The transfer of antibiotic resistance from food to humans: facts, implications and future directions

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Summary

The food chain, from production to the consumer's kitchen, can be an important contributor to the development, persistence and dissemination of antibiotic-resistant (ART) microbes, including both ART foodborne pathogens and commensal bacteria. Many factors in the food chain, such as the antimicrobial compounds used and how they were used, microbial co-selection, fitness and persistence mechanisms, host lifestyle, and food treatment conditions, influence the antibiotic resistance (AR) cycle. Targeted mitigation strategies, such as those used in the dairy processing industry, can be effective in reducing the AR gene pool.

Keywords

Antibiotic resistance – Commensal bacteria – Food chain – Gut microbiota – Horizontal gene transfer – Targeted mitigation.

Introduction

Antibiotics are widely used in human and veterinary medicine, and have been essential for ensuring human and animal health. However, the emergence of bacteria resistant to commonly used antibiotics compromises effective treatment, resulting in the need for stronger drugs and more costly therapy. The spectre of antibiotic-resistant (ART) microbes is a worldwide concern due to the public perception of an incoming post-antibiotic era. Besides clinical therapy, antibiotics have also been used to improve aquaculture and agriculture production. It is well recognised that antibiotic selective pressure has a major role in the emergence and amplification of ART pathogens; resistant pathogens derived from food animals and the environment also impact humans through the food chain and environmental exposure. Therefore, restricting both therapeutic and prophylactic uses of antibiotics in clinical settings and food animal production has so far been the primary strategy for antibiotic resistance (AR) mitigation. However, despite these efforts, the rising trend of AR continues. It is important to recognise that prudent use of antibiotics is not only about whether or not to use antibiotics, but also when and how to use them, and what to use them for. However, the knowledge and technology to practically provide the right balance is not yet available (66). Simply restricting the general use of antibiotics in clinical therapy is not the entire solution for reducing the development of more complicated and difficult-to-treat disease situations. For example, limiting the prophylactic use of antibiotics may contribute to severe biofilm-associated infections in patients (Wang *et al.*, unpublished data). The situation regarding antibiotic use in food animal production is also controversial. In the past few years, research has revealed a much more complex picture regarding AR emergence, amplification, persistence and circulation, suggesting that more targeted strategies are needed for effective mitigation. This paper will address several issues related to the food chain that are relevant to the emergence and mitigation of AR.

Antibiotic-resistant pathogens and commensal bacteria associated with the food chain

Owing to their public health relevance, several foodborne pathogens and opportunistic pathogens have been the foci of AR research and government-sponsored monitoring programmes. Many studies and reports have addressed the occurrence of ART *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococcus* spp. in food animals, food production environments and retail food samples (for a comprehensive review, see 11). These studies provided an in-depth understanding of organism-specific mechanisms in AR emergence, dissemination and persistence, and many of these discoveries have broad implications. However, foodborne pathogens account for only a very small percentage of the food microbiota, and the numbers of ART foodborne pathogens are even smaller. Moreover, raw animal food products are often subject to processing treatments which inactivate microbes. Hence, the impact of AR dissemination from ART pathogens in foods to humans is probably minimal. However, recent studies have revealed a large AR gene pool in foodborne commensal bacteria in retail foods, including many ready-to-consume food products and restaurant foods (12, 32, 64). Some food items carried as many as 10^8 copies of AR genes per gram of food (37), with a broad spectrum of foodborne bacteria identified as the AR gene carriers (10, 15, 32, 42, 55, 57, 64). Antibiotic resistance genes from foodborne bacteria were transferred in the laboratory to human resident and pathogenic bacteria by natural horizontal gene transfer (HGT) mechanisms, leading to acquired resistance in the recipient strains (16, 35, 59, 65). Because many of these foods are directly consumed without further processing, large numbers of ART bacteria are directly introduced to humans through daily food intake. Due to the limitations in methods and the cost associated with microbial ecology studies, the reported prevalence rate is probably an underestimation of the actual magnitude of AR in the microbiota associated with food products. A constant supply of ART bacteria, mostly commensals, to the human gastrointestinal tract, coupled with occasional colonisation and HGT events, will probably influence the AR of the human gut microbiota (65).

