

Evidence for the circulation of antimicrobial-resistant strains and genes in nature and especially between humans and animals

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Summary

The concern over antibiotic-resistant bacteria producing human infections that are difficult to treat has led to a proliferation of studies in recent years investigating resistance in livestock, food products, the environment and people, as well as in the mechanisms of transfer of the genetic elements of resistance between bacteria, and the routes, or risk pathways, by which the spread of resistance might occur. The possibility of transfer of resistant genetic elements between bacteria in mixed populations adds many additional and complex potential routes of spread. There is now considerable evidence that transfer of antimicrobial resistance from food-producing animals to humans directly via the food chain is a likely route of spread. The application of animal wastes to farmland and subsequent leaching into watercourses has also been shown to lead to many potential, but less well-documented, pathways for spread. Often, however, where contamination of water sources, processed foods, and other environmental sites is concerned, specific routes of circulation are unclear and may well involve human sources of contamination. Examination of water sources in particular may be difficult due to dilution and their natural flow. Also, as meat is comparatively easy to examine, and is frequently suspected of being a source of spread, there is some bias in favour of studying this vehicle. Such complexities mean that, with the evidence currently available, it is not possible to prioritise the importance of potential risk pathways and circulation routes.

Keywords

Antibiotic resistance – Environmental contamination – Food contamination – Risk pathway – Zoonotic infection.

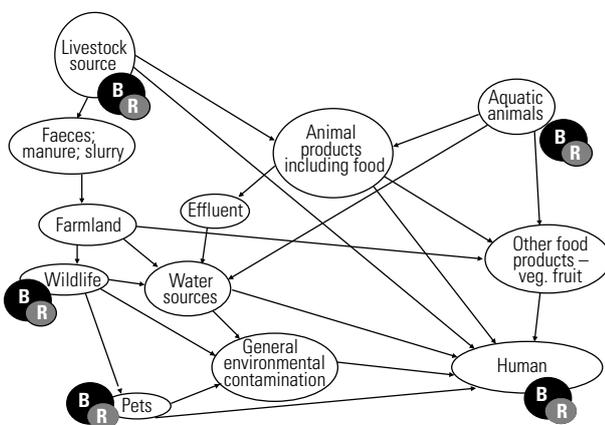
Introduction

Many bacteria found in animals are also zoonotic pathogens and can be transferred from animals to humans via a variety of routes. One way of treating human infections with pathogenic bacteria is by the use of suitable antibiotics. The definition of antibiotics used by the World Health Organization in *Tackling antibiotic resistance from a food safety perspective in Europe* (82) is 'antibacterial agents used to treat bacterial infections in both people and animals', also used here.

Antibiotic resistance describes the ability of certain bacteria to resist the effect of treatment with antibiotics, and this resistance can be innate or acquired, the latter typically associated with the use of an antibiotic that inhibits or destroys susceptible bacteria within a population, allowing resistant strains to flourish (82). This paper focuses on the routes by which antibiotic resistance can circulate in the environment, and thus by which they can potentially transfer from animals to humans, and the evidence available for such routes. There is also the potential for transfer from humans to animals, and, although less evidence is available, this aspect is also included.

Potential routes of circulation and transmission of antibiotic resistance – risk pathways

Many of the potential routes for the transmission of antibiotic resistance from animals to humans are likely to be the same as those for transmission of bacterial pathogens themselves. These include transmission by direct handling or close contact (e.g. aerosols) between infected animals (including aquatic animals) and humans, transmission via contaminated animal products, particularly but not exclusively food products, and transmission via contamination of the environment, which may be direct from animals (excretions and secretions) or indirect, via, for example, the spreading of slurry, contamination of watercourses or the involvement of fomites to spread the bacteria. In addition, bacteria may spread among the animals themselves before moving further afield, and this spread may include farmed livestock, wild animals and companion animals. Cross-contamination in the processing and preparation of animal products, including food, can occur, and fruit and vegetables can also be contaminated in this way and also by farmland or watercourse contamination. Kitchens, shops, restaurants and other food-associated areas can also become contaminated. Virtually any activity in which bacteria are present can result in contamination of the surrounding general environment. In addition, antibiotic-resistant organisms in humans, acquired either from the routes summarised here, or nosocomially, may also spread into the environment and to other animal species. Figure 1 illustrates the most significant of the potential risk pathways for the transmission of bacteria, with any resistant population that it may include, from animals to humans.



B **R** = bacterial population with resistant proportion

Fig. 1
The potential routes of transfer bacteria, with any resistant population that they may include, from animals to humans

However, antibiotic resistance has an additional route of spread, namely the transfer and exchange of genetic information. This may occur in settings in which susceptible bacteria mix with resistant bacteria, of either the same or different species and strains, and directly transfer resistance to previously susceptible populations. And, if the relevant genes are co-located in the element being transferred, this type of mechanism may also transfer resistance to a number of unrelated antibiotics at the same time (82). It is not the intention of this paper to detail genetic transfer mechanisms, but such mixing might occur in the intestines of animals or humans, or in other highly mixed settings where contact is close; for example, slurry or damp contaminated bedding litter.

In addition, antibiotics given to animals are often given orally, and may fail to be metabolised in the digestive tract. This can result in antibiotics being excreted in faeces, and their subsequent presence in manure and the environment. They may well therefore exert their effect on bacterial populations at this or later points in the bacterial transmission chains, further increasing the population of resistant bacteria before they reach the stage of human exposure.

The concern over antibiotic-resistant bacteria producing human infections that are difficult to treat has led to a proliferation of studies on the subject in recent years, including studies on livestock, food products, the environment and cases in humans. There are also some studies of human-to-animal transfer.

The World Health Organization has developed a list of antibiotics of particular importance for human medicine, and these include fluoroquinolones, cephalosporins (third and fourth generation) and macrolides (82). The World Organisation for Animal Health (OIE) has also developed a list of antibiotics categorised by the need for their use in animal treatment, an added complication here being the need to treat many species. The category 'veterinary critically important antimicrobials' includes fluoroquinolones, cephalosporins and macrolides, as well as a number of other families of antibiotics (83). Thus, these antibiotics may all affect bacteria in both animal and human treatment settings, and residues of all these antibiotics may be expected in both animal and human waste.

