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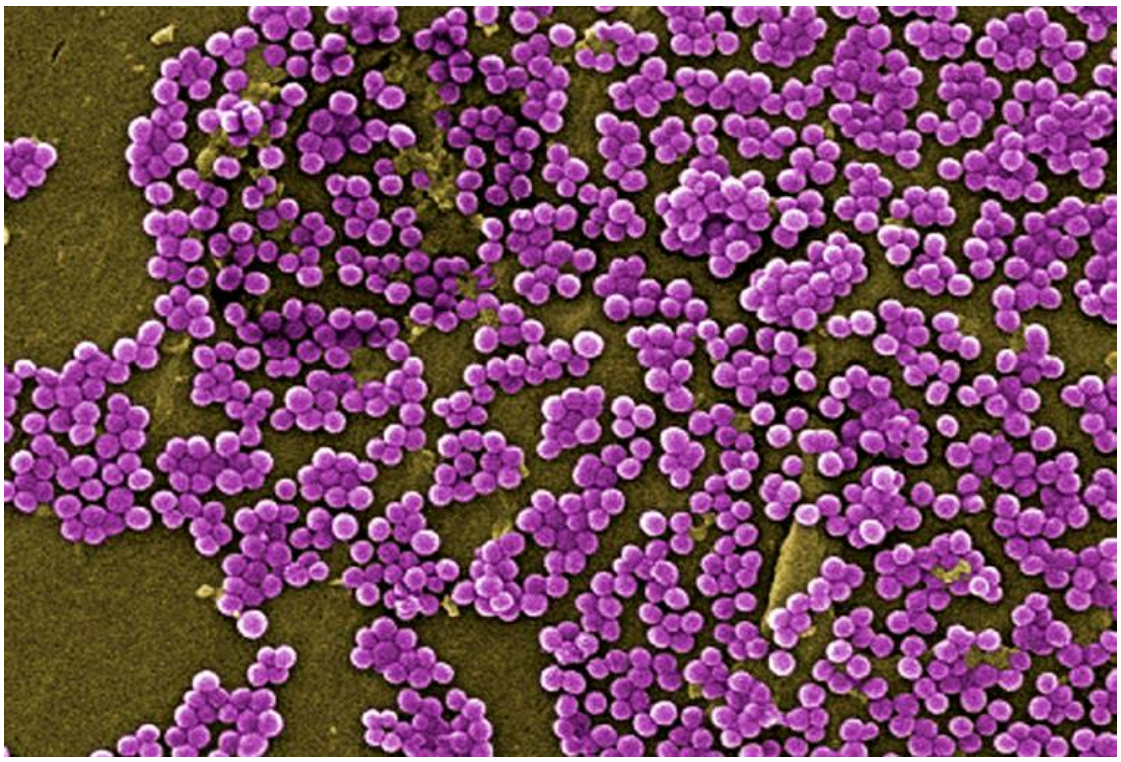

PIRU Publication
2019-25-A6

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December 2019

This report is based on independent research commissioned and funded by the NIHR Policy Research Programme through its core support to the Policy Innovation Research Unit (Project No: 102/0001). The views expressed in the publication are those of the authors and are not necessarily those of the NHS, the NIHR, the Department of Health and Social Care, its arm's length bodies or other Government Departments.

Acknowledgment

This report has been reviewed in draft form by Nicholas Mays, Policy Innovation Research Unit, London School of Hygiene and Tropical Medicine.

Contents

Acronyms

AB	Antibiotic
ABU	Antibiotic use
AM	Antimicrobial
AMU	Antimicrobial use
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CIAs	Critically important antimicrobials
DANMAP	Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals, food and humans
DDD	Defined Daily Dose
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EU	European Union
EFSA	European Food Safety Authority
EFFORT	Ecology from Farm to Fork of microbial drug Resistance and Transmission
EMA	European Medicine Agency
ESBL(s)	Extended-spectrum beta-lactamase(s)
ESCR-EC	Extended-spectrum cephalosporinase- producing *E. coli*
FPA	Food producing animals
MARAN	Monitoring of Antimicrobial Resistance and Antimicrobial Usage in Animals in the Netherlands
MDR	Multidrug resistance
MGE	Mobile genetic elements
MIC	Minimum inhibitory concentration
PFGE	Pulse field gel electrophoresis
Spp.	Species
SVARM	Swedish Veterinary Antimicrobial Resistance Monitoring
UK	United Kingdom
UTI	Urinary tract infections
VRE	Vancomycin-resistant enterococci
WBT	Whole bacterium transmission
WGS	Whole genome sequencing
WHO	World Health Organisation

Summary

Antimicrobial resistance (AMR) is a global health problem. AMR in humans is linked to AMR in animals and the environment, and bacteria carrying resistance genes can be transmitted between humans and animals by direct contact, and also through contaminated environments and food. Although AMR occurs in nature, the overuse and misuse of antimicrobials (AMs) is known to accelerate AMR.