The evolution of resistance in both pathogens and commensal bacteria is affected by the type of antibiotic exposure, the local microbial population, and other host and environmental factors. Since commensal bacteria dominate in both number and genetic diversity in natural and host environments, they can potentially be better indicators of the AR status of the microbial community than foodborne pathogens, and could provide an early warning of the emergence of AR in pathogens (64). Antibiotic-resistant pathogens are the ultimate health concern and they may persist once they have emerged, even after the removal of antibiotic selective pressure, due to various resistance-persistence mechanisms (22, 24, 32, 38, 40, 51, 54, 57, 64). Government monitoring systems using standard methods of analysis help determine the impact of agricultural practices on the development of ART pathogens. However, such measurements are of AR events that have occurred and do not predict an outcome which might subsequently be difficult to reverse. The use of an alternative system of monitoring AR in commensal bacteria may serve as an early warning protocol for the development of AR in pathogen populations.

Dominant AR gene carriage can vary among ecosystems by the antibiotics used and even by the specific AR genes present within the same host or environmental microbiota. For example, Levy *et al.* (31) studied ART *E. coli* in a farm production environment and discovered that ART *E. coli* were disseminated from the food animal production environment to humans. However, in fermented dairy products the main AR gene carriers are not *E. coli* but rather lactic acid bacteria and *Staphylococcus* spp. (65). In a study by Zhang *et al.* (70), although Tet^r bacteria and the *tetM* gene pool were found to be rapidly established in the gastrointestinal tracts of infants shortly after birth, *Enterococcus* spp., rather than *E. coli,* were found to be primary AR gene carriers in the subjects during the course of the study. These data show that ART bacteria vary greatly among microbial ecosystems. Hence, studies on commensal bacteria can help identify the main players in AR dissemination in different microbial ecosystems in order to facilitate the development of targeted mitigation strategies (66).

Commensal bacteria probably played a key role in the evolution and dissemination of AR; however, our knowledge of ART commensal bacteria is limited. Focusing future AR studies on ART commensal bacteria may be beneficial for developing meaningful risk assessments and effective mitigation strategies for AR. Compared to previous documents, a significant change in the newly released Codex *Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance* (11) is that risk assessment is no longer focused on foodborne pathogens. Instead, the entire document uses the broad term 'antimicrobial resistant microorganisms', when discussing the risks associated with the food chain, indicating recognition of the significance of commensal bacteria in the AR arena.

Antibiotic-resistant intestinal microbiota and the influence of animal and human hosts on the dissemination of antibiotic-resistant bacteria

It is well established that extensive use of antibiotics is directly related to the emergence of ART bacteria. However, in developing effective AR mitigation strategies, it is important to understand whether antibiotic selective

pressure is the only factor involved in the amplification, maintenance and transmission of ART bacteria in microbial ecosystems and to ascertain how antibiotics influence resistance of the microbiota (66). Hence, a fundamental understanding of the composition of ART bacteria in the host's intestinal microbiota is important to determine the impact on AR development of antibiotics and other antimicrobials used in the food chain.

Several studies have revealed that tetracycline- and erythromycin-resistant bacteria are prevalent in the oral and intestinal microbiota of healthy children and adults who have not recently been exposed to antibiotics (20, 47, 63). Zhang *et al.* (70) examined the development of infant intestinal microbiota, and determined that ART bacteria were established within days after birth, independent of exposure to antibiotics or the intake of conventional foods rich in bacteria. These results reveal that the mother probably plays a major role in the initial dissemination of AR to her infant, and indicate that hosts play an important role in amplifying the circulation of ART bacteria and the AR gene pool in the environment, food and other hosts, independent of antibiotic selective pressure. In addition, a study on home-bred hamsters that had never been exposed to antibiotics revealed that Tet^r bacteria were present in the faeces of hamster parents and their offspring. The AR gene pattern of bacteria in the offspring was consistent with that in the mother but not in the father, even though all of the animals were exposed to the same commercial feed and bedding (34). These findings are in line with those reported by Stanton *et al.* (55), who determined that there were large faecal populations of chlortetracycline (CTC)-resistant bacteria in organically raised swine that did not receive antibiotics. Stanton *et al.* (55) also determined that the faecal populations of CTC-resistant bacteria were low in feral swine, suggesting an influence of human activities, and possibly host specificity, on AR development. Even without direct exposure to antibiotics, animal and human wastes are one of the most significant sources of ART bacteria directly contributing to the amplification cycle of AR in the global ecosystem. Antibiotic exposure further selectively enriches ART bacteria in the host gut microbiota. Ultimately, a large number of ART bacteria in sewage and manure directly contribute to the prevalence of ART microbiota in the environment (soil, water, etc.), raw food materials (meat, milk, plant materials), food processing environments and food handlers. An estimated 335 million tonnes of animal manure dry matter (with a moisture content of approximately 90% to 95%), plus faecal waste from more than 300 million people are produced annually in the United States (61, 62), hence there is an abundance of faecal bacteria to contaminate the environment. While detailed assessments are yet to be conducted in more food- and non-food-producing animals, it is well documented that animal manure, lagoon water and human faeces are rich in ART bacteria (8, 9, 28, 41, 43, 50, 52, 53).

