COMMENTARY

Antibiotic Resistance: How Much Do We Know and Where Do We Go from Here?[∇]

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The rapid dissemination of antibiotic resistant (ART) pathogens threatens human health and may have significant social and financial impacts. It is well recognized that applications of antibiotics in human clinical therapy, aquaculture, and food animal production all contribute to the emergence and amplification of ART pathogens due to selective pressure (8, 14). Restricting the therapeutic and prophylactic use of antibiotics in clinics and food animal production has been the primary strategy for antibiotic resistance (AR) mitigation to date. However, despite these efforts, which include changes in medical practice guidelines and government policies in both the European Union and the United States, the rising trend of AR has not changed much in recent years.

In the past several years, evidence showing a much more complicated picture of AR has emerged. Data from both organism-specific and population-based studies have suggested that various mechanisms contribute to the retention of AR determinants, and in certain cases ART bacteria become the dominant microbial population, even in the absence of a corresponding antibiotic selective pressure (7, 9, 17, 18). ART bacteria have been found not only in various food products and environmental samples but also in hosts without a history of direct exposure to antibiotics (6, 20, 25). The existence of large AR gene pools in food-borne commensal bacteria present in many ready-to-consume food items suggested that human beings are constantly inoculated with large numbers of ART bacteria through daily food intake, independent of clinical antibiotic exposure (3, 19, 28). A broad spectrum of commensal bacteria, including lactic acid bacteria, have been identified as being carriers of AR genes and are able to transmit those genes to other bacteria in laboratory settings, leading to increased resistance in the recipient organisms (4, 28). While the impacts of commensal bacteria in food, host, and environmental ecosystems on AR origination, dissemination, and persistence have yet to be fully appreciated, it is evident that underestimating the roles of commensals and other AR dissemination channels in the AR picture may have hindered the development of effective mitigation (1, 26).

To fill in the critical knowledge gaps and facilitate the de-

velopment of targeted control strategies, USDA-CSREES-NIFSI and The Ohio State University cosponsored the international conference “Food Safety and Public Health Frontier: Minimizing Antibiotic Resistance Transmission through the Food Chain,” which took place 2 and 3 April 2009 in Arlington, VA. The conference brought together experts from academia, industry, and federal agencies for a balanced and scientific review of AR. Invited senior experts shared their most recent discoveries, visions of AR management, and successful experiences in AR mitigation. The resultant expert report recommended that systematic studies for a comprehensive understanding, at both the macroscopic and microscopic levels, of AR connected to the food chain are central to the design of targeted and integrated intervention strategies for effective mitigation of AR (27). The conference organizers sincerely appreciate ASM and *Applied and Environmental Microbiology* for recognizing the scientific significance of the event and providing the platform by which to systematically present related work contributed by the conference speakers. This special issue addresses several questions imperative to the fundamental understanding of AR and with direct impact on mitigation efforts.

As mentioned above, antibiotics selectively enrich ART bacteria and the corresponding AR gene pools in the microbiota. A critical issue of relevance to control strategies is whether antibiotic selective pressure is the only factor responsible for the amplification, maintenance, and transmission of ART bacteria in microbial ecosystems. To reveal the baseline of ART bacteria in host gastrointestinal tracts, Stanton et al. (24) examined chlortetracycline (CTC)-resistant *Escherichia coli*, *Megasphaera elsdenii*, and anaerobic bacteria in swine feces. Up to 10¹⁰ CTC-resistant (at 64 µg/ml) anaerobic bacteria were detected in fecal samples from organically raised swine, while the number was significantly lower in fecal samples from feral swine. The data illustrated the prevalence of ART bacteria in the gastrointestinal tracts of animals lacking direct exposure to antibiotics. Meanwhile, the results also suggested an impact of human activities, environmental factors, and also possibly host specificity on AR development. More directly, Zhang et al. (29) illustrated that levels of ART bacteria rose to between 10⁹ and 10¹⁰ CFU/g in the infant gut microbiota within days after birth, independent of exposure to antibiotics and intake of ART bacterium-rich conventional foods. The results illustrated a potentially significant route of AR dissemination from

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mother to infant and, most importantly, suggested that hosts play a critical role in the amplified circulation of ART bacteria and the AR gene pool among the environment, food, and hosts, independent of antibiotic selective pressure.