Antimicrobials can be used in animal production for therapeutic and non-therapeutic purposes. There are concerns that the widespread use of antimicrobials in the food chain constitutes an important source of AMR in bacteria that affect humans but the extent of resistant bacteria transmission from the food chain to humans is not well understood. This report provides a review of the evidence on the links between antimicrobial use (AMU) in animal production, and AMR in people and animals.

Several studies have showed that there is a link between AM consumption and the occurrence of resistance affecting both humans and animals. Moreover, interventions in animal populations to reduce the use of AMs are effective in reducing resistant bacteria in these animals. Such data are mainly available from countries where integrated surveillance strategies have been adopted for an extended time. The benefits of reduction of AMU in animals on the prevalence of resistant bacteria in humans are difficult to quantify, though an association has been reported among farm workers in contact with food producing animals. This difficulty may be explained by the contribution of factors other than the quantity of AMs used in animals on AMR in humans and the complex epidemiology of AMR.

There is evidence that AMR bacteria are present in the human food supply chain, which presents a potential exposure route and risk to public health. Food can be contaminated by AMR pathogens or resistance genes in different ways including contamination of food during agricultural production, presence of resistance genes in bacteria added during food processing, or cross-contamination with resistant bacteria during food processing. However, it is still not clear what fraction of resistant bacteria found in humans originates from food producing animals. Microbial genome sequencing has enabled the establishment of some links between the presence of resistant bacteria in humans and animals such as *E. coli* strains in urinary tract infections in Denmark and extra-intestinal *E. coli* infections resistant to expanded spectrum cephalosporin. For some AMs, no links between resistance in humans and animals have been established.

Food processing and preservation techniques can extend the shelf life of food products. The effects of these techniques on bacteria present in food vary but, in general, the number of bacteria is reduced when these techniques are applied. Raw food is not subject to any treatment and is considered to present the highest risk. Minimal processing causes stress to bacteria which can induce changes in the cells that may affect antimicrobial susceptibility and expression of resistance genes. However, there is still a paucity of data on the impact of minimal food processing on food pathogen AMR. In general, food processes that kill bacteria in food products decrease the risk of transmission of AMR.

Significant knowledge gaps remain including the exact contribution of food producing animals compared to the other pathways affecting the presence of resistant bacteria in humans, resistance genes transfer between bacteria, including non-pathogenic ones, and the impact of reducing the presence of resistant bacteria in food producing animals on humans. To address these gaps, an integrated surveillance strategy for AMR that includes samples from humans, animals and the

environment supported by collaborative analyses across species and sectors is a high priority. This would allow the identification of the links between emerging resistances, monitoring trends, assessing the impact of the changes in policies and identifying the role that other reservoirs could play in AMR in humans. In addition, the integration of whole genome sequencing in such surveillance programmes would allow a better understanding of the ecology of AMR.

1 Introduction

Antimicrobial resistance (AMR) is recognised as one of the key threats to human and animal health at global level with significant economic implications (O'Neill, 2016). Antimicrobials (AM) are defined as naturally occurring, semi-synthetic or synthetic substances that exhibit antimicrobial activity (kill or inhibit the growth of microorganisms) at concentrations attainable in vivo. Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition (OIE, 2017). Antimicrobials are used to treat infectious diseases in humans and animals, but are also used in animals in a non-therapeutic way such as prophylactic treatment (e.g. AMs administered to a herd or a flock at risk of a disease) and metaphylactic treatment (e.g. AMs administered to healthy animals belonging to the same flock of animals with clinical signs). They can also be used as growth promoters.In the EU, the use of antibiotics as growth promoters in animal feed was banned in 2006 as a response to increasing concerns about the effect of this type of use on resistance (Rushton et al., 2014). High quantities of AMs used in animal production contribute to the development of AMR.

AMR occurs when microorganisms such as bacteria change in response to the use of AMs, and, as a result, the medicines become ineffective and infections persist in the body increasing the risk of spreading to others (WHO, 2017). It is estimated that AMR is responsible for 25,000 deaths per year in the EU and 100,000 deaths per year globally. This number is estimated to reach 10 million deaths per year by 2050 if no action is taken (EC, 2016; O'Neill, 2016). AMR is also responsible for an increase in the costs of treatments and decrease in productivity due to prolonged illness. In the EU, it is estimated that AMR costs EUR 1.5 billion annually in healthcare costs and productivity losses. At a global level, it is estimated that drug-resistant infections could have a cumulative cost to global economic output of USD 100 trillion by 2050 (EC, 2016; O'Neill, 2016)

The presence of resistant bacteria in humans can be linked to AMR in animals and the environment. Bacteria carrying resistant genes from animals can be transmitted to humans directly through the food chain by consumption of inadequately cooked food, handling of raw food or by cross contamination with other foods or indirectly through the environment. Resistant bacteria can also be transmitted directly from animals in the farms (Rushton et al., 2014). While AMR development is a naturally occurring phenomenon (D'Costa et al., 2011), overuse and misuse of AMs can accelerate this process. Of particular concern is the emergence of AMR in Gram-negative bacteria that can constitute a major public health risk. There are concerns that the widespread use of AMs in the food chain constitutes an important source of AMR potentially affecting humans. However, there is limited evidence of what contribution food-producing animals make to the overall burden of resistant bacteria in humans.

