

# ANTIBIOTIC RESISTANCE VIA THE FOOD CHAIN: FACT OR FICTION?

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## ABSTRACT

The mechanisms that bacteria use to acquire additional genetic material, including genes coding for antibiotic resistance, are principally the secondary pathways that have been described as transformation and conjugation pathways. The farming industry often is reported as a hotspot for antibiotic-resistance reservoirs. In this review, we consider the exposure of food animals during the course of their lifespans to preventative, therapeutic or prophylactic treatment with antibiotic agents. In this context, zoonotic bacteria are commonly recognised as a potential threat to human health, with therapeutic treatment of pathogenic organisms on farms increasing the likelihood of selective antibiotic pressure influencing the commensal flora of the intestines. Existing literature indicates, however, that the effective impact on human health of such interventions in the food production process is still subject to debate.

## INTRODUCTION

Medini et al.<sup>1</sup> refer to the total genetic makeup of an individual bacterium species as the pan genome. They further differentiate the pan genome into the ancestral or core genome and the dispensable genome, which comprises additional or acquired genetic material and can differ between strains within a species. In situations in which bacteria carrying acquired genetic material are hosted by food animals, the question arises as to whether exposure of these food animals to therapeutic and/or prophylactic antibiotic treatments creates a potential threat to human health.<sup>2,3</sup> We review whether a specific gene encoding a particular antibiotic-resistant gene element, originally present in a bacterium derived from a food animal source, can be the same gene encoding antibiotic resistance subsequently residing in a human clinical isolate of a totally different bacterial strain, species or genus.

## TRANSFER OF ANTIBIOTIC RESISTANCE

Acquired antibiotic resistance is attributed to both direct and indirect pathways. Direct or primary pathways are mutations in the gene encoding resistance against the mechanisms of particular antibiotics. The most commonly known example is resistance of *Mycobacterium tuberculosis* to streptomycin that is associated with an adaptation of the ribosomal sites.<sup>4,5,6</sup> Indirect or secondary pathways are the gaining of small fragments of DNA coding for resistance. These indirect pathways for resistance acquisition can be further sorted into three categories: transformation, conjugation and transduction.<sup>4,7</sup>

### Transformation

Theoretically, even a dying bacterium cell may release its plasmids, or short fragments of its DNA into the environment, which allows for the possibility for a healthy bacterium cell, called a recipient, to acquire this material (and use it for its own benefit) directly through the cell wall. This kind of transfer via the cell wall is known as the transformation pathway. However, our present knowledge of this form of genetic transportation indicates that it has limitations; for bacteria, such an action can only be executed if the bacterium has the requisite genetic capacity to absorb 'loose' compatible DNA – usually in a plasmid form – and, to present knowledge, can only occur in a limited number of bacteria. However, some scientists believe that this pathway usage by bacteria is underestimated.<sup>4,7,8,9</sup>

### Conjugation

Conjugation allows transmissible plasmids and chromosomal DNA of very large sizes to be transferred from cell to cell, either within or between species, mediating the transmitted genetic material through variously specified enzyme activities. The conjugational pathway does not select DNA material simply from the environment, as in the case of transformation, but instead has a direct cell-to-cell exchange of genetic material, where one cell is the donor of the genetic material and the other the new host or recipient. The conjugation pathway uses a very specific, hair-like attachment on the surface of the bacterial cell, constructed mainly of oligomeric pilin protein, that acts as a bridge pulling the two cells together.<sup>4</sup> Enzymes further initiate the transportation of a single DNA strand to the new host, where both cells synthesise duplicates. Both the cells can now act as donor cells. Additional role players that use such pathways to assist the bacterial strain to obtain advantageous characteristics (such as antimicrobial resistance and pathogenicity) are plasmids, pathogenicity islands, transposons, integrons<sup>7,10</sup> and insertion sequences.<sup>11</sup> Conjugative plasmids that have the ability to replicate independently in the newly acquired (recipient) host have greater opportunity to spread through a bacterial flora community than those without conjugative abilities.<sup>9</sup> Gentamycin-resistant *Staphylococcus aureus*, for example, reflects the presence of aminoglycoside acetyltransferases and aminoglycoside phosphotransferases – enzymes responsible for drug inactivation – and is encoded on a gene [*aac(6')-Ie+aph(2'')*] located on the transposon Tn4001. This transposon is common in *S. aureus* isolates and is also found on conjugational plasmids. The *aac* gene is not specific to Gram-positive bacteria as it has been found to cause multi-drug resistance in *Enterobacteriaceae* isolates.<sup>12</sup> The tetracycline-resistant *tet(Q)* gene is often found simultaneously with the erythromycin-resistant *erm(F)* gene on a conjugative transposon, resulting in multi-drug resistance.<sup>13</sup> But it is unclear whether the transfer of genetic elements through conjugation occurs voluntarily or equally between both cells (that is, whether both cells actively seek to exploit the possibility of transference) or whether a scenario of cell 'hijacking' takes place for the sake of survival of a more aggressive genetic element.<sup>14</sup>