Evidence for the circulation, spread and risk pathways of antibiotic resistance

Evidence for the likely routes (risk pathways) of transfer of antibiotic resistance may emerge in a number of ways,

although absolute proof that a particular transmission route occurred 'in the field' is unlikely. It may be that resistance patterns in humans mirror similar patterns in time and space to those in certain groups of animals, or there may be similar temporospatial relationships between the use of antibiotics in animal populations and human resistance. Such information suggests an association and possible transmission of resistance, but may not indicate the specific route(s) of that transmission. More useful in this context is where a likely route of transfer can be identified; however, the evidence for a complete transmission pathway is often lacking. To construct complete potential transmission, or risk, pathways one may then have to make assumptions based on biological knowledge plus the evidence that is available. This paper examines the evidence available for the various transmission routes postulated.

Transfer of antibiotic resistance between bacteria

It is not the intention of this paper to detail the mechanisms of antibiotic resistance transfer at the molecular level. However, in a review of these mechanisms, Jayaraman (50) argues that the range of mechanisms is such that, in principle, any organism could develop resistance to any antibiotic. The potential for this transfer to occur increases greatly the complexity of the possible routes of exposure to resistant bacteria by both animals and humans.

The transfer of antibiotic resistance could, theoretically, be expected to occur in any setting in which resistant elements are present and there is sufficient bacterial mixing. Resistance genes can easily transfer between bacteria from terrestrial animals, fish and humans, and this can happen in a variety of mixing places, including barns, water sources and kitchens (56). Ecosystems in close proximity to aquaculture farms and the stability of 'naked' DNA in the soil environment are also both likely to play a part in the ease of such transfer (15, 45).

One setting that might therefore be expected to provide a suitable environment is within animals' intestines. Horizontal transfer of DNA between *Campylobacter* strains, and the transfer of plasmids carrying resistant genes, has been shown to occur in the intestines of chickens (8). In enteric bacteria in cattle, at least, there may also be a link, under antimicrobial selection pressure, between antibiotic resistance genes and niche or growth-specific fitness traits, allowing resistant bacteria to reside long term (17). Such persistence could enhance mixing opportunities. Enterococci, ubiquitous in animals' digestive tracts, frequently carry antibiotic resistance genes plus numerous

mobile genetic elements, and thus have a large potential capacity for horizontal gene transfer (36). They would therefore be an ideal group for further investigation (62).

However, transfer may also occur in the surrounding environment. The transfer of an H2 plasmid (coding for resistance to chloramphenicol, sulphonamides, streptomycin and tetracycline) is considerably reduced above 34°C and was thus assumed to occur outside an animal's body (75). This was later shown to be the case by investigating transfer of H2 between salmonellae and a range of *Escherichia coli* serotypes present in calf faeces (60). By muzzling, and then dosing calves with donor and recipient bacteria, it was found that they were colonised by transconjugants only after they were allowed to lick their environment, no transconjugants being excreted while they were muzzled, implying that the transfer took place in excreted faeces, which were then re-ingested. Further, transfer appeared to be more effective if the calves lay for some time on the excreted manure, keeping it warm.

In a review of the contribution of manure to resistance levels in agricultural soils (43), the authors state that manure has become a reservoir of resistant bacteria. They conclude that its application to agricultural soils significantly increases the prevalence of antibiotic-resistant genes and thus the selection of resistant bacterial populations in soils. They also conclude that horizontal transfer of mobile genetic elements, such as broad host-range plasmids, integrons and transposable elements, to bacteria adapted to soil supports their environmental transmission independent of the original host – and that human exposure to soil-borne resistance is likely to be severely underestimated.

Microbes normally found in water can contain their own genes for antibiotic resistance. In addition to this, organisms containing antibiotic-resistant genes can enter aquatic environments from both animal and human sources (especially farms and hospitals), where they are able to spread their genes to the indigenous bacteria. Risk assessment methodology to investigate this possibility, based on improved tracking of antibiotic-resistant microbes, is being developed (9).

Using a number of experimentally simulated, but commonly encountered, food preparation environments has demonstrated the potential for multiply-resistant plasmid transfer. Examples include transfer between a resistant strain of *Aeromonas salmonicida*, from raw salmon, and a susceptible human *E. coli* strain on a cutting board, and between a resistant bovine pathogenic *E. coli* and susceptible human *E. coli* on a milk-contaminated hand towel (56). The transfer of genetic material associated with vancomycin resistance, from *Enterococcus faecalis* to a human clinical isolate of *Staphylococcus aureus*, has been documented (81), indicating the potential importance of horizontal gene transfer in a human clinical setting.

These examples demonstrate transfer of resistant genetic elements between bacteria in animals in a variety of environments, including farm, watercourse and food preparation, and in human clinical settings, greatly facilitating the spread of resistance, and increasing the difficulties in collecting and interpreting evidence of circulation patterns.

Evidence for bacteria and antibiotic resistance in animals and their close environment

Bacteria are a normal and significant part of intestinal contents in all species, as is the presence of some innate antibiotic resistance in bacteria. However, it is generally accepted that the use of antibiotics, for whatever reason, will result in an increase in antibiotic resistance. It is not the purpose of this paper to detail antibiotic usage, or the biological mechanisms by which it can increase resistant populations; however, changes in levels of resistance are frequently associated with changes in levels of their use. This needs to be borne in mind when considering the levels of resistance found in bacteria taken from animals and their surroundings. And, as many isolates are from faecal samples obtained from those surroundings, there may be little ability to differentiate between the situation within the animal and that surrounding the animal, unless faecal samples are taken directly from the animal.

There is also a lack of data on what factors, other than antibiotic use, are responsible for the levels of resistance. Possible factors include management factors; for example, the size of the herd or flock, the levels of hygiene, and the potential for contact between animals from different groups (43).

Farmed animals

In 1995 Denmark developed a monitoring programme for antibiotic use and resistance in animals, food and humans known as the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) – the first country to do so. In due course systems broadly similar to DANMAP were developed by a variety of other countries including Norway, Sweden, the Netherlands, Canada and the United States (USA) (39).