The aim of this report is to review the evidence on the links between anti-microbial use (AMU) in the food chain and the occurrence of AMR bacteria in people and animals.

2 Methods

To provide an overview of the evidence on the linkages of AMU and AMR in the food chain, a narrative literature review was conducted. The findings were used to provide information on the links between AMU and AMR in the food chain and AMR in humans as illustrated in Figure 1.

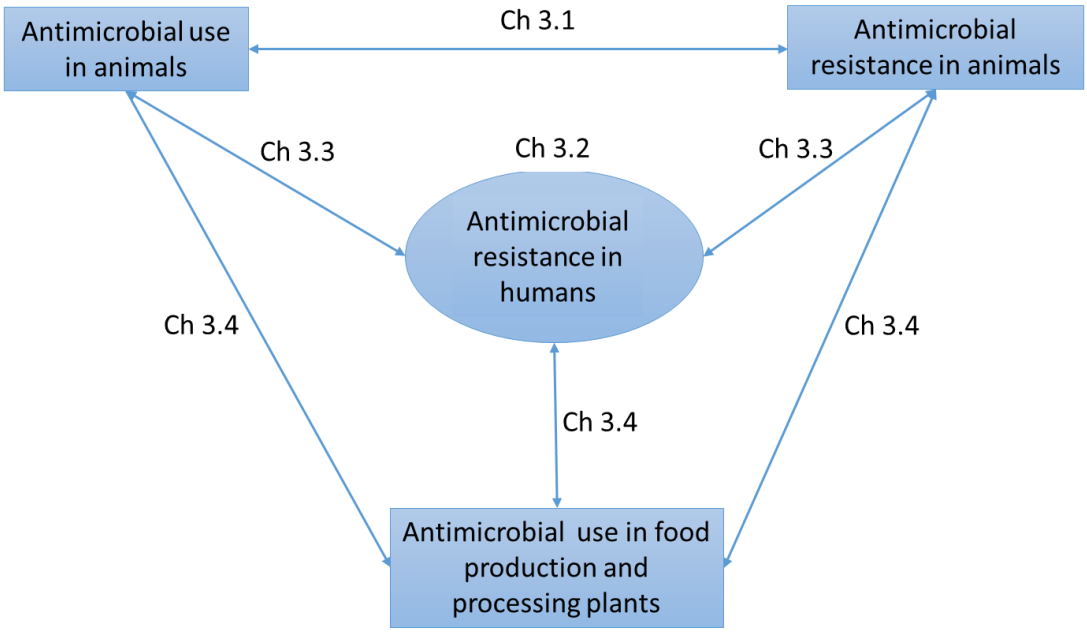

Figure 1. Links between different fields of data identified on the relationship between AMU and AMR in the food chain and people. The numbers refer to the chapters (Ch) where relevant information on this link is presented and discussed.

A literature search was conducted using a title and abstract search in Pubmed and Science Direct. A Google search was also conducted to identify grey literature of relevance. The search terms used were: "antimicrobial resistance", "antimicrobial use", "antibiotic resistance", "antibiotic use", "food chain", "food producing animals", "agriculture" and "livestock". Apart from the general search in the databases mentioned above, a targeted search was performed to identify grey literature in the Netherlands, Denmark and Sweden where integrated AMU and AMR surveillance data in humans, animals and food have been collected for many years. No restrictions about publication type or year were applied. Only terrestrial animals were considered (poultry, sheep, goats, cattle, calves, pigs). The references selected were screened and the following exclusion criteria applied: 1) the reference did not refer to an association between AMU and AMR; 2) the reference referred to animals other than terrestrial animals (e.g., wildlife, companion animals); 3) The reference was in a language other than English. The remaining articles examining the links between AMU and AMR in the food chain and AMR in humans were reviewed in full and the relevant information was extracted.

3 Results

3.1 Antimicrobial use and antimicrobial resistance in animals

The studies investigating links between AMU and AMR in animals were mainly national surveillance reports from countries where integrated surveillance has been conducted for a long time, namely Denmark, Sweden and the Netherlands. Other studies were designed to investigate statistical associations between AMU and AMR for different combinations of ABs and bacteria using data from national surveillance programmes or from European surveillance programme for AMR.

Available data suggest that there is a correlation between the quantity of AMs used in animals and the development of resistance in bacteria present in these animals. This link has been demonstrated for a range of combinations of specific pathogens, commensals, antimicrobial substances and livestock species as detailed below.

