Bacterial Antibiotic Resistance, Animal Agriculture, and Human Health: No Simple Answer at the Interface of Three Complex Systems

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Infectious disease is the second-leading cause of death worldwide and the third-leading cause of death in the United States (US) (Spellberg et al., 2008). The therapeutic use of antibiotics to treat bacterial infections is often cited as the most important medical innovation of the 20th century, and it has been estimated that the incidence of human premature death due to bacterial infection would be 40% higher if antibiotics did not exist (WHO, 2000). The increasing occurrence of antibiotic-resistant infections in humans, including infections caused by methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant Mycobacterium tuberculosis, multidrug-resistant gram-negative bacteria (Escherichia, Klebsiella, and Acinetobacter species), multidrug-resistant Neisseria gonorrhoeae, cephalosporin-resistant Salmonella, and fluoroquinolone-resistant Campylobacter has lead many organizations (governmental, non-governmental, professional, medical, and scientific) to declare antibiotic resistance the most critical threat to public health (EASAC, 2007, Frieden, 2010, Davies, 2013, Spellberg et al., 2008, WHO, 2013). The goals of these declarations are to call attention to and prevent a widely feared "return to the pre-antibiotic era in medicine." Due to complex scientific, regulatory, and business factors, the introduction of novel classes of antibiotics for therapeutic use in the next 20 years is highly unlikely (Davies and Davies, 2010, Projan, 2003). Because the problem of antibiotic-resistant infections will not be solved by the introduction of "new antibiotics", attention has been focused on preserving the effectiveness of existing antibiotics, monitoring antibiotic resistance, and understanding the complex processes that contribute to the prevalence of antibiotic-resistant infections.

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Bacterial antibiotic resistance is an ancient, natural, and dynamic process that pre-dates the human use of antibiotics. Antibiotic resistance genes have been identified in bacteria isolated from environments not exposed to human activity, and genetic analysis indicates that antibiotic-inactivating enzymes have been produced by bacteria for more than 2 billion years (D'Costa et al., 2011, Hall and Barlow, 2004, Hall et al., 2004). Although the specific genes and mutations that confer antibiotic resistance are innumerable, antibiotic resistance occurs by four general processes: inactivation of the antibiotic, removal of the antibiotic, alteration of the antibiotic target, or increased production of the antibiotic target. Antibiotic resistance may arise in an organism through either mutation of endogenous genes or the acquisition of exogenous genes encoding resistance. The acquisition of exogenous antibiotic resistance genes is known as horizontal gene transfer (HGT) and can occur by three general methods: conjugation (direct transfer of DNA contained in plasmids, transposons, or other genetic elements between bacterial cells, regardless of relatedness), transformation (incorporation of DNA from the extracellular environment), and transduction (transfer of DNA via bacteriophage, generally between closely related bacteria). Genetic elements including conjugative plasmids, transposons, integrons, and bacteriophage that facilitate HGT are known collectively as mobile genetic elements (MGEs). It is thought that HGT is a dominant contributor to the spread of antibiotic resistance because highly conserved antibiotic resistance genes contained within MGEs have been isolated from distantly related bacteria (Djordjevic et al., 2013).

Concerns that antibiotic use in animal agriculture contributes significantly to human antibiotic-resistant infections are longstanding, beginning at least as early as 1969 with the release of the Swann Report (Swann, 1969). Antibiotic use increases or "selects" bacteria that are resistant to the applied antibiotic because these resistant bacteria survive exposure to the antibiotic and may multiply to replace the susceptible bacteria killed by antibiotic exposure (Salyers and Amabile-Cuevas, 1997). In addition,

the frequent isolation of MGEs harboring genes conferring resistance to unrelated antibiotics demonstrates that the use of one antibiotic can co-select for resistance to other antibiotics (Bass et al., 1999). The use of antibiotics in agriculture might impact human health either directly or indirectly. A direct impact can occur by antibiotic use that selects for an antibiotic-resistant zoonotic pathogen that contaminates food, is consumed, and results in human illnesses for which treatment is complicated by the antibiotic resistance. An indirect impact may occur by antibiotic use that selects for a commensal antibiotic-resistant bacterium that transfers a MGE containing antibiotic resistance genes to a zoonotic pathogen, which contaminates food, is consumed, and results in illnesses for which treatments are complicated by resistance. An indirect impact also may occur by selection of commensal antibiotic-resistant bacteria that contaminates food, is ingested, colonizes the human gastrointestinal system, and then transfers the MGE harboring antibiotic-resistance genes to other bacteria, including pathogens, leading to illnesses for which treatments are complicated by resistance (McDermott et al., 2002). However, several quantitative assessments have demonstrated that the risk posed to human health from antibiotic use in animal production is very low (Hurd et al., 2004, Singer et al., 2007, Hurd and Malladi, 2008, Hurd et al., 2010).