Building on such initiatives, the European Union (EU) ‘Community Summary Report on antimicrobial resistance in zoonotic agents and indicator bacteria from animals and food in the European Union for 2004 to 2007’ contains a huge amount of information on antibiotic resistance to

Salmonella spp., *Campylobacter* spp., *E. coli* and indicator enterococci in poultry, pigs, cattle and turkeys, as reported by EU Member States (plus two non-Member States). The report focuses wherever possible on quantitative data, and maps are given in some cases. Resistance to antibiotics was common in all these bacteria, but there were large differences between Member States (for some combinations of antibiotic and species, ranging from 0% to almost 100% resistance). Considering those antibiotics of particular importance in human treatment, fluoroquinolone resistance was commonly reported in *C. jejuni* and *C. coli* isolates and, in some countries, resistance to third-generation cephalosporins at low level was found in salmonellae and *E. coli*. Resistance to vancomycin was found in indicator *E. faecium* and *E. faecalis* from chickens, pigs and cattle. Differences between Member States may be real or may be related to differences in the monitoring systems used (31).

Such monitoring systems and summary reports provide a large amount of evidence for the presence of antibiotic resistance within farmed animals.

Intestinal bacterial loads are normally high. Findings indicate that intestinal populations of bacteria in ruminants often exceed 10^{11} colony-forming units (CFU) per gram of faeces (85). For pigs, one estimate is that a typical pig bred for meat produces approximately 1,500 kg of manure in its normal 5- to 6-month life, and that each gram of such manure typically contains between 10^{11} and 10^{15} bacteria (45).

In an investigation into what constituted the normal bacteria within the digestive tract (76), the investigators found that in farm animals the most widespread bacteria were *E. coli* and enterococci. However, in an evaluation of the microbial diversity in the guts of cattle the same investigators state that, despite the development of techniques utilising DNA and related analysis, and their application in humans (see, for example, reference 40), there is still a lack of data on bacterial diversity within animal intestines (27). This is said to be, in large part, because of the expense of these methods. They also suggest that, although there is bias in DNA-based methods (e.g. their ability to detect both viable and non-viable bacteria), traditional methods of identification involving culturing have tended to select for easy-to-culture organisms such as *E. coli*. Their own DNA studies identified 274 different bacterial species in faecal samples from 20 healthy cattle; one of the predominant genera was *Enterococcus*, and although *E. coli*, *Salmonella* and *Campylobacter* were found, they were not considered predominant.

As well as the large number of bacteria excreted daily, some of which already demonstrate the presence of antibiotic resistance genes, animal faeces and urine also contain antibiotic residues; for example, as much as 50–90% of

erythromycin is excreted in faeces and 10% in urine, which is thus still available to affect bacteria and increase the resistant proportion in the manure (19, 69, 84).

Some examples indicating the wide variety of types of individual studies, on which summary reports and monitoring systems may rely, now follow. In the People's Republic of China (hereafter referred to as China), a study investigating plasmid-mediated quinolone resistance measured this in *E. coli* isolates in pigs (four farms) and chickens (one farm). Of the 73 pig isolates, 40 (55%) were positive, and for chickens the figures were 25 isolates with three (12%) positive (86).

As part of a study to compare antibiotic resistance in food and farmed animals in six states in the USA, faeces of healthy chickens, turkeys, cattle and pigs were examined for gentamicin resistance genes in isolates of enterococci. A variety of resistance genes, some at high percentages, were found in all species, the enterococci tested being *E. faecalis*, *E. faecium*, *E. casseliflavus* and *E. gallinarum* (26). A similar type of study in central Italy examined the faeces of chickens and pigs from slaughterhouses supplying four food production plants. Samples were examined for 11 different resistance genes, effective against a wide variety of antibiotics. For both chickens and pigs, all samples contained at least some of the resistance genes, and, overall, pig faeces had a higher prevalence of antibiotic resistance genes (35).

Avoparcin is a glycopeptide antibiotic that is included in EU monitoring. It is similar to the glycopeptide vancomycin, which is important in human use. One finding of the EU monitoring is the trend in glycopeptide resistance which, following the ban on the use of avoparcin as a growth promoter, showed a marked decrease in resistant isolates of *E. faecium* in both broiler chickens and pigs. By 2005 the percentage of resistant isolates was in very low single figures in both species, down from between 20% (pigs) and 80% (broilers) ten years earlier (39).

Although the level of resistance tends to be lower in small and organic pig farms (11), evidence suggests that, in general, antibiotic-resistant bacteria are found associated with pigs at higher frequencies than in cattle or sheep, and this also correlates with the amounts of antibiotics generally used in pig farming (29, 61).

Intensive fish farming is increasing globally, and this has resulted in an increase in bacterial infections, generally treated with antibiotics added to fish feed (15, 82). As with many terrestrial livestock, fish are unable to fully metabolise these antibiotics, which they then excrete untransformed into ponds and holding tanks, and it is suggested that, as water from these sites is likely to be disseminated widely and easily, this source may well have an impact on the environment and human health that is

equal to or greater than antibiotics used in terrestrial livestock farming (15).

Wild and companion animals

Studies of wild animals are less common than those of livestock, with less routine monitoring. A comparatively early study of wild baboons (*Papio cynocephalus*) in Kenya (66) found that the group that ranged over an area including human latrines and a refuse pit, from which the baboons scavenged daily for food, had significantly higher levels of resistance to enteric coliform bacilli than did the two groups with much lower human-associated contact. This resistance was also frequently based in transferable plasmids. It was concluded that contaminated human food was the source of the resistance in the baboons. Another study of baboons reported lower frequencies of resistance but otherwise findings were broadly similar (67).

An investigation of wild animals (including birds) in Portugal (24) examined 72 faecal samples for susceptibility to 16 antibiotics. *Escherichia coli* isolates were obtained from 56 (78%) samples, and resistance levels ranged from 0% (for amikacin, cefoxitin and imipenem) through to between 19% and 35% for tetracycline, streptomycin, ampicillin and trimethoprim-sulphamethoxazole (SXT), with a variety of genes being identified. The authors concluded that the intestines of wild animals were reservoirs of antibiotic resistance genes, especially for ampicillin, tetracycline, streptomycin and SXT, and that some animals carried multi-resistant *E. coli* isolates.