In the Netherlands, AMU in food-producing animals has been reduced considerably in the last few years due to a government policy to reduce AMU after the country had been identified as one of the highest consumers of AMs among EU countries in 2007 (EMA, 2011). Between 2007 and 2013, there was a 63% reduction in AMU in animals from 565 tonnes per year to 217 tonnes per year accompanied by a reduction of the general levels of AMR in animals (MARAN, 2015). A reduction in resistance was observed in commensal *Escherichia coli (E. coli)* isolated from broilers, slaughter pigs and veal calves between 2009 and 2014, and a reduction in resistance in *E. coli* isolated from meat from poultry, beef, pork and veal. Another analysis conducted by Dorado-Garcia et al. (2016) to quantify the association between AMU and acquired resistance in indicator *E. coli* in the Netherlands concluded that the policy implemented to reduce AMU had had an impact in decreasing *E. coli* resistance in pigs and veal calves but the impact on dairy cattle and poultry was less clear. Resistance of *E. coli* to cefotaxime (a 3rd generation cephalosporin) in broilers increased after 2003 to reach a level of more than 20% in 2007 (percentage of *E. coli* isolates resistant to cefotaxime); this resistance prevalence decreased sharply after the ban on the use of ceftiofur (also a 3rd generation cephalosporin) in hatcheries in 2010 to reach a level of 2.9% in 2014 (MARAN, 2015; Havelaar et al., 2017).

Data from Denmark on the percentage of resistant isolates in indicator *E. coli* from healthy pigs, cattle and broilers between 2001 and 2008 showed that the highest level of resistance (to streptomycin, sulphonamide, tetracycline and ampicillin) was found in pigs; a sub-sector that had the highest AMU both in treatment and prevention of infection (DANMAP, 2015). Another study found a significant impact of the 2010 voluntary ban of cephalosporin in Danish pig production on the prevalence of extended-spectrum cephalosporinase (ESC)-producing *E. coli* in pigs and pork. The occurrence of ESC-producing *E. coli* declined in pigs at slaughter from 11.8% in 2010 to 3.6% in 2011, and from 11% in 2010 to 0% in 2011 in pig farms (Agersø and Aarestrup, 2013).

Different antimicrobial substances that belong to the same drug class can favour survival of bacteria that harbour genes for the same AMR mechanism. Avoparcin is a glycopeptide, previously used in veterinary medicine as growth promoter, and belongs to the same antimicrobial class as vancomycin - a critically important antimicrobial for human medicine according to the World Health Organisation (WHO, 2016). Denmark was the first country to ban avoparcin as a growth promoter in 1995 after it discovered that the use of this AM selected for the occurrence of vancomycin resistant *Enterococci* (VRE) (DANMAP, 2015). The ban of avoparcin led to a marked decrease of VRE isolated from faecal samples of broilers from 72.7% in 1995 to 5.8% in 2000 and <3% in 2005 (Hammerum et al., 2007; Aarestrup et al., 2001). Resistance to erythromycin among *E. faecium* and *E. faecalis* isolates from pigs

was almost 90% between 1995 and 1997; this level decreased to 46.7% and 28.1% for *E. faecium* and *E. faecalis*, respectively, following a sharp decrease in tylosin use in 1998-1999[1] (Aarestrup et al., 2001).

The European Centre for Disease Prevention and Control (ECDC) with the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) produced integrated reports in 2015 and 2017, analysing the potential relationships between the consumption of AMs and the occurrence of AMR in bacteria isolated from humans and food-producing animals. In the first report, 28 countries reported AMU data in humans and 26 in animals; the data used were from 2011 and 2012. For the second joint report, 30 countries reported data for humans and 29 for animals; the data covered the years 2013, 2014 and 2015. This integrated analysis was based on the "One Health" approach and considered particular combinations of AMs and foodborne zoonotic bacterial strains (e.g., *Salmonella* spp., *Campylobacter* spp.) considered important to public health but also commensal indicator bacteria (i.e., *E. coli*, *E. faecalis* and *E. faecium*) (ECDC/EFSA/EMA, 2015 and 2017). In these reports, a significant association was observed between the consumption of fluoroquinolones and other quinolones in animals and resistance to fluoroquinolones in indicator *E. coli*, *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli* from food-producing animals. The category food producing animals included broilers, pigs and cattle for 2013 and broilers, turkeys, pigs and calves for 2014-2015.

In a joint scientific opinion by EMA and EFSA on measures to reduce the need to use AMs agents in animal husbandry in the European Union and the resulting impact on food safety (RONAFA), it was concluded that it was difficult to assess the impact but that overall it was "reasonable to assume" that a reduction in AMU would result in a reduction in AMR in bacteria from food producing animals and food (EMA/EFSA, 2017).

3.2 Antimicrobial use and antimicrobial resistance in humans

The studies included in this part came up during the literature search although the search terms selected did not target human studies specifically.

Gossens et al. (2005) investigated AMU in outpatient settings and its association with AMR in 26 European countries between 1997 and 2002. This study showed significant variation between countries with low AMU in northern regions, and moderate to high use in eastern and southern regions. The highest prescription rate was in France with 32.2 DDD (Defined Daily Dose) per 1,000 inhabitants daily and the lowest was in the Netherlands with 10 DDD per 1,000 inhabitants daily. The countries that had high consumption of AMs also had high rates of resistance.