Major food vehicles of antibiotic-resistant bacteria

As mentioned previously, a variety of ART bacteria have been isolated from raw food materials, of both plant and food-animal origin. Although data on ART commensal bacteria are limited, it is thought that ART commensal bacteria would be present in samples positive for ART pathogens.

Dairy products

Frequently, between 10^2 and 10^4 colony-forming units (CFU) of ART bacteria per ml are detected in raw milk samples. *Staphylococcus* spp., *Leuconostoc* spp., *Lactococcus* spp., *Streptococcus uberis* (65), *Streptococcus thermophilus*, *Enterococcus* spp., and *Pseudomonas* spp. (Li and Wang, unpublished data) are among the Tet^r- and Em^r-encoding gene carriers that have been identified. Many of these bacteria belong to the natural microflora associated with the animal host.

Fermented dairy products made from raw milk can potentially be contaminated with ART bacteria that survive the manufacturing process. The ART *Lactococcus lactis* subsp. *lactis* strain, K214, was first isolated from a soft cheese made from raw milk (44). This strain carries a plasmid, pK214, which has multiple resistance-encoding genes, including chloramphenicol acetyltransferase (*cat*), streptomycin adenylase (*str*), tetracycline *tet*(S), and a multidrug efflux gene mef214 (45). A *Lactobacillus plantarum* isolate from raw-milk cheese, M345, carries an erythromycin resistance-encoding plasmid, pLFE1, which has a broad host range, including *Lactobacillus rhamnosus*, *Lactococcus lactis*, *Listeria innocua*, the opportunistic pathogen *Enterococcus faecalis*, and the pathogen *Listeria monocytogenes* (16).

Pasteurisation is effective in minimising microbial counts in milk and limiting contamination with ART bacteria. Antibiotic-resistant bacteria were not detected in properly pasteurised liquid milk samples using a published screening method (33). However, a study assessing 14 retail cheese products during 2004 and 2005 revealed that 12 samples (multiple brands and varieties) contained tetracycline- and/or erythromycin-resistant bacteria, with eight containing ART bacteria ranging from 10^3 to 10^7 CFU/g of food (65). Antibiotic resistance gene carriers isolated from the cheese products included *Lactococcus* spp., *Leuconostoc* spp., *Streptococcus* spp. (*S. thermophilus* in particular), *Enterococcus* spp., *Pseudomonas* spp., *Staphylococcus* spp. (33, 49, 65) and *E. coli* (21). Manuzon *et al.* (39) reported the detection of 10^4 to 10^8 gene copies (mean of 10^7) of *tetS/M* per gram in all 11 cheese samples.

Antibiotic resistance genes from ART *Lactococcus* spp. and *Enterococcus* spp. isolates were transmitted to *Streptococcus mutans* by natural transformation and to *Enterococcus faecalis* by electroporation, which led to resistance in the recipient strains (65). Both *Staphylococcus* spp. and *Enterococcus* spp. have been used as starter cultures in certain traditional fermented products, and AR-encoding genes have been reported in such microbes (48). Horizontal transmission of AR genes can occur not only in pathogens and commensal bacteria, but also in beneficial bacteria, including those used as starter cultures and probiotics. For example, a *Bifidobacterium* spp. strain once used to supplement yogurt products carried a Tet^r-encoding gene. Lactic acid bacteria are also prone to HGT mechanisms, although this feature in lactic acid bacteria was considered to be beneficial for bioengineering strains with industrial applications. Besides serving as an AR gene reservoir, commensal bacteria may also serve as an AR gene transmission amplifier. Luo *et al.* (37) reported that a laboratory *Lactococcus* strain containing an intrinsic high-frequency conjugation mechanism could facilitate the dissemination of a broad-host-range drug-resistance-encoding plasmid by up to 10,000 times. Since this high-frequency conjugation system has also been reported in *Enterococcus* spp., *Lactobacillus* spp., and *Bacillus* spp., this finding may have broad implications. On the other hand, AR gene transmission from foodborne *Streptococcus thermophilus* isolates to recipient strains could not be demonstrated under experimental conditions (Li and Wang, unpublished data). These data suggest that ART bacteria vary in their ability to disseminate AR genes. Therefore, besides the size of the AR gene pool carried by bacteria, the genetic characteristics of bacteria carrying the AR genes are important parameters for assessing the potential for HGT.