In Ontario, Canada, a study included the examination of faecal samples from 302 small wild mammals from four environments: pig farms, residential areas, landfill sites and natural habitats. Patterns and prevalences for resistance genes from generic *E. coli*, isolated from 163 (54%) samples, and from *S. enterica* (four samples, 1%) were examined. Although the highest prevalence of resistance was found in pig farms, *E. coli* isolates from all four environments showed antimicrobial resistance (6).

Houseflies (*Musca domestica* L.) develop generally in decaying organic material, for example manure. They feed by regurgitation, are attracted to human food and are able to fly long distances. Samples from the digestive tracts of 260 houseflies collected from five restaurants in Kansas in the USA were examined for enterococci: 97% were positive with a mean of more than 10^3 CFU per fly, the dominant organism being *E. faecalis*. Testing in this organism for a variety of antibiotic resistance genes was undertaken, and resistance was found to tetracycline (66.3%), erythromycin (23.8%), streptomycin (11.65%), ciprofloxacin (9.9%) and kanamycin (8.3%) (62).

Although the information available is comparatively sparse, it is clearly possible for resistance to a wide range of

antibiotics in a number of different bacteria to be present in, and therefore excreted from, wild animals, and thus contaminate their surroundings in settings from the farm through to the dining table. In addition, there is some information about the possible sources of that resistance, including livestock and farm environments and contaminated human food or waste materials.

As for farmed livestock, the normal bacteria within the digestive tract in companion animals were found to be *E. coli* and enterococci (76). Domestic companion animals have historically been given little attention as sources of zoonotic antibiotic-resistant bacteria, and yet they are routinely prescribed antibiotics in clinical practice, at least in the 'developed' world. However, studies have shown that strains resistant to various antibiotics have emerged in isolates from companion animals, including meticillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, and multidrug-resistant *S. enterica* subsp. *enterica* serovar Typhimurium DT104 (37, 70).

Although there are clear potential transmission routes, through direct contact and through environmental contamination of homes and gardens, more data are needed to assess the actual risk of transmission of antibiotic resistance from companion animals to humans – and vice versa.

Evidence for the potential for environmental risk pathways in the spread of antibiotic resistance

That there is the potential for antibiotic-resistant bacteria to be spread through environmental pathways can be demonstrated by finding resistant organisms in different environmental settings. In recent years, there has been a gradual increase in environmental sampling and testing, sometimes linked to the testing of animals, food or humans in the same region. However, with the wide variety of both bacteria and resistance patterns that may be tested for, inference rather than proof of a particular pathway is often necessary.

An extensive review of antimicrobial resistance found in bacteria on farms, published in 2006 (2), concluded that farm ecosystems provide an environment in which resistant bacteria and genes can emerge, amplify and spread. Soil typically contains around 10^7 bacteria per gram (34), and enterococci have been identified as an important reservoir of antibiotic resistance in the environment (36). The development of molecular methods

has been instrumental in identifying many bacteria, plus genetic resistance elements, in environmental samples and ecosystems, for example sludge and marine waters (45). The exact effects of the various treatments of manure on reduction of bacteria and bacterial resistance have not been thoroughly investigated, but neither composting nor anaerobic digestion and storage has been shown to be particularly efficient (20, 21).

Thus, despite typical manure treatment regimes, antibiotic residues are still frequently present in slurry at the point of disposal (54) and can also move into the wider environment through spills and leakages. In addition, faecal bacteria can survive for weeks or months in the environment, depending upon their species and the temperatures experienced, and genetic elements can persist regardless of cell viability (19). Frequently, slurry is disposed of by being spread onto farmland as a fertiliser, and, if any resistant bacteria or antibiotic residues remain after treatment, these will also be spread onto the soil and may then leach out to contaminate ground or surface waters (12).

A survey of 17 types of environment within Europe, including farm soil; waste water; cattle, chicken and pig manure; fish farm sediment; and some arable crops, was able to detect a number of different types of generic resistance elements, using a polymerase chain reaction-based detection method. Resistance was identified to gentamicin, sulphadiazine and amoxicillin. Such surveys provide insights into the persistence, stability and ubiquity of these elements and can indicate where resistance transfer and recombination are likely to be at the higher levels (10, 42, 44, 74).

Environmental detection of antibiotic resistance is not a localised phenomenon; evidence is reported from regions worldwide. For example, in Guangdong Province, China, environmental isolates of *E. coli* were tested for quinolone resistance. Samples came from pig and chicken farms and included soil, sewage, drinking water and pond water. Of the 34 isolates from pig farms, 16 (47%) were positive, and from the chicken farms two of seven isolates (29%) were positive (86).

One study examined in detail the contribution of antibiotic residues and antibiotic resistance genes found in livestock operations to the residues and resistance genes in groundwater, both 'upstream' and 'downstream' of pig farms, 'upstream' water serving as the control for 'downstream' water. The study also investigated samples from land fertilised by manure from a treatment pit (45). The farms had routinely used tetracycline and tylosin, and resistance genes for these (*tet* and *tlr*, respectively) plus erythromycin (*erm*) were targeted.

Manure treatment and storage pits always contained *tet* genes, and frequently also *erm* genes. Groundwater wells near manure stores also had high frequencies of both, and the different patterns between upstream and downstream wells enabled the gene pool in the control environment to be differentiated from that associated with pig production. Soil sampling also showed the effects of manure application; no *tet* genes were detected prior to application, whereas, after this, detection was possible for up to seven months. Exposure to pig waste was considered the most important factor in determining spatial and temporal patterns of resistance genes, rather than the persistence of antibiotic residues.

Beef and dairy cattle are major reservoirs for enterohaemorrhagic *E. coli* (EHEC), and watersheds in areas with high cattle densities may contain *E. coli* O157:H7 (52). Watercourses not closely associated with the farm environment have also been shown to contain potentially zoonotic bacteria. A study of watersheds in Baltimore, Maryland, testing 1,303 samples from 19 sites, had a mean frequency of 50% positivity for *E. coli* O157. Virulence genes were identified in 26% of samples tested (72). The source of the bacteria was unknown, but, as there are few farm animals or areas of manure application in the region, more likely sources were assumed to be wildlife, companion animals or leaky sewers upstream. Although pathogenicity was indicated, resistance testing was not reported in either of these studies.