## Transduction

Transduction is the virus-like injection of genetic material into a host cell after attachment. To the best of current knowledge, transduction is a feature mainly of bacteriophages commonly acting as bacterial viruses,<sup>9</sup> and is not directly relevant to the present discussion.

## ANIMAL RESERVOIRS OF ANTIBIOTIC RESISTANCE

The farming industry is often alluded to as a hotspot for reservoirs of antibiotic resistance. During their lifespan, production animals are often subjected to preventative, therapeutic or prophylactic treatment with antimicrobial agents. Although subtherapeutic use of antibiotics in animal production is commonly considered to be a major contributor to the proliferation of antibiotic resistance amongst environmental bacteria, Ghosh and LaPara<sup>6</sup> concluded that the clearance of the animal pens of manure, followed by its subsequent disposal, had a greater impact on the lateral transfer (also known as horizontal transfer) of *tet* genes coding for antibiotic resistance amongst soil bacteria. In their study, manure that was allowed to accumulate on soil produced greater antibiotic resistance in natural soil bacteria. In addition, bacterial isolates from soil samples collected closer to the animal pens produced higher antibiotic resistance than those from a further distance. Generally, animals also have a high level of exposure to their environment, especially the soil environment, making it easier for them to be infected with bacteria that carry problematic genetic elements. D'Costa et al.<sup>15</sup> undertook a study on the antibiotic-resistance profiles of strains of soil bacteria that were chiefly from the familiar antibiotic-producing *Streptomyces* genus. They highlighted the high antibiotic resistance and multi-drug resistance found in the natural soil environment and concluded that it was possible that these soil sources could act as a reservoir for resistant elements.<sup>15</sup> Natural antibiotic-producing organisms mutate for the sake of survival in competition with other organisms sharing the same ecological environment. It was shown that erythromycin-resistance in *S. aureus*, caused by a plasmid, and corresponding resistance in soil bacteria, *Streptomyces erythraeus*, have a similar genetic mutation of the 23SrRNA. Methylation of this region is a natural process for the latter erythromycin-producing organism, suggesting that it could have originated from this source.<sup>16</sup>

As with humans, animals also are susceptible to diseases that necessitate therapeutic treatment. However, treatments of pathogenic organisms in a farming context heighten selective antibiotic pressure that influences the overall commensal flora of the intestines. While treating for mastitis caused by *S. aureus* in cattle (in Meylan, France), researchers located an R-plasmid (resistant plasmid), pTMS1 in *Escherichia coli*, hosted in the cattle and in the local veterinarian and some of the other animal handlers.<sup>17</sup> These findings were very similar to those reported in a groundbreaking study by Levy et al. in 1976,<sup>18</sup> in which the intestinal bacterial flora of farm personnel were studied after introduction of an antibiotic supplement to the animals' feed. The antibiotic was a low-dose, broad-spectrum tetracycline; within six months of its introduction, 31.3% of weekly faecal samples taken from farming personnel contained more than 80% tetracycline-resistant bacteria, compared with 6.8% of samples taken from neighbouring farm personnel who were not exposed to the farm animals.<sup>18</sup> Since then, it has been shown that tetracycline resistance may be caused by efflux proteins, 'ribosomal protection genes', or antibiotic inactivation enzymes, expressed by any one of a wide range of *tet* genes, which have been sequenced for both Gram-positive and Gram-negative bacteria.<sup>12,13</sup>

## ZOONOSES

Bacteria, normally associated benignly with animals, can also have natural qualities that allow them to replicate in humans with deleterious disease consequences. These bacteria have a greater chance of being vectors of antibiotic-resistant genes.