A study from Denmark analysed data on ciprofloxacin use in primary health care and ciprofloxacin resistance in *E. coli* from urine samples between 2000 and 2015 (DANMAP, 2010 and DANMAP, 2015). The results showed a statistically significant association between the increase in ciprofloxacin use and increase in resistance to ciprofloxacin between 2000 and 2009. The rapid increase in ciprofloxacin use during the 2000s was due to a marked reduction in the price of ciprofloxacin after the market was opened to generic ciprofloxacin in 2002 (DANMAP, 2015). Consequently, fluoroquinolone

[1] The government banned the use of avoparcin in 1995 and virginamycin in 1998 and producers voluntarily stopped all use of AMs for growth promotion at the end of 1999.

consumption increased from 0.17 DDD/1,000 inhabitants per day in 2001 to 0.57 DDD/1,000 inhabitants/day in 2010. The percentage of ciprofloxacin-resistant *E. coli* isolates increased from 1% in 2001 to 11% in 2010. After 2009, resistance rates levelled off in line with a stabilising of the rate of consumption of fluoroquinolone from 2009 onwards.

In the second ECDC/EFSA/EMA joint report, a significant association was observed between the total consumption of third and fourth generation cephalosporins in humans and the occurrence of resistance to third generation cephalosporins in invasive *E. coli* from humans. A significant association was also observed between the total consumption of fluoroquinolones in humans and the occurrence of fluoroquinolone resistance in invasive *E. coli* from humans. No association was found between fluoroquinolone consumption in humans and resistance in *Salmonella* spp. or *Campylobacter* spp. from humans (ECDC/EFSA/EMA, 2017).

3.3 Antimicrobial use in animals and antimicrobial resistance in humans

The relationship between AMU in animals and resistant bacteria in humans has been investigated using a variety of methods. The majority of studies focus on the transmission pathways of resistant bacteria from animals to humans (i.e. AMR ecology studies). Very few studies investigated direct effects of usage in animals on the occurrence of resistance in bacteria and its impact on humans. A summary of the findings is described in the following sections.

3.3.1 Molecular evidence supporting a link between resistant bacteria in animals and their transmission to humans

The role of animal derived *E. coli* strains in urinary tract infections (UTI) in humans was investigated in a study in Denmark. A sample collection of *E. coli* strains from cattle, pigs, poultry and meat products from these animals was compared to a collection of *E. coli* isolates from healthy humans and those with UTI. These strains had been identified previously as exhibiting virulence genotypes. Comparison of virulence genes, phylotypes, pulse-field gel electrophoresis (PFGE) and antimicrobial susceptibility testing (AST), demonstrated a clonal link between *E. coli* from animal or meat and humans. This led to the conclusion that *E. coli* UTI in humans could be the result of zoonotic transmission (DANMAP, 2015).

Lazarus et al. (2015) conducted a systematic review to investigate whether extra-intestinal *E. coli* infections resistant to expanded spectrum cephalosporins (ESCR-EC) originated from food-producing animals. Thirty-four studies were identified for inclusion. Six molecular studies supported the transfer of resistance via whole bacterium transmission (WBT) which was well characterised among poultry in the Netherlands but it was not clear if this was a geographic phenomenon or due to limited research in other parts of the world. Thirteen molecular studies supported the notion of transmission of resistance via mobile genetic elements (MGEs) and these studies had greater diversity of geography and host species of food producing animals, thus strengthening the relevance of this observation. Seventeen studies did not support WBT and two did not support MGE transmission. Four observational studies supported the hypothesis of zoonotic transmission. The review concluded that a proportion of human ESCR-EC was attributed to food-producing animals, with poultry being the most likely source, but the quantitative and geographical extent of the problem was not well understood.

In a study of multi-drug resistant *Salmonella typhimurium* DT104, whole genome sequencing was used to investigate the phylogenetic relationship of the bacterium and its AMR genes through the course

of an epidemic (Mather et al., 2013). A total of 142 isolates from humans and 120 from animals (70% of the animal isolates were of bovine origin) from Scotland were sequenced covering the years 1990 to 2011; an additional 111 international isolates were added to the sample. The results showed that the bacterium and its resistance genes were maintained separately within animal and human populations with limited spill-over in both directions. It was also reported that there was greater diversity of AMR genes in the human isolates compared to the animal isolates; this indicated that other sources of *S. typhimurium* DT104 could have contributed to the human resistance, such as imported food, foreign travel and environment reservoirs.

3.3.2 Presence of AMR bacteria in food products from animal origin available to consumers

Antimicrobial resistant bacteria are present in the food chain; this constitutes a potential route for human exposure to AMR bacteria or resistance genes. This can occur due to contamination of food during agricultural production, presence of resistance genes in bacteria added during food processing or cross-contamination with AMR resistant bacteria during food processing (Verraes et al., 2013).

Two systematic reviews were conducted between 1999 and 2016 in the UK and in Switzerland, respectively, to investigate the occurrence of AMR in bacteria present in food at retail level in these countries (FSA, 2016; Jans et al., 2018).