In a European environmental study, a large number of resistance genes for gentamicin were identified in sea water collected close to a waste-water outflow (42). In developing countries waterborne diseases are a serious concern, and drinking water may be one route for infection. An investigation of 1,000 drinking water samples from sources including wells, hotels and restaurants found 424 contaminated with *E. coli*, and resistance was found to a range of antibiotics (78). A study undertaken in South Africa investigated resistance patterns found in *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus* and *V. fluvialis*) isolated from waste-water effluents in a 'typical' peri-urban community. Multi-resistance to between eight and ten antibiotics was common, with high (up to 100% in some cases) levels of resistance against erythromycin and up to 95% against cerufoxime and cefalotin; associated genetic elements were also detected (49).

A recent review of the role of 'megacities' as sources for pathogenic bacteria in rivers and their downstream fate concluded that there is a lack of information; in particular, as to how long introduced pathogens can survive and exactly what niches they occupy, although biofilms will facilitate gene transfer, including genes for antibiotic resistance (1). A study in Brazil looked at the antibiotic

resistance profiles of *Pseudomonas aeruginosa* from hospital waste water and compared them with those of water samples from watercourses receiving this discharge. The results showed that the antibiotic resistance levels were higher in hospital waste water, and that the resistance profiles were different in the two sample types, demonstrating genetically different populations. The authors concluded that it was most likely that a source other than the hospital waste water was contributing to the contamination in the 'downstream' watercourses (33).

Although no resistance testing was reported, results of a study looking at colonisation and viability of *S. enteritica* on a variety of potential food preparation work surfaces, including granite, marble and stainless steel, showed that all materials were prone to bacterial colonisation (65). In a retail setting in Karachi, Pakistan, swabs from meat processing equipment and the surrounding environment were found to be contaminated, mainly with *S. aureus* and *Shigella* spp., with resistance rates to a variety of antibiotics of up to 75% (5).

Kitchens can also be contaminated. Isolates from 15 of 200 restaurant kitchens sampled in Ireland were positive for *Salmonella* spp., and a variety of antibiotic resistance patterns were found. Profiles also suggested that isolates from geographically adjacent restaurants were related (14). Hospital kitchens are not immune from contamination either. Following a hospital outbreak of extended-spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae*, up to 35% of hospital kitchen surfaces or food in them were found to be colonised, and 6 of 14 (44%) of the food handlers were faecal carriers (wards and healthcare workers remained clear of infection) (16). In water accumulated in the bottom of restaurant freezers during a power cut in Nigeria, a variety of enteric pathogens were found, with 'alarming' levels of antibiotic resistance. The result was a 'soup' of pathogens facilitating resistance transfer (28). Refrigerators can also harbour resistance. In samples from 137 household fridges in Tennessee in the USA a variety of bacteria were isolated, the most common being *K. pneumoniae* (24.3% of fridges), antibiotic resistance most commonly being to erythromycin (39.9% of samples) and ampicillin (33.8%). None were resistant to ciprofloxacin or gentamicin (53).

The spread of environmental contamination can also be via fomites. A study in India found *S. aureus* contaminating paper currency collected from a wide variety of places, including shops, hospitals and restaurants, and showed that the bacteria could survive for up to eight days on the money. Virulence genes and antibiotic sensitivity to nine antibiotics were both investigated, and those samples with all tested virulence genes had higher levels of antibiotic resistance (57).

Evidence for the potential for food chain risk pathways in the spread of antibiotic resistance

To investigate the proposed link between antibiotic usage in animals and human antibiotic treatment failures, a plethora of studies have looked for bacteria and associated antibiotic resistance in food products derived from animals; a search on 2010/2011 articles alone revealed almost 1,000 potentially relevant publications. There is a much smaller number investigating non-animal-derived food (arable crops, for example), which may have become contaminated in other ways.

Systems for monitoring bacteria and antibiotic resistance in animals and animal-derived food products in Denmark (DANMAP), Norway, Sweden, the Netherlands, Canada and the USA have been referred to earlier (39). For the EU, for example, the Community Summary Report, also referred to earlier, contains a large amount of detailed information on antimicrobial resistance found in broiler meat, pig meat and meat from cattle for *Salmonella* spp., *Campylobacter* spp., *E. coli* and indicator enterococci. Summarising resistance findings to crucial antibiotics for human use, resistance to fluoroquinolone in *C. jejuni* and *C. coli* isolates was found in broiler meat, as was resistance to third-generation cephalosporins in salmonellae from meats in some Member States. There are considerable differences across the EU in the patterns and prevalence of antibiotic resistance in food products, in part associated with the animals from which the product is derived (31).

These systems and reports provide and summarise a large amount of evidence for the presence of antibiotic resistance. They rely on a variety of individual surveys and studies to provide the data and elucidate links in the risk pathway, and examples of different types of product are given.

Enterococci are recognised human pathogens, and studies have isolated enterococci from a number of foods, including milk, cheese and meat (62). A study of six states in the USA (26) examined food from grocery stores, and compared the gentamicin resistance genes found in 360 enterococci isolates with those from the faeces of healthy chickens, turkeys, cattle and pigs. The study demonstrated that, when the genes were highly prevalent in the animals' faeces, there was an equally high prevalence in food samples from the same species, suggesting that the food-producing animals were the source.

Another grocery store study in the USA (51) examined poultry, pig and cattle meat for antibiotic-resistant isolates

of *E. coli*, comparing them with susceptible *E. coli* from the same species, and found that they were similar within a species but different from those from other species, indicating that the resistance in meat was derived from the live animal rather than from cross-contamination. Collignon (23) states that it is obvious from the evidence available that much antibiotic resistance in humans, at least for *E. coli*, is derived from animals via the food chain, despite the reluctance of many people associated with agriculture or the pharmaceutical sector to accept this.

