red meat and products (Harrington <em>et al.</em> 2001). Assumed that majority is purchased as raw and as meat cuts.</td>
<td>Assumption.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption. Lower probability than for fresh or raw ‘other’ meat.</td>
<td>Assumption. Same as fresh or raw ‘other’ red meat.</td>
<td>Assumption.</td>
<td>Assumption.</td>
</tr>
</tbody>
</table>

\(^{21}\) hare, rabbit, venison, goat, mutton

\(^{22}\) Little *et al.*, (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin
<table>
<thead>
<tr>
<th>Food category</th>
<th>Food sub-category</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed&lt;sup&gt;13&lt;/sup&gt;</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Poultry meats</td>
<td>4.1 Broiler</td>
<td>A. Fresh or raw</td>
<td>In large UK study, 50% of chickens sampled (5394) were positive for <em>Campylobacter</em> spp. [56% fresh chicken; 31% frozen chicken]. Of 1636 isolates tested, 74% were <em>C. jejuni</em>. (Food Standards Agency, 2003). 187/301 (62.1%) whole birds and 896/1477 (60.7%) portions were positive for <em>Campylobacter</em>. 163/239 (68.2%) were <em>C. jejuni</em> (Little et al., submitted). In Northern Ireland study 141/412 (34%) of local whole chickens were <em>C. jejuni</em> positive. From imported (frozen) chicken breasts 33/150 (22%) were <em>C. jejuni</em> positive. (Wilson, 2003). 156/198 (77.3%) of chilled chicken breast or thigh portions were positive for <em>C. jejuni</em>. (Kramer et al, 2000).</td>
<td>Assumption. Lower probability than for fresh or raw ‘other’ meat. Assumption. Same as fresh or raw ‘other’ red meat.</td>
<td>Assumption</td>
<td>84.3 % of participants in Irish survey consumed broiler meat and products (Harrington &lt;i&gt;et al&lt;/i&gt;. 2001). Assumed that majority is purchased as raw.</td>
</tr>
</tbody>
</table>

<sup>13</sup> Little <i>et al</i>, (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin
<table>
<thead>
<tr>
<th>Food category</th>
<th>Food sub-category</th>
<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed&lt;sup&gt;13&lt;/sup&gt;</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption.</td>
<td>Assumption. Same as fresh or raw broiler meat.</td>
<td>Assumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. Processed</td>
<td>0/121 ready to eat chicken (cold) positive for <em>Campylobacter</em> (Elson et al., 2004) 0/495 VP-MAP ready to eat chicken positive for <em>Campylobacter</em> (Sagoo et al., 2007) 0/449 chicken sandwich samples were positive for <em>Campylobacter</em> (Little et al., 2002).</td>
<td>Assumption. Same as fresh or raw broiler meat.</td>
<td>Assumption</td>
</tr>
<tr>
<td>4.2 Turkey</td>
<td>A. Fresh or raw</td>
<td></td>
<td>1/2 (50%) of whole birds and 71/212 (33.5%) portions were positive for <em>Campylobacter</em>. 7/15 (46.7%) were C. jejuni. (Little et al., submitted)</td>
<td>16.7% of <em>C. jejuni</em> resistant to nalidixic acid; 16.7% resistant to ciprofloxacin (6 isolates tested) (Little et al., submitted)&lt;sup&gt;27&lt;/sup&gt; Note - low sample size.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td></td>
<td>Assumption. Lower probability than fresh or raw turkey meat</td>
<td>Assumption. Same as fresh or raw turkey meat.</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

<sup>24</sup> Anon. 2005 used the HPA breakpoints, i.e. 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

<sup>25</sup> Wilson, 2003 used the following concentrations of antimicrobials in the discs susceptibility testing: 30µg for nalidixic acid; 1µg for ciprofloxacin.

<sup>26</sup> Kramer et al. 2000 used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.