The review produced in the UK looked at studies from the UK and countries exporting to the UK. It showed that there was a lack of data on British-produced food and to a lesser extent on countries exporting to the UK, with the exception of northern European countries. For poultry meat in the UK, an increasing trend of fluoroquinolone resistance in *Campylobacter jejuni* isolates had been observed since 2001. Resistance levels to ciprofloxacin and nalidixic acid in 2001 were 12.6% and 15.6%, respectively. These levels increased to 21.7% and 23.7%, in 2005 and were up to 50% and 51% in 2014-2015for ciprofloxacin and nalidixic acid. High levels of resistance to ciprofloxacin and nalidixic acid in *C. jejuni* for poultry meat were also observed in studies from the Netherlands (63.4% in 2014) and Poland (up to 100%). For Denmark, an increase in ampicillin resistance in bacteria isolated from pork was observed in *Salmonella* isolates (up to 73% in 2013) and in *E. coli* isolates (up to 33% in 2012) (FSA, 2016).

The review produced in Switzerland (Jans et al., 2018), targeted studies linked to Switzerland and the Swiss retail sector, i.e. studies from Switzerland as well as countries exporting food to Switzerland. The largest number of AMR positive samples was observed in raw meat products, which can be partly explained by an overrepresentation of studies on raw meat (of the 313 studies included in the review, a total of 160 studies contained data on testing of raw meat). Major resistances among Gram-negative foodborne pathogens were observed in *Campylobacter* against fluoroquinolones and tetracyclines. In *Salmonella*, the main resistances were detected against aminoglycosides, cephalosporines, fluoroquinolones, penicillins, sulphonamides and tetracyclines (Jans et al., 2018).

AMR genes in microbes in food can – provided that the DNA is not digested – spread to other bacteria in the human gut. The public health relevance of such gene transfer has yet to be quantified. To reduce the risk of human exposure to bacteria carrying AMR genes, good hygiene practices need to be put in place, including cleaning, chilling, and avoiding cross contamination. In addition, sufficient cooking is crucial as it destroys bacteria present in food.

3.3.3 Association between AMU in food animals and resistant bacteria in humans

A study from Canada showed a positive correlation between ceftiofur-resistant *Salmonella. Heidelberg* isolated from retail chickens and incidence of ceftiofur-resistant *S. Heidelberg* infections in humans across Canada. After a voluntary withdrawal of ceftiofur use in hatcheries in Canada in 2005, a decrease in ceftiofur resistance *S. Heidelberg* in chickens and humans was observed, followed by an increase in resistance levels in both species after reintroduction of its use in young chicks to control omphalitis in 2007 (Dutil et al., 2010). This is a rare example indicating a direct temporal association between AMU in animals and AMR in humans indicating a high likelihood of the association being causal. In the US, enrofloxacin was withdrawn from use in poultry in 2005 after it was associated with an increase in human infections with fluoroquinolones-resistant *Campylobacter* species (Nelson et al., 2007).

The analysis of the data from the second ECDC/EFSA/EMA joint report showed a significant association between the total consumption of fluoroquinolones and other quinolones in food-producing animals and the occurrence of resistance to fluoroquinolones in invasive *E. coli* from humans. In addition, a significant association was observed between resistance to fluoroquinolones and other quinolones in *Salmonella* spp. and *C. jejuni* from humans and resistance in bacteria from food-producing animals (ECDC/EFSA/EMA, 2017).

In a recent systematic review and meta-analysis, Tang et al. (2017) investigated the associations between restricting the use of ABs in food-producing animals and its association with AMR in such animals and humans. A total of 179 animal studies were found to be relevant; 81 of them were included in the meta-analysis. Twenty-one human studies were found with 13 included in the meta-analysis. A total of 19 studies reported outcomes from both humans and animals, so were counted in both human and animal studies. The results showed that interventions to reduce ABU in animals have an impact on reducing AB resistance in these animals with an overall reduction of AB resistance by about 15% and MDR bacteria between 24% and 32%. The evidence of the effect on humans was limited and less robust; the analysis of the 13 studies showed a reduction of 24% in AB resistant bacteria in humans due to the reduction of AMU in animals. This impact was mainly observed in people directly in contact with livestock; source attribution to the food chain was not possible. The authors acknowledged the limitations of the review and the high level of heterogeneity observed between the studies. They concluded that these results cannot be extrapolated to the human population as a whole because of the limited number of studies available.

The impact of restricting AMU in food-producing animals on resistant bacteria in humans was also investigated using a mathematical model (Van Bunnik and Woolhouse, 2017). The objective of the modelling study was to understand better the dynamics of the relationship between AMR in humans and animals, and to identify the model parameters that have the greatest impact on the model results, i.e. the reduction of transmission risk. The results showed that reducing the amount of AMU in food-producing animals had little impact on resistant bacteria in humans if used in isolation and that reducing the rate of transmission of resistance from animals to humans may be more effective.