The high prevalence of ART bacteria (mean of 10^7 copies per gram [39]) in most of the retail cheese samples made from pasteurised milk assayed in 2004 and 2005, as mentioned above, revealed that there was probably a systemic issue. A recent retail study revealed that the mean size of the AR gene pool of 11 retail cheese samples purchased in 2009 and 2010 decreased to slightly above the detection limit (10^4 copies per gram). This indicates that recent targeted mitigation strategies by the dairy and starter-culture industry to reduce dissemination of AR genes are effective (33). Although starter cultures from major suppliers are now carefully screened and selected to avoid AR genes, the lack of AR gene screening of starter and adjunct cultures at the processing plant, as well as occasional ART bacterial contamination during cheese making, probably contributes to the sporadic occurrence of cheeses contaminated with ART bacteria (33). This observation is in agreement with recent reports by others on the isolation of ART bacteria from dairy foods (mostly from speciality cheeses) (10, 12, 49, 58).

Produce

Fresh produce is susceptible to chemical/pesticide exposure from plant disease and insect treatments and to contamination by foodborne pathogens and ART bacteria, either from the production environment (i.e. soil, water, manure, wild animals) or as a result of human handling from farm to fork. Many products are consumed raw, either with minimal or no processing or without sufficient treatment that would kill harmful microbial contaminants. Therefore, consuming these products increases the risk of foodborne illnesses. A case in point is a lettuce-associated outbreak of *Salmonella typhimurium* DT104 infection in 2000. The etiological agent *Salmonella typhimurium* is resistant to multiple antibiotics (23), and the outbreak resulted in 361 cases of illness.

Boehme *et al.* (6) examined ART enterococci in agricultural foodstuffs and concluded that vegetable foods, although not as well studied as animal products, could serve as a vehicle of the AR gene pool. Enterococci that were phenotypically resistant to multiple antibiotics were isolated from fresh produce samples in the southwest of the United States (25). A multidrug-resistant (MDR) *Staphylococcus* spp. strain was isolated from garden salad, and the strain contained a pSTE2-like plasmid with *tetK* and *ermC* genes (30). In a study of fresh salad vegetables in Canada, AR genes and self-transmissible plasmids were commonly detected, mainly in oxidase-positive, Gram-negative bacteria such as *Pseudomonas*, *Sphingobacterium*, and *Acinetobacter* (4). Lee *et al.* (29) determined that 27% of the ready-to-consume vegetables sampled in South Korea were positive for *Yersinia* strains that were highly resistant to ampicillin, cephalothin, and carbenicillin.

A small-scale study of ready-to-eat fresh produce samples from retail stores and fast-food restaurants in Columbus, Ohio, between 2004 and 2005 revealed that 10 of 11 samples contained tetracycline-resistant bacteria, with the mean value for resistant bacteria count being 10^5 CFU/g of food. Representative isolates carrying the AR-encoding genes were confirmed and identified (30). A follow-up study of fresh produce sampled in 2006 from the same area revealed a lower level of contamination with tetracycline-resistant bacteria, i.e. 10^2 CFU/g (Rattanaprasert and Wang, unpublished data).