The European Union (EU) and Denmark have taken numerous actions to regulate antibiotic use in animal agriculture with the goal of reducing the risk posed to humans by antibiotic-resistant infections. These actions are frequently cited by consumer groups and US government reports as examples that should be considered for implementation in the US (GAO, 2011). The first actions occurred in 1995 in Denmark and in 1997 in the EU, when each banned the growth-promoting use of avoparcin, an antibiotic similar to vancomycin. It was concluded that the use of avoparcin in animal agriculture for growth promotion was responsible for the increased levels of the opportunistic pathogen vancomycin-resistant Enterococci (VRE) observed in EU food animals, meat, and human commensal flora compared to similar samples from the US, where avoparcin was never approved for agricultural use but the clinical use of vancomycin was higher than in the EU. The stated goal of the avoparcin ban was to reduce VRE, and following implementation of the ban, VRE levels in EU food animals, meat, and human commensal flora decreased (van den Bogaard et al., 2000, Bonten et al., 2001).

Subsequently, additional restrictions on the use of antibiotics for growth promotion were enacted by Denmark and the EU, culminating in 2006 with an EU ban on all growth-promoting uses of antibiotics in animals. In contrast to the ban on avoparcin, the goals of the subsequent bans were not reductions of specific resistant bacterial groups but were based on the "Precautionary Principle", which states that "where there are threats of serious or irreversible damage, lack of scientific certainty should not postpone cost-effective measures to reduce risks to humans" (GAO, 2011, Casewell et al., 2003). The restrictions on growth-promoting uses of antibiotics in animals have not had the desired effect on human health as, with few exceptions, decreases in antibiotic-resistant infections in humans have not been observed in Denmark's comprehensive antibiotic resistance monitoring program, DANMAP, in the years following the enactment of the restrictions (Phillips, 2007, GAO, 2011, DANMAP, 2011). In addition, following the Danish enactment of restrictions on growth-promoting antibiotics in animals, the use of therapeutic antibiotics in animals in Denmark increased (GAO, 2011, DANMAP, 2011). In response to the increased use of therapeutic antibiotics, Denmark has increased regulation on all antibiotic uses in production farms and increased veterinary oversight (GAO, 2011).

Advocacy groups in the US continue to campaign for the enactment of more restrictive regulations similar to EU and Danish regulations regarding agricultural antibiotic use (CSPI, 2013). Recently, a federal judge ordered the Food and Drug Administration (FDA) to initiate withdrawal proceedings for growth-promoting uses of antibiotics including penicillin and tetracycline (Katz, 2012). In 2012, the FDA issued guidelines for the judicious use of medically important antibiotics (FDA, 2012). Perhaps most significantly, in 2011 the US Government Accountability Office (GAO) issued a report (GAO-11-801) entitled "Antibiotic Resistance: Agencies Have Made Limited Progress Addressing Antibiotic Use In Animals (GAO, 2011)." Specifically, the GAO found that the current surveillance program monitoring antibiotic resistance in foodborne pathogens known as the National Antimicrobial Resistance Monitoring System (NARMS) is inadequate because samples are not collected in a representative manner. NARMS is an interagency program led by the FDA involving the Centers for Disease Control (CDC) and the United States Department of Agriculture (USDA). Each agency samples different matrixes and cultures for different sets of bacteria. CDC samples clinical isolates of foodborne pathogens from all 50 states in a representative manner. The GAO report found that FDA sampling of retail meat products (chicken breasts, pork chops, ground turkey, and ground beef) from 10 states is not representative. In addition, the GAO report concluded that USDA sampling of carcasses (chicken, turkey, cattle, and swine) and ground products (chicken, turkey, and beef) at processing plants is not representative of production environments. Furthermore, the GAO report found that the samples collected for the USDA portion of NARMS are unsuitable for the analysis of trends because the USDA samples are obtained from targeted, non-representative sampling. The USDA samples are obtained during Hazard Analysis and Critical Control Points (HACCP) verification testing performed by the Food Safety and Inspection Service (FSIS). Finally, the GAO report found that NARMS does not collect data pertaining to antibiotic use and resistance at animal production facilities (GAO, 2011).