3.3.4 Studies where no association could be established between AMR in food-producing animals and humans

In Denmark, CTX-M-15 is the dominating resistance genotype for Extended Spectrum Beta-Lactamases (ESBLs) in *E. coli* and *Klebsiella* spp. in humans (around 50-70% of all ESBLs). Yet, this gene has rarely

been found in Danish production animals, indicating that there is little transfer of these strains from livestock to humans in Denmark (for example in pigs and pork, the most common ESBL type was CTX-M-1) (DANMAP, 2015). A similar result was found in a study conducted in Sweden to investigate food as a potential source and dissemination of ESBL-producing *E. coli* to humans (ESBL producing *Enterobacteriaceae* including *E. coli* is the most commonly reported resistance type in Sweden). The study analysed data from approximately 5,300 samples taken from foods (domestic and imported), farm animals, healthy volunteers, severely ill patients, the environment and sewage water. The comparison of the genes encoding ESBL showed that there are three separate populations of genes encoding ESBL in Sweden, one in Swedish foods and farm animals, one in imported foods, and one in humans and the environment. The results indicated that food had a limited contribution to the occurrence of ESBL-producing *E. coli* within the healthcare sector in Sweden (SVARM, 2014), though the exact level of contribution was not reported.

Another example relates to the resistance to carbapenems that is emerging in humans and constitutes a public health concern. Carbapenems are classified by the WHO as critically-important antibiotics because they are used for the treatment of serious infections in humans and are considered the last line therapy for infections caused by multi-drug-resistant Gram-negative bacteria (EFSA, 2013). Although this class of AM is not authorised for use in animals, carbapenem resistance in bacteria from animals has been reported in a very few cases, indicating that dissemination from humans to animals directly or through environmental routes may occur (ECDC/EFSA/EMA, 2015).

3.4 Antimicrobial use in food production and processing plants

The use of disinfectants in food production plants is important in decreasing the risk of contaminated food products reaching consumers. There have been concerns that bacteria exposed to disinfectants could develop resistance to disinfectants and consequently have a higher risk of developing AMR. In order to examine the prevalence of biocide resistant *Salmonella* spp. and to assess if there was a correlation between susceptibilities to biocides and ABs, and the impact of cleaning and disinfection on the selection of isolates with changed susceptibility, Gantzhorn et al. (2014) conducted a study in six Danish pig slaughterhouses. The susceptibility toward three different biocides, triclosan and two commercial disinfection products: Desinfect Maxi, a quaternary ammonium compound, and Incimaxx DES (an acetic compound), was determined. The study found no resistance towards the biocides tested but found that isolates obtained after cleaning and disinfection had an increased resistance toward one of the disinfectants (Incimaxx DES) compared to isolates obtained before cleaning and disinfection. This indicated the possibility of selection of strains that were more tolerant to biocides due to the cleaning and disinfection. Also, a weak correlation was observed between susceptibilities to biocides and some antibiotics, for example a negative correlation between triclosan and plymixin B and a positive correlation between Desinfect Maxi and tobramycin. This indicates that resistance to biocides and antibiotics may be genetically coupled and resistance to one could incur resistance to the other.

Other studies conducted in laboratories found correlations between exposure to biocides and decreased resistance to antibiotics. Alonso-Hernando et al. (2009) tested *Salmonella Enterica* and *Listeria monocytogenes* strains against sub-inhibitory concentrations of decontaminants (trisodium phosphate, acidified sodium chlorite, citric acid, chlorine dioxide or peroxyacetic acid) applied in poultry processing. The AMR patterns were compared before and after exposure, and an increase in

resistance to various ABs after exposure to chemicals was observed. Condell et al. (2012) investigated the tolerance of a collection of susceptible and multi-drug resistant *Salmonella Enterica* strains to seven food-grade biocide formulations and explored their ability to adapt. The results showed that after exposure, a high level of tolerance was selected for a number of *Salmonella* serotypes and that most tolerant isolates displayed changes in their patterns of susceptibility to AMs. However, in a review on biophysical parameters affecting gene transfer in the food chain that was conducted as a part of the project "Ecology from Farm to Fork of microbial drug Resistance and Transmission" (EFFORT), it was concluded that no common pattern of reduced resistance/decreased susceptibility or creation of cross-resistance could be deducted from the laboratory-scale experiments and that this depended on the biocide-antimicrobial-strain combination and the concentrations applied under laboratory conditions (personal communication K Staerk)..

To investigate the impact of food processing on the transfer of AMR bacteria to humans, a review was conducted by Verraes et al. (2013). The results showed that the effects of food processing and preservation techniques (such as heat treatment, cooling, acidification, modified atmosphere packaging, freezing, mild pasteurization and ultra-violate radiation treatment) on bacteria were variable but in general there was a decrease in the number of bacteria when these techniques were applied appropriately. Dead bacteria are not able to perform conjugation and heat treatment that kills bacteria reduces the risk of AMR gene transfer. Raw food was considered to be the source of highest risk because resistant bacteria are not killed by any treatment. This study also showed that minimal processing or preservation treatments resulted in stressed bacteria that could be maintained in the food and which could increase the probability of AMR transfer. Stress in bacteria can cause changes in the cells that may lead to antimicrobial susceptibility and expression of resistance genes (Poole, 2012). There is still a lack of information on the effect of these techniques on the risk of AMR. It was also reported that microorganisms intentionally added to foodstuffs, such as starter cultures, probiotics, and biopreserving microorganisms, may contain AMR genes and may transfer them to bacteria (Verraes et al., 2013).