Meat products

Antibiotic-resistant *Salmonella*, *Campylobacter*, *E. coli* and multidrug-resistant *Staphylococcus* have been detected in many different types of retail meat and poultry products, as well as in farm animals and the farm environment (5, 14, 17, 68). A recent outbreak of salmonellosis caused by multidrug-resistant *Salmonella* Heidelberg was associated with ground turkey products; the outbreak caused one death and more than 70 illnesses (7). The 2009 retail meat

survey carried out by the Food and Drug Administration in the United States, as part of the National Antimicrobial Resistance Monitoring System (NARMS), revealed that over one-quarter of *Salmonella* isolates from ground turkey were multidrug resistant (18). In comparison, nearly half (48.4%) of *Salmonella* isolated from chicken breasts were resistant to three or more classes of antibiotics and more than 30% were resistant to five or more classes of antimicrobials. This is significant because different antibiotic classes often have different mechanisms of action. The 2009 NARMS data also revealed a marked increase in multidrug-resistance compared to previous years, when only 20% to 38.2% of *Salmonella* from chicken isolates were resistant to three or more classes of antibiotics. Multidrug-resistance is uncommon in *Campylobacter*, and less than 1% of the *Campylobacter* isolates detected in the NARMS retail meat study were resistant to three or more antimicrobial classes. Approximately 70% of the retail meat samples were positive for commensal *E. coli*, although the percentage of isolates resistant to antibiotics was generally between 5% and 10%, with the exception of ampicillin-resistance, which was seen in more than half the ground turkey isolates (18). Although not pathogenic, ART commensal bacteria can serve as a reservoir for AR determinants.

Many standard pathogen isolation procedures involve an enrichment step(s), hence most of the reported pathogen prevalence data only reflect the presence, not the magnitude, of ART foodborne pathogen contamination. Lehman (30) examined several retail samples (ground beef, ground turkey, pork chops, breakfast sausage patties, ground pork) and detected Tet^r and Em^r commensal bacteria, mostly in the range of 10^3 to 10^6 CFU/g of food. However, most animal muscle products are subject to cooking before consumption, so the level of ART bacteria in prepared food would be less than those indicated above, if the products were properly cooked, handled and stored.

Seafood

Duran and Marshall (15) examined imported ready-to-eat shrimp originating from four different countries, and recovered many ART bacteria representing 162 species. Tran *et al.* (60) detected ART *Pseudomonas putida* on frozen imported shrimp samples sold in the United States. Two isolates harboured plasmids with *qnr*A and *qnr*B genes. Polymerase chain reaction and DNA sequencing of the quinolone resistance-determining regions revealed novel substitutions in the GyrA and GyrB regions. Lehman (30) tested several retail raw and cooked shrimp products and detected up to 10^3 CFU Tet^r and Em^r bacteria per gram in most samples. Studies by Li and Wang (35), and Zhang and Wang (unpublished data) of retail raw and cooked shrimp, raw fish and sushi from Columbus (Ohio) and Honolulu (Hawaii), have detected a variety of ART bacteria, including *Brochothrix* spp., *Enterococcus* spp., *Lactococcus garvieae, Streptococcus* spp., *Carnobacterium* spp., *Pseudomonas* spp., and *Acinetobacter* spp. Recent studies by Zhang *et al.* (unpublished data), have detected ART bacteria in aquacultured fish and shrimp products from South China, including *Lactococcus* spp., *Enterococcus* spp., *Macrococcus* spp., *E. coli*, *Exiquobacterium* spp., *Bacillus* spp., *Citrobacter* spp., *Kurthia* spp., and *Providencia* spp. Because some of the bacteria of these genera (e.g. *Carnobacterium* spp., *Lactococcus* spp., *Enterococcus* spp. and *Bacillus* spp.) may be used as probiotics, the potential involvement of such microbes in AR dissemination would need to be carefully evaluated before they could be used in bio-control applications.

Antibiotic-resistant pathogens have also been obtained from seafood products. For example, Kadlec *et al.* (26) studied the trimethoprim-sulfamethoxazole (SXT) resistance of fish pathogen aeromonads isolated from seafood products in Germany. They associated SXT-resistance with class 1 integrons, which also carry gene cassettes for other resistance properties. The results suggested that there was a risk of co-selection and persistence of other resistance genes under selective pressure imposed by the use of trimethoprim/sulfonamide combinations. Antibiotic resistance was also detected in *Vibrio parahaemolyticus* isolated from shellfish from Georgia and South Carolina (2), in *E. coli* O157:H7 from retail shrimp from India (56), in *V. parahaemolyticus* and *V. alginolyticus* from farmed fish from Korea (42), and in *Salmonella* from imported seafood (27). The observation that similar or even identical gene cassettes have been detected in bacteria from fish, humans, food-producing animals and/or companion animals suggests that there is an inter-connection among these microbial ecosystems, with the sharing of a common AR gene pool, thereby influencing the evolution of ART bacteria (26).