South African veterinarians working in constant close proximity with farm animals have a three-fold greater likelihood of being infected with a zoonotic disease than their counterparts in the small-animal or other research fields.<sup>19</sup> This figure is highly significant when we consider how many people in South Africa, and in Africa generally, live in secluded or rural areas in close proximity to farm animals, with possible exposure to zoonotic bacteria. Zoonotic bacteria with pathogenic capabilities are generally a threat to the human health system, and more so if they become resistant to the antibiotics normally dictated for treatment. Geonaras et al.<sup>20</sup> undertook a susceptibility study of *E. coli* strains collected in poultry, measured against five antimicrobial agents used in the South African poultry industry. All strains showed susceptibility to danofloxacin and colistin, while 96% were resistant to two tetracyclines.<sup>20</sup> Moreover, in figures for antibiotic susceptibility of potentially human pathogenic bacteria isolates clinically, as well as from healthy farm animals, published in the first report of the South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs (SANVAD), both *E. coli* and *Enterococcus* species showed increased antibiotic resistance, but equivalent to that of comparative profiles from European counterparts. The highest resistance was reported for tetracycline and sulphonamides and the lowest resistance for ceftiofur (a third-generation cephalosporin). Highlighted in the report was evidence of *E. coli* isolates obtained from abattoir chickens, which showed higher resistance for tetracyclines, fluoroquinolones and sulphonamides, in particular, than isolates obtained from clinically ill chickens.<sup>21</sup>

In addition to zoonotic bacteria acquiring antibiotic resistance, pathogenicity can also be increased by the addition of virulent genetic elements. For example, in Ireland, a potential epidemic clone of *Salmonella enterica* serotype Typhimurium DT104 was detected in human gastroenteritis isolates, veterinary isolates, and food samples, with 78% of the isolates showing simultaneous resistance against ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline.<sup>22</sup> Similar resistances were found in the United States, France, Sweden, Russia and Canada for *Salmonella* serotypes as well as for *E. coli* and *Campylobacter* species.<sup>23</sup> The step-wise partial or complete banning in various countries of antibiotics used in food animal production was primarily in response to results reported on clinical zoonotic bacteria. However, the reported bacterial resistance profiles varied from country to country. Aquino et al.<sup>24</sup> relate the inconsistencies between antibiotic resistance profiles to the way therapeutic and prophylactic treatment of humans and animals was exercised in different demographical and geographical areas. The clinical resistances found were correlated with each country's regulations on the use of specific drugs for certain groups of food animals. Australia, for example, did not allow fluoroquinolones to be used in commercial food production and only cooked meat products were allowed to be imported, a fact which might explain why clinical fluoroquinolone-resistant *Campylobacter*s were rarely detected in Australia, in comparison to what was experienced by other countries at the time.<sup>25</sup> The European Union consequently banned the use of all antibiotics as growth promoters as from 01 January 2006. In the United Kingdom, antibiotics used to improve growth efficiency have been restricted as prescription-only medicines.<sup>26</sup>

## FOOD ANIMALS AS A SOURCE OF ANTIBIOTIC RESISTANCE

It is difficult to prove that a specific gene (e.g. coding for antibiotic resistance) has been transferred from a food animal source and is now residing in a pathogenic human clinical isolate in a different bacterial strain, species or genus. However, two general opinions exist as to whether food animals can act as a source of a human pathogenic antibiotic-resistant bacteria.

The first opinion is that food animals exposed to additives such as the antibiotics used for growth promotion may serve as a reservoir of resistant bacteria and/or resistance genes that may

spread to the human population, thereby limiting the medical value of antimicrobial drugs.<sup>3</sup>

The initial efficacy of antibiotics in food animal production and, specifically, as growth promoters, was achieved through (1) stabilising the gut microflora by suppressing subclinical pathogenic populations, (2) enhancing protein metabolism by improving digestibility and nitrogen upkeep, (3) enhancing vitamin production by gastrointestinal microorganisms, and (4) increasing intestinal absorption of nutrients by the host.<sup>13,27</sup> The practice of mass treatment of animals such as poultry by supplementing their food or water supply is of particular concern when considering the development of antibiotic resistance because, as the animals compete for food sources, the doses received differ between individuals. The consequence of some individuals receiving higher doses than others introduces another differential in the selective pressure on commensal bacteria<sup>28</sup> and opens the possibility for the transfer of genes encoding for antibiotic resistance and multi-drug resistance. For example, *E. coli* strains from commensal environments act as reservoirs of genes, such as class 1 integrons, with both antibiotic-resistant and transferable qualities; these genes can be horizontally transferred throughout a bacterial population and carry several antimicrobial resistance cassettes.<sup>10</sup>