Jans et al. (2018) also confirmed the presence of AMR bacteria in fermented products and starter cultures and concluded that systematic surveillance needs to be applied to collect the data needed to assess the public health risk from this type of exposure.

4 Discussion and conclusions

There is evidence of a link between AM consumption and the occurrence of AMR affecting humans and animals. Interventions to reduce the use of AMs in animals were effective in reducing AMR in these animals in Denmark and the Netherlands. This indicates that measures to reduce the use of AMs as far as possible (e.g. campaigns for responsible use) should remain a priority for both human and animal populations to preserve the efficacy of AMs and reduce the pool of resistance. Data showing the impact of reduction of AM use on resistance were available mainly from countries that had pursued integrated surveillance strategies for a long time. Hence, without long-term monitoring, changes in AMU and AMR as well as the effect of specific interventions are difficult to capture. The benefits of reduction of AMU in animals for AMR in humans are difficult to quantify with an association reported mainly for people in contact with food-producing animals (Tang et al., 2017). This may be explained by the complexity of AMR and the contribution of factors other than the quantity of AMs

used in animals on AMR in humans, such as AMU in humans both in hospitals and at community level, exposure to resistant bacteria present in the environment and in fresh food products. Also, different bacteria utilise different genetic resistance mechanisms and are transmitted by different pathways. Importantly, reducing resistant bacteria in food producing animals is not only necessary because of potential transmission risks to humans, but also to safeguard the health and welfare of animal populations and thereby secure production of animal source foods and contribute to food security.

AMR bacteria exist in the food chain and therefore present an exposure route for humans but it is not clear what fraction of AMR bacteria in humans originates from food producing animals. Microbial genome sequencing has established some links between resistance in humans and animals such as *E. coli* strains in urinary tract infections in Denmark and extra-intestinal *E*. *coli* infections resistant to Expanded Spectrum Cephalosporins (DANMAP, 2015; Lazarus et al., 2015). For some AMs, no link between humans and animals could be found (DANMAP, 2015; SVARM, 2014; EFSA, 2013).

Whole genome sequencing constitutes an important advance in technology allowing better understanding of AMR ecology. However, to obtain valid results, there is a need for representative sampling of bacteria from humans, animals and the environment. As many surveillance systems currently incorporate whole genome sequencing to improve the diagnosis and control of infectious diseases, there will be opportunities for investigating the presence of resistance genes in these samples.

The impact of food processing and preservation techniques is variable but, in general, the number of bacteria is reduced when these techniques are applied. Raw food presents the highest risk because it is not subject to any treatment. There is still a paucity of data on the impact of minimal food processing on food pathogen-related AMR. Food processes that kill bacteria decrease the risk of transmission of AMR. In terms of the health consequences for consumers exposed to resistance genes in foods, current evidence suggests that the health impact of the presence of resistance genes in processed foods is likely to be limited. There remains uncertainty regarding the biological consequences of ingested resistance genes in bacteria that are able to survive digestion, such as gastrointestinal pathogens.

The main aim of this review was to assess the evidence on the links between AMU in the food chain, and AMR in people and animals. There are still significant evidence gaps, including the exact contribution of food-producing animals and food products compared to the different other sources of AMR in humans, better understanding of resistance genes transfer between bacteria including non-pathogenic (commensals) ones and the impact of reducing AMR in food producing animals on AMR in humans.

The development and spread of AMR in the environment is also of increasing concerns and there is a lack of data on the role of environmental factors in the transmission of resistance. Antimicrobials can contaminate the environment through animal waste, human waste and manufacturing waste. This can provoke the development of resistance in bacteria present in soil, crops and water sources, and therefore may potentially constitutes a significant pathway for transmission of AMR to humans and animals (O'Neill, 2016).

For a better understanding and management of AMR, it is important to develop integrated surveillance strategies with harmonised designs across human and animal populations with linkages to samples from the environment. Integrated surveillance will be useful in monitoring trends,

identifying the links between emerging resistances, assess the impact of the change in policy and identify the role that other reservoirs could play in AMR in humans.

Control strategies need to include a specific plan for targeted studies with appropriate designs and methodologies that would address the gaps in knowledge and the weaknesses of the existing studies. These studies need to use a variety of methods and include experts from different disciplines, such us microbiologists, veterinarians, doctors, genetic specialist, epidemiologists and social scientists, to be able to capture the different factors involved in the use of AMs in animal production that could pose a risk to humans. In particular, it will be critical to use study designs that allow gaining information on source attribution and to demonstrate linkages across several steps, i.e. investigate whether the reduction in AMU in animals translate to a positive effect in the human population through the food chain.

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