Ready-to-consume, deli and restaurant foods

Compared to raw foods, ready-to-consume products – including processed dairy, meat, seafood and vegetable products and deli and restaurant foods – are usually ingested directly without further processing. Therefore, the number of ART microorganisms in these products gives an indication of the actual risk of oral exposure to ART microbes through food intake. Lopez *et al.* (36) determined that about 39% of *Bacillus cereus* isolates obtained from honey were resistant to tetracycline, of which 77% contained at least one of the resistant determinants: *tetK, tetL, tetM, tetO, tetW, otrA* or *otrB*. Li and Wang (35) obtained Tet^r bacteria from 20 out of 26 food samples from salad bars at grocery stores and restaurants in Columbus, Ohio. Approximately one-third of the samples contained at least 10^3 CFU of Tet^r bacteria per gram of food. Out of 740 Tet^r isolates examined, more than 15% carried one or

more of the *tetM, tetL, tetS*, or *tetK* genes. The most prevalent genotype was *tetM*, which was detected in 57% of the Tet^r gene carriers, followed by *tetL* (37.8%), *tetS* (9.6%) and *tetK* (3.0%). Bacterial genera identified as AR gene carriers included *Enterococcus, Lactococcus, Staphylococcus, Brochothrix, Carnobacterium*, and *Sphingobacterium*. Some of the isolates carried AR-encoding plasmids that persisted after being consecutively transferred for more than 400 generations in bacterial media without tetracycline, suggesting the presence of plasmid stability mechanism(s). Shen *et al.* (51) determined that *Listeria monocytogenes* were present in 3% of more than three thousand ready-to-consume foods (deli-style sandwiches, smoked turkey, beef, and ham) sampled in Florida, and 78% of these isolates exhibited multidrug-resistance to ciprofloxacin, tetracycline and others. The microbiota of ready-to-consume foods are affected by the microorganisms associated with raw materials, as well as by post-harvesting processing, handling and storage procedures before consumption.

The influence of production practices, processing treatments and consumption style on the development and circulation of antibiotic-resistant bacteria

Antibiotic uses in agriculture and aquaculture production

Animal and human faeces are important sources of ART bacteria. According to data from the United States Department of Agriculture (USDA), approximately 8.4 billion chickens (broilers), 264 million turkeys, 103 million hogs and more than 37 million head of cattle were produced in 2004 for food, and 9.12 million cattle were in dairy production (2002 data) in the United States (14). These animals produce more than 335 million tonnes of dry matter manure waste every year (14). In addition to the already substantial population of ART microbes acquired through birth, food and environmental contacts, the estimated 30 to 50 million pounds of antibiotics used annually in agriculture (21), mostly in food animal production, further selectively enrich the ART bacteria population in microbial ecosystems. Therefore, besides foods serving as important vehicles in disseminating ART bacteria to hosts through food intake, another important contributor of the food chain to AR dissemination and circulation is the impact of ART bacteria-contaminated manure and other animal wastes on the environmental microbiota. Hence, proper manure and human waste treatment should be one of the most important critical control points for AR reduction (64). Products from organic farming, particularly fresh produce for direct consumption, can be contaminated by ART bacteria from manure, soil, water, and even dust in the air.

Although aquaculture is not a large industry in the United States, worldwide aquaculture production in 2005 reached over 40 million tonnes (19). Antibiotics are commonly used in aquaculture production, especially in developing countries, for disease prevention and treatment. In addition, integrated aquaculture, which is a popular practice in many countries, uses animal manures as nutrients for growing fish and shellfish. These production practices can facilitate the dissemination of ART bacteria and AR genes within the production environment, in the aquacultured creatures, and in humans exposed to such environments and food products.

Microbial intervention strategies

Probiotic microbes are becoming more widely used globally as food supplements as consumers become increasingly interested in their potential health benefits. In addition to human consumption, probiotics are also being used in food animal and aquaculture production, in part to reduce the use of antibiotics as growth promoters to improve production efficiency. However, beneficial bacteria, including fermentation starter cultures and probiotic bacteria, are also susceptible to HGT mechanisms. In fact, lactic acid bacteria and *Bacillus* spp. have been found to be AR gene carriers in aquaculture products from China, and these types of bacteria are commonly used as probiotics in aquaculture production (Zhang *et al.*, unpublished data).