The existence of transformational and conjugational pathways was demonstrated when *qnr* genes, coding for quinolone resistance, were transferred in the laboratory from human clinical non-Typhi *S. enterica* serotypes to an *E. coli* J53 strain. These variants of the *qnr* were found to be widely distributed across different states of the United States and found in a variety of animal hosts.<sup>29</sup> Kruse et al.<sup>30</sup> demonstrated the transfer of R-plasmids by conjugation after preparing an *Aeromonas salmonicida* subsp. *salmonicida* NVH4133 (recipient)-infected fish (salmon) on a plastic cutting board infected with *E. coli* DH5 cells (donor). They were also able to demonstrate the transfer between pathogenic bacteria in various other environments, including a hand towel and pig faeces.<sup>30</sup>

Meat and meat products seem to be particular loci of antibiotic-resistant bacteria. Contamination of meat can occur during carcass processing if the rumen is accidentally cut. In the case of poultry, the intestine may rupture when the carcass is gutted during processing, releasing its contents into the thoracic and abdominal cavities.<sup>31</sup> The gut contents of chickens, especially of the caeca, are considered to be peak environments for bacterial colonisation, providing a ready platform for the transfer of genetic entities<sup>7,8</sup> with subsequent spreading to other meat products that emanate from commercial abattoirs. The Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) reported laboratory studies of the exchange of *vanA* genes in the intestines of mice and humans that code for vancomycin resistance. Special concern was raised for meat contaminated with enterococci transferring their *vanA* genes to human pathogenic *E. faecium* isolates.<sup>32</sup> Retail 'ready-to-eat' foods could present particular risk because they are consumed as purchased, rather than undergoing subsequent cooking.

Products containing poultry, beef and pork in Minneapolis, United States, were found to be contaminated with resistant *E. coli* and extraintestinal pathogenic *E. coli* carrying additional virulent factors. These findings corresponded closely with the prevalence of contamination found for animal carcasses exposed to immediate contamination from the intestines, as well as for processed meat further along the production line.<sup>33</sup>

This form of meat contamination was also found in Addis Ababa, Ethiopia, where 8% of the beef samples collected were contaminated with *E. coli* O157:H7, but less than 2.5% of sheep and goat meat samples were contaminated. This lower contamination rate was attributed to the lower faecal prevalence of *E. coli* O157:H7 in sheep and goats. The meat contamination

rates thus corresponded closely with the prevalence rates in the respective hosts. The samples collected from butcheries also yielded higher contamination rates than those from abattoirs, including an export abattoir.<sup>34</sup> *E. coli* O157:H7 is a well-recognised pathogenic invasive strain of *E. coli*; Mizan et al.<sup>35</sup> have demonstrated the strain's susceptibility to the acquisition of resistant plasmids in laboratory conditions using bovine rumen fluid.

Following ingestion, either the complete bacterium starts to colonise the human digestive system or the genetic resistant elements are transferred to commensal or pathogenic *E. coli* strains in the human intestines, causing food-borne disease. The antibiotic-free labelling of production animal food sources apparently fails to take into account potential subsequent contamination during the processing of the food products.<sup>33,36</sup> Generally, bacteria with or without resistant genes present in the meat are killed in the course of cooking the meat. But the potential for infection still persists in contamination of the preparation environment, if, for example, hands are not washed properly or the same utensils are used to prepare both meat and salad dishes. In Malaysia, Chai et al.<sup>37</sup> frequently isolated *Campylobacter jejuni* and *Campylobacter coli* from raw vegetables used for popular salad dishes, probably attributable to animal-contaminated irrigation water.<sup>37</sup> The latter instance signals how easily bacteria normally associated with animals can be sustained in non-animal environments.

The second general opinion is that concerns of food animals being the major source of antibiotic-resistant genes are unfounded. Phillips et al.<sup>38</sup> believe that the likelihood that bacteria from food origin have the ability to colonise the human gut and transfer resistance genes is low, and that, if the possibility did exist, the clinical consequences would be insignificant.<sup>38</sup>

Food animal production usually incorporates some form of antibiotic usage, either therapeutic or prophylactic. But horizontal transfer of genes coding for antibiotic resistance happens with ease, even without selective pressure imposed by the presence of antibiotics.<sup>6</sup> According to Kruse et al.<sup>30</sup>, the transfer of plasmids in faeces indicates that conjugational transfer of R-plasmids takes place in the digestive tract, irrespective of antibiotic usage. It appears that transfer can occur between bacteria of diverse origin – humans, food animals, fish and even sea water.<sup>30</sup> According to reviews by Kelly et al.,<sup>7,8</sup> the transfer of genetic entities, including those carrying antibiotic-resistance qualities, becomes all the more probable in environments with high bacterial loads.