<sup>27</sup> Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin.
<table>
<thead>
<tr>
<th>Food category</th>
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<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
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<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>0/411 ready to eat turkey (cold) positive for <em>Campylobacter</em> (Elson et al., 2004) 1/523 VP-MAP ready to eat turkey positive for <em>Campylobacter</em> (Sagoo et al., 2007)</td>
<td>Assumption. Same as fresh or raw turkey meat.</td>
<td>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.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>4.3 Other</td>
<td>A. Fresh or raw DUCK: 2/7 (28.6%) of whole birds and 37/70 (52.9%) portions were positive for <em>Campylobacter</em>. 8/19 (42.1%) isolates were <em>C. jejuni</em>. (Little et al., submitted) OTHER: 11/23 (47.8%) of whole birds and 1/12 (8.3%) portions were positive for <em>Campylobacter</em>. 2/4 (50.0%) were <em>C. jejuni</em>. (Little et al., submitted) Note - low sample size</td>
<td>DUCK, 0% of <em>C. jejuni</em> resistant to nalidixic acid; 0% resistant to ciprofloxacin (9 isolates tested) (Little et al., submitted) OTHER: 0% of <em>C. jejuni</em> resistant to nalidixic acid; 0% resistant to ciprofloxacin (3 isolates tested) (Little et al., submitted)&lt;sup&gt;29&lt;/sup&gt; Note - low sample sizes. Assumption. Same as fresh chicken &amp; turkey.</td>
<td>2.3% of participants in Irish survey consumed other poultry meat and products (Harrington et al. 2001). Assumed that majority is purchased as raw.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Minimally processed (cured and/or smoked)</td>
<td>Assumption.</td>
<td>Assumption. Same as fresh or raw 'other' poultry meat.</td>
<td>Assumption</td>
<td>Assumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption.</td>
<td>Assumption. Same as fresh or raw 'other' poultry meat.</td>
<td>Assumption</td>
<td>Assumption</td>
</tr>
</tbody>
</table>

<sup>28</sup> grouse, guinea fowl, ostrich, partridge, pheasant, poussin, quail, wood pigeon

<sup>29</sup> Little et al., (submitted) used a breakpoint of 16mg/litre for nalidixic acid and 1 mg/litre for ciprofloxacin
<table>
<thead>
<tr>
<th>Food category</th>
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<th>Processing factors</th>
<th>Probability of bacteria being present in food at retail</th>
<th>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</th>
<th>Probability food purchased and consumed</th>
<th>Probability of AMR bacteria being in food purchased that will be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Aquaculture and marine</td>
<td>5.1 Fish</td>
<td>A. Fresh or raw</td>
<td>Assumption</td>
<td>Assumption.</td>
<td>65.1% of participants in Irish survey consumed fish and fish products (Harrington et al. 2001). Assumed that significant proportion is purchased as raw.</td>
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<td>B. Minimally processed (pickled and/or smoked)</td>
<td>Assumption</td>
<td>Assumption.</td>
<td>Assumption</td>
<td>Assumption.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption</td>
<td>Assumption.</td>
<td>65.1% of participants in Irish survey consumed fish and fish products (Harrington et al. 2001). Assumed that significant proportion is purchased as processed.</td>
<td></td>
</tr>
<tr>
<td>5.2 Crustaceans, shellfish, molluscs</td>
<td></td>
<td>A. Fresh or raw</td>
<td>In Irish study, 3/117 (2.5%) of seafood sampled (oysters and mussels) were \textit{C. jejuni} positive (Whyte et al. 2004). 47% of 331 mixed bivalves (cockles, mussels, scallops) shortly after harvesting were positive for \textit{Campylobacter spp}. Only 2% of these were \textit{C. jejuni}. (Wilson &amp; Moore, 1996).</td>
<td>Assumption.</td>
<td>8.8% of participants in Irish survey consumed crustaceans, shellfish and molluscs (Harrington et al. 2001). Assumed that small proportion is purchased as processed.</td>
<td>Very low</td>
</tr>
<tr>
<td>Food category</td>
<td>Food sub-category</td>
<td>Processing factors</td>
<td>Probability of bacteria being present in food at retail</td>
<td>Probability that bacteria present in food at retail is resistant to antimicrobial class of interest</td>
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<td>3/49 (6%) of depurated oysters were positive for <em>Campylobacter spp.</em> None were <em>C. jejuni</em>. (Wilson &amp; Moore, 1996). Note - low sample size for <em>C. jejuni.</em></td>
<td>Assumption.</td>
<td>8.8% of participants in Irish survey consumed crustaceans, shellfish and molluscs (Harrington et al. 2001). Assumed that majority is purchased as processed.</td>
<td></td>
</tr>
<tr>
<td>6. Vegetables, cereals, fruits</td>
<td>6.1. Vegetables and juices</td>
<td>A. Fresh or raw</td>
<td>0/2870 open ready to eat salad samples positive for <em>Campylobacter</em> (Sagoo, et al., 2003a) 0/3827 ready to eat salad vegetable samples positive for <em>Campylobacter</em> (Sagoo et al., 2003b) 0/151 unprepared non-UK whole lettuce positive for <em>Campylobacter</em> (Little et al., 1999) 0/2883 ready-to-eat organic vegetables positive for <em>Campylobacter</em> (Sagoo et al., 2001)</td>
<td>Assumption. Animal/human wastes spread onto land</td>
<td>99.9% of participants in Irish survey consumed vegetables &amp; juices and products (Harrington et al. 2001). Assumed that majority purchased as raw.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>B. Minimally processed (pickled vegetables)</td>
<td>Assumption. Lower probability than for fresh or raw vegetables.</td>
<td>Assumption. Same as fresh/raw</td>
<td>Assumption. Same as fresh/raw</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption. Lower probability than for minimally processed vegetables.</td>
<td>Assumption. Same as fresh/raw</td>
<td>99.9% of participants in Irish survey consumed vegetables &amp; juices and products (Harrington et al. 2001). Assumed that significant proportion are purchased as processed.</td>
<td></td>
</tr>
<tr>
<td>Food category</td>
<td>Food sub-category</td>
<td>Processing factors</td>
<td>Probability of bacteria being present in food at retail</td>
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<tr>
<td>6.2 Cereal</td>
<td></td>
<td>A. Fresh or raw</td>
<td>Assumption.</td>
<td>Assumption. Animal/human wastes spread onto land</td>
<td>100% of participants in Irish survey consumed cereal products (Harrington et al. 2001). Assumed that large proportion are purchased as fresh/raw.</td>
<td></td>
</tr>
<tr>
<td>products</td>
<td></td>
<td>B. Minimally</td>
<td>Assumption. Lower probability than for fresh/raw cereals</td>
<td>Assumption. Same as fresh/raw</td>
<td>Assumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>processed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption. Lower probability than for fresh/raw cereals</td>
<td>Assumption. Same as fresh/raw</td>
<td>100% of participants in Irish survey consumed cereal products (Harrington et al. 2001). Assumed that majority are purchased as processed.</td>
<td></td>
</tr>
<tr>
<td>6.3 Fruit,</td>
<td></td>
<td>A. Fresh or raw</td>
<td>0/143 strawberries were positive for <em>Campylobacter</em> spp.; 0/162 melons were positive for <em>Campylobacter</em> spp. (Williamson et al., 2003)</td>
<td>Assumption.</td>
<td>91.4% of participants in Irish survey consumed fruits, berries, juices and products (Harrington et al. 2001). Assumed that majority are purchased raw.</td>
<td></td>
</tr>
<tr>
<td>berries and</td>
<td></td>
<td>B. Minimally</td>
<td>Assumption.</td>
<td>Assumption.</td>
<td>Assumption</td>
<td></td>
</tr>
<tr>
<td>juices</td>
<td></td>
<td>processed</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>C. Processed</td>
<td>Assumption.</td>
<td>Assumption.</td>
<td>Assumption</td>
<td></td>
</tr>
<tr>
<td>7. Herbs and</td>
<td></td>
<td>A. Fresh or raw</td>
<td>Assumption.</td>
<td>Assumption. Animal/human wastes spread onto land</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>spices</td>
<td></td>
<td>B. Minimally</td>
<td>Assumption.</td>
<td>Assumption. Same as fresh/raw herbs and spices</td>
<td>Assumption</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>processed</td>
<td></td>
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</table>
## Draft Opinion on antimicrobial resistance in food