Historically, AR genes were not part of the standard screening assays for starter cultures and probiotic bacteria used in foods or as food supplements. Both starter cultures and probiotic bacteria are consumed live and in large amounts, and one of the preferred features of probiotics is that these bacteria colonise the gastrointestinal tract. Consuming products containing ART starter cultures or probiotic bacteria could be detrimental, instead of beneficial, to gut and public health. It is important that bacterial cultures intended for use in fermentation or probiotic applications be characterised at the strain (isolate) level for not only the absence of AR genes but also for the potential of both acquisition and dissemination of such genes via HGT mechanisms. While this has become a standard practice by major microbial culture companies in the United States and the European Union, small culture suppliers and those in developing countries should also adopt such practices.

Recently, clustered regularly interspaced short palindromic repeats (CRISPRs), a diverse family of DNA repeat sequences, have been found in 90% and 40% of the archaea and bacteria genomes examined, respectively. Emerging evidence has revealed that CRISPRs are part of DNA-encoded immunity mechanisms that protect host microorganisms against the invasion of foreign genetic elements such as phages and plasmids (3). For instance, when exposed to bacterial phage invasion, the host bacteria can generate phage-resistant derivatives containing invasion phage sequence-specific CRISPR repeat-spacer units at the leader end of the CRISPR loci. The CRISPR-spacer sequence is correlated with resistance to the invasion agent. Because CRISPR loci can hinder horizontal transfer of foreign genetic materials, such as phage and even AR genes to microbial cells, they can be used in selecting and engineering microbes suitable for food fermentation and food supplement applications.

Recently, bacterial phages have emerged as a tool for pathogen mitigation in the food chain, and have received regulatory approval for use in specific foods and have been proposed as treatments of live animals and manures. However, phages are frequently associated with molecular evolutionary changes in bacteria, including the acquisition of the Shiga-toxin-producing virulence factor by *E. coli* O157:H7 and other enterohaemorrhagic *E. coli*. Hence, the risk of using phages as antimicrobials needs to be carefully evaluated, especially regarding their potential involvement in the evolution of ART bacteria and even the emergence of new 'superbugs' (66).

Processing treatments

During the journey from farm to fork, foodborne microbes are susceptible to many different antimicrobial agents, e.g. nisin, lysozyme, lactoferrin, essential oils, and organic acids. Sanitisers and disinfectants are often used in the food system, in production, processing and household environments. These exposures might also contribute to an increase of ART bacteria due to cross-resistance, co-selection and stress adaptation (71). Mutations at a common cellular target and acquiring a general drug efflux pump can lead to resistance to several antibiotics. In the case of co-selection, the microbe may become resistant to a number of different antibiotics, heavy metals, biocides or disinfectants, each with distinct mechanisms of action due to the same or related genetic determinant(s). Besides integrons and plasmid-encoded resistance determinants, activation of the MDR operon by antimicrobial compounds commonly used in the food industry, such as chlorine, sodium nitrite, sodium benzoate, or acetic acid, also leads to increased resistance to multiple antibiotics in a number of pathogens, including *E. coli* O157:H7 and *Salmonella* Enteritidis (46).

Consumer habits

Data from several studies have revealed that most ART bacteria are as susceptible to conventional heat treatments as the antibiotic-sensitive population of otherwise similar bacteria (1, 69). Therefore, proper cooking is effective in reducing the number of ART bacteria in foods before final consumption. However, the increased popularity of consuming raw vegetables, and certain raw meat and seafood (such as sushi) products, due to the growing trend of 'healthy' eating, may increase the risk of ART bacteria exposure through food intake.

Knowledge gaps and future directions

Antibiotic resistance is a complicated issue, and effective mitigation will require targeted strategies built upon a comprehensive understanding of AR emergence, amplification, dissemination, persistence and circulation. Antibiotic use is a double-edged sword. While the type of drug used is a key factor in the selective enrichment of an ART population, the lack of early treatment to effectively stop the development of more severe and complicated disease conditions in both animal and human hosts, including biofilms and polymicrobial infections that can be more resistant to conventional treatments, may lead, or have already led, to significant loss in host health and increases in healthcare costs.