In a study of 88 samples of Italian-produced chicken and pork meat (including raw and fermented), bacterial DNA was examined for genes encoding resistance to tetracycline, erythromycin, vancomycin, aminoglycosidase, meticillin and β -lactams (35). *Tet* and *erm* genes, indicating resistance to tetracycline and erythromycin, were both prevalent in the meat samples. The results were compared with those found for isolates taken from chickens and pigs at the slaughterhouses that fed the four meat-processing plants, and from a comparison with these results the authors concluded that the contamination was likely to be associated with the animals themselves rather than contamination of the processing plant at a later stage.

Dairy products have also been shown to contain antibiotic resistance genes. In a study of European cheeses (48) it was found that 24% of 187 enterococci isolates were resistant to tetracycline, and 4% had multiple resistance to tetracycline, erythromycin, and chloramphenicol. The study also demonstrated that this resistance was transferable to recipient bacteria, although a later study (47) showed that the presence of antibiotic resistance genes did not always mean that they were 'active' and able to confer resistance, and that phenotypic testing is necessary to confirm this. In a completely different environment, Assiut City in Egypt, 30 samples each of raw buffalo, cow, goat and sheep milk were taken from both farmers' houses and dairy shops and examined for *Klebsiella* spp. The prevalence of *K. pneumoniae* was 10% in buffalo milk and 13% in cow milk, and, although most isolates were sensitive to norfloxacin, gentamicin and cefotaxime (weakly), resistance to other antibiotics was also found (55).

A study in Trinidad tested table eggs from both farms and retail outlets: of 131 bacterial isolates, 125 (95.4%) were positive for resistance to one or more antibiotics, in particular streptomycin (90.1%), tetracycline (51.9%) and kanamycin (30.5%). Shells and contents were both tested, and the prevalence of bacteria in the contents was generally higher for eggs that had cracks or blood or faeces on the shells than for those without (4).

Frozen shrimps from markets and department stores in Dhaka City, Bangladesh, were found to contain *Vibrio* spp.,

Salmonella spp. and *Shigella* spp., almost all isolates being resistant to penicillin and bacitracin. The highest frequencies of contamination were found in the department stores (64).

The presence of antibiotic-resistant bacteria and genes in manure and slurry applied to arable land as a fertiliser, and the presence of these organisms in watercourses, led researchers to consider and examine the effects on the crops grown in this environment. In addition, there is the possibility that excreted antibiotics themselves would be present in fertiliser and irrigation water. Crops may be grown for human food, providing a direct risk pathway to humans, or for animal feeds, which is then likely to lead to an increased prevalence of resistant infection in farmed livestock. Investigating this possibility, studies demonstrated the bioaccumulation of the antibiotic chlortetracycline in crops (corn, green onion and cabbage) that had been fertilised with manure, which suggests that this type of effect may enhance antibiotic resistance in humans who eat such vegetables (58).

However, before this, Levy (59) had found that large numbers of bacteria isolated from fruits and vegetables (carrots, celery, cucumber, lettuce, peppers and tomatoes) were of the type associated with faecal origin, and many also demonstrated antibiotic resistance. Combining his findings with data on average portion sizes, he estimated that around 10^9 bacteria would be ingested in a single meal and that many of these would carry transmissible plasmids. Such findings formed the basis of one explanation as to why those on a vegetarian diet might be colonised by resistant bacteria (38).

Many studies that include non-animal-derived products also include meat items. A Turkish study examined 100 samples of ready-to-eat food, purchased from retail sources, for the presence of *Listeria* spp. Twenty samples were taken from each of five different food types: green salad, mayonnaise-based salad, fried meatballs, fried liver and rice-stuffed mussels. Six (30%) of the green salad samples were positive, as were four (20%), one (5%), one (5%), and one (5%) of the other food types respectively. Thus, the two salad dishes (although one type could have contained raw egg in the mayonnaise) had notably higher *Listeria* frequencies; however, the other dishes were all cooked dishes. All *Listeria* isolations were resistant to rifampicin but susceptible to a variety of other antibiotics (73). Ready-to-eat foods from street vendors and shops have also been studied for antibiotic-resistant *S. aureus* in Nigeria, the foods including moin-moin (a steamed black bean pudding containing onions and peppers) and various meat and fish dishes. Of the isolates found, 13.4% were from the moin-moin, and all isolates were resistant to two or more antibiotics, most commonly to streptomycin and tetracycline (3).

Potential links and sources for antibiotic resistance found in human cases

Antibiotic resistance in humans is now very well documented and routinely the subject of investigation; a search for relevant publications in 2010/2011 produced close to 9,000 results. The potential link between antibiotic resistance in animals and humans has long been recognised. In 1986, Linton (60) described work in the UK in which identified strains of antibiotic-resistant *E. coli* were traced from the gut contents of pigs, calves and chickens to carcasses at slaughter, and from there shown to colonise the gut of a human volunteer handling and eating the meat, the identity of the strains being confirmed by molecular studies. The same author describes the isolation of antibiotic-resistant strains of *Salmonella* Typhimurium phage type DT204c from both humans and calves, indicating a possible zoonotic spread, but there was uncertainty on any precise route of spread. It has been suggested too that both skin contact and oral ingestion can transmit antibiotic-resistant bacteria to humans.

A recent review, produced jointly by a number of European expert groups, concluded that the majority of resistant *Salmonella* and *Campylobacter* isolates that have been identified as causing infections in people originate with healthy carrier animals (especially poultry, pigs and cattle) and are spread as faecal contaminants via the food chain; however, further work is needed to identify the source(s) of infections caused by antimicrobial-resistant *E. coli* strains, which are becoming more common (30).

Based on work comparing susceptible and resistant *E. coli* in meat products, the argument for a similar food chain route to humans for most resistant *E. coli*, at least in countries where prudent use of antibiotics in farmed livestock is not practised and where human faecal material is generally prevented from entering water supplies, is presented. In essence, findings show that resistant isolates found on poultry products were very similar in properties, other than this resistance, to susceptible isolates found on poultry products, but different from both resistant and susceptible isolates from pork and beef products. Thus, it is highly likely that resistant strains found in, for example, poultry meat actually originated with the poultry (and are presumed to be due to the use of antibiotics) rather than from humans or other cross-contamination. In addition, many of the isolates possessed virulence factors associated with causing disease in people, and many of the susceptible strains carried by humans have very different characteristics from the resistant strains they carry (23, 51).