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<tbody>
<tr>
<td>C. Processed</td>
<td>Assumption</td>
<td>Assumption</td>
<td>Assumption. Same as fresh/raw herbs and spices</td>
<td>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).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Mixed or buffet meals*  

9. Other foods*  

10. Tap water, including well-water  

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>A. Not chlorinated</td>
<td>Assumption</td>
<td>Assumption. Private water supplies may be contaminated by run-off from nearby land</td>
<td>Assumption</td>
<td></td>
</tr>
<tr>
<td>B. Chlorinated</td>
<td>Assumption</td>
<td>Assumption. Same as unchlorinated water.</td>
<td>94.2% of participants in Irish survey consumed tap water (Harrington et al. 2001). Assumed that majority is chlorinated.</td>
<td></td>
</tr>
</tbody>
</table>

* 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.
References for Table C:


de Louvois, J., and Rampling, A., 1998. One fifth of samples of unpasteurised milk are contaminated with bacteria. B.M.J., 316, 625


Little, C.L., Barnes, J. and Mitchell, R.T., on behalf of the Food Standards Agency (FSA) and the Public Health Laboratory Service (PHLS), 2001. Microbiological examination of ready-to-eat burgers sampled anonymously at the point of sale in the United Kingdom. Communicable Disease and Public Health, 5, 289 - 298.


Little, C.L., Gillespie, I.A., Mitchell, R.T. on behalf of the Local Authority Co-ordinating body on Food and Trading Standards (LACOTS) and the Public Health Laboratory Service (PHLS), 2001. Microbiological examination of ready-to-eat burgers sampled anonymously at the point of sale in the United Kingdom. Communicable Disease and Public Health, 4, 293 - 299.


Opinion on antimicrobial resistance in food