Several papers in this special issue also exemplify resistance stabilization and induction mechanisms, independent of a corresponding antibiotic exposure. Shen et al. (22) reported that salicylate, a common nonsteroidal anti-inflammatory drug, induced the production of the CmeABC multidrug efflux pump in *Campylobacter* isolates by binding to the transcriptional repressor and led to decreased susceptibility to antibiotics and increased emergence of fluoroquinolone-resistant mutants. Kadlec et al. (10) examined the trimethoprim-sulfamethoxazole (SXT) susceptibility status of fish-pathogenic aeromonads from Germany and the resistance-encoding genes. They reported the association of SXT resistance with class 1 integrons and the risk of coselection and persistence of other resistance genes due to applications of trimethoprim/sulfonamide combinations. In another study, Li et al. (15) identified a plasmid, pM7M2, with two Tet resistance-encoding genes from a persistent dairy isolate. Plasmid characterization revealed the presence of a toxin-antitoxin system-independent plasmid stabilization mechanism, likely prevalent in a series of AR-encoding plasmids. Results from these studies further support the notion that multiple factors have contributed to the emergence, amplification, maintenance, and dissemination of ART bacteria.

So far, several food-borne pathogens, including *Escherichia coli* and *Enterococcus* spp. as opportunistic pathogens, have been the foci for AR research and government-sponsored monitoring programs due to their immediate public health impacts. Extensive investigation of these organisms enabled the establishment of an in-depth understanding of AR mechanisms and prevalence, and many of the discoveries have broad implications. For instance, Schink et al. (21) examined extended-spectrum β -lactamase (ESBL) genes in *E. coli* isolates from companion and farm animals with defined disease conditions. They concluded that the presence of identical *bla*_{CTX-M-15} genes in diverse plasmid backgrounds in different members of the *Enterobacteriaceae* from human and animal sources in different countries highlighted the incredible flexibility of resistance plasmids to undergo structural changes in response to evolutionary needs. Furthermore, the finding of *bla*_{CTX-M-1} genes with similar genetic environments on IncN plasmids in *E. coli* isolates from different human and animal hosts suggested potential plasmid transfer among *E. coli* strains from human and animal sources and a common gene pool for transferable ESBL genes. Karczmarczyk et al. (12) examined fluoroquinolone-resistant *E. coli* isolates from food-producing animals in Ireland and reported the finding of multiple mechanisms of fluoroquinolone resistance associated with the efflux pump and various mutations within the quinolone target genes in these isolates.

The impact of commensal bacteria on the emergence, amplification, dissemination, and maintenance of the AR gene pool has been recognized by the scientific community in recent years. The well-studied bacteria *E. coli* and *Enterococcus* spp. again have been used as representative commensal bacteria to illustrate the impact of such organisms on AR dissemination. For instance, Karczmarczyk et al. (13) conducted molecular