In China, comparison of quinolone resistance in *E. coli* isolates from five farms, from pigs and chickens, environmental samples and farm workers, showed similar patterns, with higher levels on pig farms for all groups and no resistance found in the chicken farm workers. Overall, the total isolates and numbers positive were: for animals 98 and 43 (44%); for the environment 41 and 18 (44%); and for farm workers 33 and 11 (33%) (86). These farm workers had had no previous antibiotic treatment or hospital admissions, and the authors suggest that their resistance is due to dissemination from the livestock on the farms.

Enterococci are recognised as important nosocomial human pathogens, and two species of enterococci are responsible for most human infections, *E. faecalis* and *E. faecium* (62). In the USA, data on gentamicin resistance in enterococci suggested that there were similarities in resistance in enterococci isolated from farm animals, retail food and humans, from geographically diverse areas, which provides evidence of spread of gentamicin-resistant enterococci from animals to humans through the food chain (26).

It is more difficult to confirm exposure to zoonotic organisms via water sources as they can be easily transported away in flowing water, or diluted below detectable levels (72), leading to under-reporting, and this will apply equally to bacteria and genetic elements carrying antibiotic resistance. Nevertheless, it is sometimes possible to confirm the source; one large waterborne outbreak of EHEC was documented in Ontario, Canada, in 2000 (46).

Resistance to antibiotics has been found in a wide variety of bacterial samples obtained from humans, both in the UK and in other regions of the world. Many of the bacteria examined are not commonly or specifically associated with animal sources or generally considered zoonotic, and in fact some are specifically associated with human-to-human contact, for example, sexually transmitted pathogens and hospital-acquired infections (41). However, there exists the potential for such bacteria to acquire and exchange genetic resistance elements with those from animal, food and environmental sources wherever mixing occurs, including within human communities and hospitals; thus, the identification of resistance *per se* does not confirm or refute the initial source.

A review of resistance profiles of bacterial pathogens that differ in host specificity (80) found that pathogens that are found in both human and animal hosts appear to have higher incidences of resistance, but the most severe resistance problems were found to occur in pathogens exclusively infecting humans. The authors concluded that ascribing many of the problems of antibiotic resistance in humans to veterinary use of antibiotics may oversimplify a very complex issue.

When dealing with bacteria which are associated with animal sources, the potential for food chain contamination as a source of antibiotic resistance spread is frequently considered the most likely route, and animal-derived food products are therefore often now routinely tested for resistance and reported (see, for example, reference 31), and this may lead to bias when attempting to attribute sources and routes of spread.

Evidence for the potential for the transfer of antibiotic resistance from humans to animals

As indicated previously, there are also potential risk pathways for the transfer of antibiotic resistance, as either bacteria or genetic material, from humans to other species. Many of these potential pathways are essentially a reversal of those from animals to humans, including direct contact and environmental routes. Environmental routes that have been suggested, or implied, often include sewage or effluents from hospitals or other large institutions that themselves may drain into the sewerage system. From there, the flow may be into watercourses or seas. Animals (including marine animals) could come into contact at a variety of points, and this possibility is implicit in some of the studies already described (42, 49, 66, 67).

One study of zoonotic organisms in marine vertebrates from the coastal waters of the north-west Atlantic tested 797 isolates for antibiotic resistance; 468 (59%) showed at least some level of resistance. The specific findings and patterns led the authors to conclude that, although the marine mammals and birds may provide a reservoir for potentially zoonotic pathogens, resistance may have been acquired from coastal waters contaminated by medical waste and sewage, as well as from agricultural waste (13). In another study, the statistical analysis of antibiotic profiling was used to assist in tracing the origin of faecal contamination in water sources, comparing isolates from the watercourses with isolates of known domestic and wild animal origin. By statistically grouping the watercourse isolates into subsets, the profiles obtained were able to correctly classify 60% of the *E. coli* isolates derived from a human origin and 95% of those of non-human derivation. This indicates that different sources were present, and provides a potential method for identifying the original source of contamination (63).

However, not many confirmed or probable incidents of human-to-animal transfer are described or available. One

case in 1999, in which such transmission was believed to be the likely route, involved 11 equine patients at a veterinary hospital, all of which developed post-procedural MRSA infections. One of the staff members involved in their care was also found to be colonised with MRSA, and the epidemiological and strain-typing features led the authors to speculate that the hospital staff were the source of the infection (71). In 2004, a probable case of human-to-dog transmission of MRSA was described. Strain typing indicated that MRSA cultured from the nose of a healthy dog was identical to that colonising the owner, who worked in a nursing home (79). A second report of transfer of MRSA from a human owner to his dog in North America was published in 2007, and in this case both sustained infections that did not respond well to treatment, and the dog was eventually euthanised (68). And, in Norway, a suspected case of MRSA transfer from farm workers to pigs has been described (77). MRSA appears to be the most commonly documented of the resistant organisms that are transmitted from humans to animals, and, in a recent paper looking at the relationship between MRSA in humans (in whom it is common) (22), and in other species (in which it is considered less common), Catry and colleagues describe the 'human contact hazard' for livestock, horses and companion animals and potential safeguards (18). But other organisms can travel in this direction too: for example, the authors of a study on the emergence of human pandemic O25:H4-ST131 CTX-M-15 ESBL-producing *E. coli* among companion animals in Europe concluded that transmission of this organism from humans to animals is also possible (32).

Conclusions

The identification and development of antibiotic resistance, with its attendant implications for human and animal health, has resulted in much activity to elucidate causes of spread and circulation patterns of resistance.

The evidence for potential environmental risk pathways indicates that it is probable that animal-derived bacteria, including resistant bacteria, resistant genetic elements and the means of creating further resistant populations, can all be present in manure, slurry, soil and the general farm environment. However, there is still a lack of information on the totality of antibiotic-resistant bacteria and genetic elements, and their interactions, in the environment affected by agricultural activities (25). There is also evidence developing from diverse regions of the world that pathogenic and resistant bacteria can be found in watercourses, including drinking water, and environments associated with food processing and preparation, and on fomites. Sometimes these bacteria can be linked to potential sources, either explicitly (see, for example, references 45 and 52) or implicitly (for example,

refrigerators and freezers are likely to have contained food), but sometimes links in the chain are unclear (72), and in certain cases the direction of spread is also equivocal (16). Overall, however, the potential for, and probability of, a wide variety of complex environmental risk pathways for the spread of antibiotic resistance seems clear.