In order to address critical knowledge and technology gaps, and to facilitate the development of targeted mitigation strategies and ensure truly prudent use of antibiotics, an expert group drew up a report (67) resulting from the 2009 conference, 'Food Safety and Public Health Frontier: Minimizing Antibiotic Resistance Transmission through the Food Chain'. The report recognised that systematic studies for a comprehensive understanding, both at the macroscopic and microscopic levels, of AR associated with the food chain are central to designing targeted and integrated intervention strategies for effective mitigation (67). Recommendations of the report included investment in '*fundamental studies at the ecosystem level to: i) develop novel experimental and systematic approaches and systematically investigate the AR ecology such as main resistance gene reservoirs and key microbial players within the ecosystems, at and between various links in the food chain from farm to table, including pre- and post-harvest environment (agriculture and aquaculture farms, surrounding soil, air and water, processing, transportation, storage, retail chain, etc.), raw and processed foods, as well as animal and human hosts; (ii) reveal the impact of natural and implemented factors (such as the application of antibiotics, compost, processing treatment, etc.) including dosage effect on the evolution and mitigation of AR in the corresponding ecosystems*'.

The expert group further stressed the equal importance of the need for '*fundamental studies at the individual microorganism (both pathogen and commensal) level to: (iii) reveal molecular mechanisms involved in AR origination, dissemination, persistence and environmental fitness as related to the food system, and (iv) investigate the impact of natural and implemented factors on the evolution and persistence of AR in such organisms as well as ecologically relevant bacterial species*'.

Finally, the report concluded that developing effective mitigation strategies and outcome measurements requires one to: '*(i) identify critical control points for AR based on sophisticated and ecological measures and risk assessment outcomes, and develop and implement agriculture, aquaculture and industrial practices to minimize and contain the spread and persistence of AR in the pre- and post-harvest food environment, products and host ecosystems; (ii) conduct studies with a focus on disease prevention and biosecurity, such as developing vaccines or alternatives for subtherapeutic uses of antibiotics in animal production; (iii) develop and implement integrated research, education, and outreach programs engaging academic, government agencies, industry and consumers including the lay public for effective mitigation; (iv) design and implement studies to measure the impact or effect of potential interventions on existing AR at the macro and micro levels*'. The newly released Codex guidelines further outline a comprehensive approach to conducting a foodborne AR risk assessment throughout the food chain (11).

Le transfert de l'antibiorésistance à l'homme par l'intermédiaire des aliments : les faits, les conséquences et les orientations futures

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Résumé

Depuis les lieux de production jusqu'à la cuisine du consommateur, la chaîne alimentaire contribue de manière importante au développement, à la persistance et à la dissémination de microbes résistants aux antibiotiques, qu'il s'agisse de pathogènes résistants d'origine alimentaire ou de bactéries commensales. Plusieurs facteurs de la chaîne alimentaire ont une influence sur le cycle de résistance aux antibiotiques, par exemple les composés antimicrobiens utilisés, la co-sélection microbienne, les mécanismes d'adaptation et de persistance, le mode de vie de l'hôte et les conditions de transformation des aliments. Les stratégies ciblées d'atténuation appliquées notamment par l'industrie de la transformation laitière se sont révélées efficaces pour réduire le fonds génétique de la résistance aux antibiotiques.

Mots-clés

Bactérie commensale – Chaîne alimentaire – Mesure ciblée d'atténuation – Microbiote intestinal – Résistance aux antibiotiques – Transfert horizontal de gènes.

Transferencia de la resistencia a los antibióticos de los alimentos al hombre: datos, consecuencias y orientaciones futuras

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Resumen

La cadena alimentaria, que abarca desde las instalaciones de producción hasta la cocina del consumidor, puede contribuir sustancialmente a la aparición, persistencia y diseminación de microbios antibiorresistentes, ya se trate de patógenos transmitidos por los alimentos o de bacterias comensales. En el ciclo de la antibiorresistencia influyen numerosos factores que forman parte de la cadena alimentaria, como los compuestos antimicrobianos utilizados, la coselección bacteriana, los mecanismos de adaptación y persistencia, el modo de vida del anfitrión o las condiciones de tratamiento de los alimentos. Para reducir la reserva genética de la antibiorresistencia pueden ser de utilidad estrategias selectivas de atenuación como las empleadas en la industria de transformación de productos lácteos.

Palabras clave

Atenuación selectiva – Bacteria comensal – Cadena alimentaria – Microbiota intestinal – Resistencia a los antibióticos – Transferencia génica horizontal.

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