characterization of multidrug-resistant (MDR) *E. coli* isolates from Irish cattle, which displayed considerable diversity with respect to the genes identified. These findings highlight the importance of the commensal microfloras of food-producing animals as reservoirs of transferable MDR. The same group also characterized MDR *E. coli* isolates from hospitalized animals, further illustrating that animal-associated commensal *E. coli* strains possess a wide array of transferable genetic determinants (11). A similar conclusion was also derived by Schink et al. (21) through the study of *bla*_{CTX-M-1} genes from *E. coli*. It is worth noting, however, that the evolution of resistant bacteria varies depending on the antibiotic and in many cases is affected by host and environmental factors as well as the local microbial population. While commensal bacteria may be a hidden reservoir for AR genes, which can serve as an early and potentially more accurate indicator of the resistance status of the microbiota, dominant AR gene carriers vary among ecosystems, antibiotics, and even the specific AR genes within the same host or environmental microbiota. For instance, the main AR gene carriers in fermented dairy products were lactic acid bacteria and *Staphylococcus* sp., not *E. coli* (28), and while Tet^r bacteria and the *tetM* gene pool were rapidly established in the infant gastrointestinal tract shortly after birth, no Tet^r *E. coli* strains were recovered from the subjects during the study period (29). Feßler et al. (5) reported the prevalence of multiresistant and enterotoxinogenic MRSA in food animal products. The results not only suggested a potential impact of the food chain on the evolution and dissemination of ART bacteria but also indirectly illustrated the susceptibility of *Staphylococcus* sp. to AR development and a potential involvement in horizontal gene transmission. Therefore, the true rationale for commensal-oriented AR studies, instead of defining commensals and pathogens, is to identify the main players in AR dissemination in microbial ecosystems through ecosystem-based studies, which in turn provides scientific justification for the selection of proper AR indicator organisms. Beyond simply reducing antibiotic selective pressure, an effective mitigation strategy should also widely cover critical control points throughout the AR emergence, amplification, and dissemination cycle, including food, birth, and fecal/manure routes.

Beneficial bacteria have become a popular supplement to improve gut health. Probiotics are further used as a growth promoter in food animal production. However, certain beneficial bacterial isolates are also susceptible to AR gene dissemination. Supplementing strains can be detrimental instead of beneficial if they are AR gene carriers or they have the potential to be involved in horizontal gene transfer events. In the past few years, the prevalence of ART bacteria in fermented dairy foods has been reduced significantly, illustrating the effectiveness of targeted mitigation (16). While strains intended for human consumption that are supplied by major companies in the United States and the European Union are carefully screened, the risks and benefits of introducing probiotic strains into food animal and aquaculture production systems worldwide need to be carefully assessed in terms of AR mitigation (16). Stanton and Humphrey (23) evaluated the effectiveness of using antibiotic-sensitive *Megasphaera elsdenii* strains as probiotics to prevent colonization of swine by ART strains. The group concluded that dosing newborn piglets with antibiotic-sensitive strains of *M. elsdenii* delayed but did not prevent

colonization by maternal resistant strains. *M. elsdenii* subspecies diversity likely contributed to the persistence of resistant strains in the absence of antibiotic selection.

Clearly, AR in microbial ecosystem is a complex issue. A thorough understanding of the molecular mechanisms involved in the emergence and dissemination of AR, careful experimental validation, and enhanced surveillance systems are essential for assessing the safety and effectiveness of AR mitigation approaches. For example, bacterial phages have emerged as a tool for pathogen mitigation in the food chain, with approved usage in selected foods and proposed treatments of live animals and manures. However, phages are frequently implicated in bacterial evolution, including that of *E. coli* O157:H7. Thus, the risk of using phages as antimicrobials must be carefully evaluated, particularly with regard to their potential involvement in the evolution of ART bacteria and even the emergence of new “superbugs.” Revealing the role of the CRISPR system in protecting microbes from horizontal gene transfer events (2) will likely impact the use of proper organisms for microbial intervention. Finally, it is important to recognize that prudent use of antibiotics does not simply mean a ban of but also what, when, and how to use the antibiotics. While bacterial infections can be easily managed by the proper use of antibiotics at early stages, delayed therapy may lead to the development of biofilm-associated infections or other complicated health conditions in both humans and animals and, subsequently, the need for more-extensive treatments. Carefully planned microbial investigations at both the ecosystem and microorganism levels are greatly needed to close the knowledge gaps for AR emergence, amplification, dissemination, and persistence and to truly achieve a prudent use of antibiotics and effective, targeted AR mitigation.

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