The GAO report recommendations for action included directing US government agencies to "…identify potential approaches for collecting detailed data on antibiotic use in food animals, including the species in which antibiotics are used and the purpose for their use…", and "to enhance surveillance of antibiotic-resistant bacteria in food animals…modify NARMS sampling to make the data more representative of antibiotic resistance in food animals and retail meat throughout the US (GAO, 2011)." The GAO report suggests that DANMAP, Denmark's comprehensive antibiotic resistance monitoring program, could serve as a model for improving NARMS. The DANMAP program provides both human and animal data on antibiotic use and antibiotic-resistant bacteria that are sufficiently detailed to allow analysis of trends and effects of policies regarding antibiotics (GAO, 2011, DANMAP, 2011). With the goal of improving the NARMS program, the FDA has funded several pilot studies to investigate sampling methods to obtain samples representative of production environments. In addition, FSIS has published Public Notice #13-13, which provides instructions to Public Health veterinarians at slaughter establishments on procedures for NARMS sampling of cecal contents for antibiotic-resistant bacteria (FSIS, 2013).

The existence of bacteria resistant to antibiotics of critical importance to human medicine will continue to be a prominent global issue, and questions regarding the contribution of antibiotic use in food animal production agriculture to antibiotic resistance in bacteria will continue to be directed at the meat industry. Regardless of potential improvements in governmental antibiotic resistance monitoring programs, additional scientific research will be required to address the issue of antibiotic-resistant foodborne pathogens because no monitoring program can provide all of the data required to improve the scientific understanding of antibiotic-resistant bacteria. For example, both the NARMS and DANMAP programs report on the prevalence of phenotypic antibiotic resistance in selected pathogens but do not explore the genetic basis of resistance. It is increasingly apparent from the experiences of Denmark and the EU that the complex issue of antibiotic resistance will not be solved by simple actions such as the imposition of severe restrictions on agricultural uses of antibiotics. Thus, science must endeavor to better understand the ecology of antibiotic resistance (i.e., the factors that contribute to the amplification, transmission, and persistence). The molecular mechanisms responsible for the physical transfer of MGEs that harbor antibiotic resistance genes are very well understood because they have been the subject of 50 years of intense laboratory study, but owing to a lack of sustained inquiry, scientific understanding of the ecology of antibiotic resistance is very poor and has been largely inferred from the behavior of laboratory-adapted bacterial strains during laboratory experiments (Djordjevic et al., 2013, Aminov and Mackie, 2007). Thus, the potential for the study of antibiotic resistance in agriculture to increase scientific knowledge is large and may impact food safety, animal health, and human health.

Research also is needed to better inform regulators. For example, the European Food Safety Authority (EFSA) recently recommended restriction or elimination of the therapeutic use of the expanded-spectrum cephalosporin ceftiofur in animal production because they concluded that ceftiofur use in animal production was at least partially responsible the increasing occurrence of expanded-spectrum cephalosporin-resistant Salmonella infections (EFSA, 2011). However, studies performed at the United States Meat Animal Research Center have demonstrated that when cattle are shipped to harvest levels of expanded-spectrum cephalosporin-resistant E. coli (the proposed reservoir of the plasmid that confers expanded-spectrum cephalosporin resistance) do not differ between ceftiofur-treated and untreated cattle (Schmidt et al., 2013). In conclusion, well-designed comprehensive studies of antibiotic-resistant bacteria throughout the meat animal production system will better define the impact of agricultural antibiotic use and increase scientific understanding of the ecology of antibiotic resistance. Commitment to the study of antibiotic-resistant bacteria present in meat production and processing environments will demonstrate that the food animal production and processing industries are contributing significantly to combating the problem of antibiotic resistance.

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