The evidence for potential food chain risk pathways from animals to humans, especially via animal-derived products, is considerable. Antibiotic resistance has also been found in arable crops, fruit and vegetables, and prepared food products derived from non-animal sources, indicative of contamination, either in the field, most likely from application of farm wastes (59), or during preparation and processing, where the source of the contamination is often less clear. Further work in this area is needed to clarify dominant pathways. Transfer of resistant genetic material to recipient bacteria has also been demonstrated, further adding to the complexities in elucidation of specific risk pathways. However, food products derived from animals, including meat, dairy products, eggs and aquatic products, may all harbour antibiotic-resistant bacteria, and, in many parts of the world, recently developed monitoring systems are able to demonstrate a probable association with the live animal source from which the product was derived (see, for example, references 31 and 39), making evidence for this route of transfer particularly compelling.

There is also some evidence of both indirect and direct spread of antibiotic resistance from humans to animals (see for example, references 13, 42, 49 and 63). Evidence for direct spread is available primarily for those who care directly for animals, with a high likelihood of close and frequent contact. Such reports also largely involve MRSA (see, for example, references 68, 71 and 79), a pathogen of great importance in human medicine (22), and this probably reflects an investigative bias for this organism. It would be prudent to assume this potential for other antibiotic-resistant organisms too.

However, several studies presented here (see, for example, references 16, 30, 72, 80) indicate that more information is required to fully elucidate the complex patterns of circulation and ascribe priorities to risk pathways. A recently published report on antibiotic resistance from the American Academy of Microbiology concluded that the reasons controlling the development and spread of antibiotic resistance are complex, multi-factorial, and largely unknown. Although veterinary and medical practice and use of antibiotics is a part of the story, it does not explain the whole issue, and other factors include industrial practice, economics, politics and the complexities of biology itself (7).



Indices de la circulation de souches bactériennes résistantes aux agents antimicrobiens et de gènes de la résistance dans la nature, et plus particulièrement entre les animaux et l'homme

M. Wooldridge

Résumé

L'inquiétude suscitée par la résistance des bactéries aux antimicrobiens et par la difficulté qui en résulte de traiter certaines infections chez l'homme a conduit à multiplier depuis quelques années les études consacrées à la résistance chez les animaux d'élevage, dans les denrées alimentaires, dans l'environnement et chez l'être humain, ainsi qu'aux mécanismes de transfert entre bactéries des traits génétiques codant pour la résistance et aux configurations ou voies de risque favorisant la propagation de la résistance. Le risque de transfert entre bactéries d'éléments génétiques codant pour la résistance dans des populations mixtes peut ouvrir des voies de propagation diverses, nombreuses et complexes. À l'heure actuelle, de nombreux éléments indiquent que le transfert direct à l'homme des traits de la résistance, via la chaîne alimentaire et les denrées d'origine animale constitue l'une des voies de propagation de la résistance. L'utilisation des déjections animales comme fertilisants en agriculture et leur subséquente infiltration dans les cours d'eau représentent également un risque potentiel, quoique moins connu, de propagation de la résistance. Néanmoins, les routes spécifiques empruntées par les bactéries résistantes pour atteindre les sources d'eau, les produits alimentaires transformés ou divers sites environnementaux sont souvent mal connues et impliquent certainement une intervention humaine. En particulier, il est difficile de contrôler les sources d'eau en raison des problèmes de dilution et de débit. En outre, la viande étant comparativement très facile à contrôler, en plus d'être souvent suspectée d'être à l'origine des contaminations, on constate une tendance à privilégier fortement l'étude de cette source de propagation. En raison de la complexité de ces différents aspects et malgré les éléments de preuve actuellement disponibles, il s'avère impossible de classer par ordre d'importance les voies de risque et de circulation potentielles de la résistance.

Mots-clés

Antibiorésistance – Contamination de l'environnement – Contamination des denrées alimentaires – Infection zoonotique – Voie de risque.



Pruebas de la circulación de cepas y genes resistentes a los agentes antimicrobianos en la naturaleza, y especialmente entre humanos y animales

M. Wooldridge

Resumen

La preocupación generada por bacterias antibiorresistentes que provocan infecciones humanas de difícil tratamiento ha dado lugar en los últimos años a una proliferación de estudios e investigaciones sobre las resistencias presentes en el ganado, los productos alimentarios, el medio ambiente y las personas,

sobre los mecanismos de transferencia entre bacterias de los elementos genéticos que confieren resistencia y sobre las vías, o procesos de materialización del riesgo, por las que se puede extender una resistencia. La posibilidad de transferencia de elementos genéticos de resistencia entre bacterias de poblaciones mixtas añade otras muchas y complejas vías de propagación posibles. Una considerable cantidad de datos demuestra ahora que una posible vía de propagación es la transferencia directa a través de la cadena alimentaria desde los animales destinados al consumo humano hasta las personas. También se ha observado, aunque los datos al respecto son menos concluyentes, que la aplicación de desechos animales a las tierras de cultivo y su subsiguiente filtración a las aguas subterráneas es una posible vía de propagación. Pero a menudo, en el caso de fuentes de agua, alimentos transformados u otras zonas del medio natural, las rutas específicas de circulación no están claras, y es muy posible que se trate de contaminación de origen humano. El análisis de cursos de agua, en particular, puede resultar dificultoso debido a la dilución y a la corriente natural. Además, dado que la carne es relativamente fácil de analizar y suele formar parte de los sospechosos habituales, existe cierta proclividad a estudiar esta vía de propagación. Todos estos factores vienen a complicar el tema y llevan a concluir que, con los datos actualmente disponibles, no es posible jerarquizar la importancia de las distintas vías de circulación y materialización del riesgo.

Palabras clave

Contaminación ambiental – Contaminación de los alimentos – Infección zoonótica – Proceso de materialización del riesgo – Resistencia a los antibióticos.



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