In rural areas in developing countries, there is a high risk of exposure to food animals, and thus exposure to zoonotic bacteria,<sup>19</sup> even though the use of antibiotic growth promoters may not be a normal farming practice. The most common poultry production systems in these localities consist of indigenous-species animals in small numbers (i.e. less than 50) that rely on scavenging as a feed source.<sup>39</sup> Dhlamini<sup>40</sup> reported that 87% of family poultry systems studied in KwaZulu-Natal used traditional remedies originating predominantly from plant material, although commercial products, in particular Terramycin and, to a lesser degree, potassium permanganate, also were used for treating the animals. Thus the resistance found in clinical samples from developing countries may have different origins to that found in developed countries. The 70th Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)<sup>41</sup> presents evaluations of certain veterinary drugs for subsequent drug residues in food products destined for human consumption. The report states that within a few hours of oral administration, more than 30% of ingested tylosin (a macrolide antibiotic) was bound to the faeces inside the intestinal tract of the animal, reducing the antimicrobial activity of the antibiotic. The antibiotic itself undergoes chemical breakdown as it passes through the digestive system, further reducing its antimicrobial activity.<sup>41</sup> So although tylosin is in the same antibiotic class as

erythromycin, which is used in human treatment, it does not remain active in the digestive system to initiate bacterial stress.

### THE HUMAN CONTRIBUTION

In recent decades, veterinary and agricultural practices have come under frequent censure for their role in establishing and spreading antibiotic-resistant bacteria. At the same time, as noted in the Danish DANMAP report<sup>32</sup>, there has also been increased clinical use of antimicrobials in human health care, probably because of an improved understanding of the pharmaceuticals of antibiotic drugs, which has often resulted in higher dosages, increased hospitalisations, but shorter hospital stays.<sup>32</sup> In addition to this, the quantity of antimicrobials being used in human health systems is further augmented by drugs prescribed in the treatment of syndromes (e.g. AIDS), where the antimicrobials are not necessarily pathogen-specific.<sup>42</sup> European human epidemiological studies have shown a wide presence of integrons, including integron 1, with additional gene cassettes coding for various forms of resistance.<sup>12</sup> The origin of the host, namely humans, is suspected to contribute to the way gene transfer occurs. For both humans and other animals, the bacterial flora in the gut is significantly determined from birth by the host's diet and environment (prior to birth, the digestive tract is bacteria-free). Not only is the eventual composition of the gut flora in adults dictated by this early colonisation of the gut, but it is also uniquely shaped for each individual by the present and future welfare, and environmental hygiene to which a newborn is exposed.<sup>43,44,45,46</sup> Adlerberth et al.<sup>45</sup> reported that breastfeeding and environmental exposure resulted in increases in pathogenic Gram-negative bacteria in the gut flora of newborns in Sweden (a developed country) and Pakistan (a developing country).<sup>47</sup> This initial establishment of the eventual composition of the gut flora dictates the host's future immune response and prevents colonisation of foreign bacteria.<sup>43</sup> Mechanisms, not yet well defined, seem to assist in the establishment of the microbiota and it is speculated that there is a very specific relationship relating to the host-microbe interaction.<sup>48</sup> There is a clear likelihood that this relationship, founded in infancy and specific to each individual, could also be disturbed by the early administration of antibiotics and even probiotics. It has been suggested that clinical treatment should be based on the unique microbiota of each individual<sup>48</sup> – a possibility with future technology development.

### CONCLUSION

A large amount of information on the transfer of antibiotic-resistance genes has been derived from developed countries, including the prevalence of antibiotic resistance in food animals, the possible correlation to human health, subsequent antibiotic management strategies and ongoing surveillance programmes that have often led to new legislation and regulations. Molecular tools have highlighted genes linked to known, unknown and undefined bacteria.<sup>27</sup> The influence of these unknown organisms on the transfer of antibiotic resistance is speculative. Early-infancy colonisation of the gut determines the future bacteriological welfare of an individual. Taking into account the divergences in healthcare systems (including the prevalence of acute and chronic diseases with accompanied short-term and long-term antibiotic therapy), economic welfare and cultural habits, the average gut bacteria composition for African people is likely to be distinct from their Western counterparts. In all probability, bacterial evolution is a continual process determined by a survival strategy. Our focus on the continually changing micro-environment of bacteria has undoubtedly become sharper as we view it through 'the lens of antibiotic resistance'.

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