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Agricultural ceftiofur use and the dissemination of bacterial resistance: Genes of public health concern

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Humans as well as their companion and food animals all commonly receive antimicrobial drugs therapeutically in response to perceived or diagnosed bacterial infections. It is well accepted that this treatment provides selection pressure on the target organisms as well as non-target organisms, including intestinal flora. This selection pressure may lead to the proliferation of resistant clones, which can ultimately limit the therapeutic value of the antimicrobial drug in individuals and populations.

This inherent negative side effect of antimicrobial treatment has long been recognized as a "necessary evil" which must be tolerated and managed in order to reap the great benefits of these drugs. Recently however, the therapeutic use of antimicrobial drugs in food animals, particularly important "front line" human drugs, has been challenged as a public health risk. The premise is that therapeutic drug use in food animals during production selects for resistant bacteria in the intestinal flora, which may subsequently contaminate meat products during harvest and processing.

The zoonotic food-borne transmission of pathogenic enteric bacteria to humans is frequently reported. If these pathogens are resistant to antimicrobial drugs, then treatment failure may occur and outbreak severity may increase. In addition, normally non-pathogenic enteric bacteria such as *E coli* and *Enterococcus spp.* may contaminate meat products, and potentially provide a reservoir of bacterial resistance genetics to be transferred to the intestinal flora of humans that consume them. Current industry standards for the processing and distribution of fresh ground meat provide the opportunity for rapid and widespread dissemination of resistant enteric bacteria in human populations.

Clearly, there is the potential for a public health risk resulting from the therapeutic use of antimicrobial drugs in food animals.

Recent, highly publicized reports in the medical literature claim to have attempted to establish that the therapeutic treatment of food animals with one "front line" human drug, the third-generation cephalosporins, poses a significant public health risk. Ceftiofur (Naxcel and Excenel) is the only third generation cephalosporin approved by FDA for use in food animals, and is available by veterinary prescription only. It is widely perceived by dairy producers and their veterinarians to be an effective treatment for a variety of common health conditions including respiratory disease, metritis, and bovine foot rot. Dairy producers widely and routinely use this drug because it has no withholding time for milk and a very short withdrawal time in meat.

Although ceftiofur is only used in animals, other third generation cephalosporins are also widely and commonly used in human medicine in the form of ceftriaxone and as well as several other drugs, including ceftizoxime and cefixime.

Ceftriaxone is used to treat moderate to severe Salmonella infections, and is the treatment of choice for children with Salmonella infections because of its safety, low levels of bacterial resistance, and because fluoroquinolone drugs are not available for use in children under 12 years old. In addition, this class of drug is indicated for a variety of other important human health conditions including bone and joint infections, skin and soft tissue infections, and bacterial septicemia.

The first domestically acquired infection with a ceftriaxone resistant Salmonella was

observed only five years ago in 1998. Since that time, the emergence of ceftriaxone resistant Salmonella as an important public health concern has been documented. In 2002, the Centers for Disease Control investigated an epidemic of *Salmonella newport* infections that occurred in a five-state area between January and April. A similar epidemic of *Salmonella newport* infections was concurrently observed in dairy cattle in the same general geographic region.

These *Salmonella newport* isolates had a distinct and unique characteristic in that they were all resistant to ceftiofur, and had at least reduced susceptibility to ceftriaxone. In addition, these isolates were resistant to a wide variety of additional antimicrobial drugs of importance to both veterinary and human medicine.

A follow-up investigation revealed that human cases were associated with direct exposure to dairy farms, ill cattle, unpasteurized milk and cheese products, as well as undercooked ground beef products. In only four short years, Salmonella isolates resistant to third generation cephalosporins went from unknown to epidemic, with an implied but unproven zoonotic component.

Incredibly, while anecdotal reports are commonly cited as evidence that antimicrobial drug use in food animals is an important public health risk, in each case the role of animal drug use is only hypothesized and never actually established or measured. Proposed restrictions on antimicrobial drug use in food animals have the potential to greatly impact agricultural productivity and veterinary medicine, in addition to the obvious effects on animal health and well-being. Therefore, a more complete scientific assessment of the potential for antimicrobial drugs used therapeutically in food animals to impact public health is seriously needed before national policy decisions are made.

Science-based risk assessments

Highlighting the importance of a science-based risk policy, FDA has recently released a guidance document with recommendations for *"Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Public Health Concern."* The risk assessment includes estimation of exposure, release, and consequence assessments, with risk being defined in qualitative measures (high, medium, low).

Release assessment encompasses measurement of selection pressure of the antimicrobial on bacterial populations in the target animal; exposure encompasses the risk of human consumption of these resistant bacteria; and consequence assessment considers the negative health outcomes as a result of human consumption. Although the guidance document currently only recommends a qualitative assessment, a quantitative approach would more accurately reflect the risk to human health by assessing the quantitative response by the microbiological flora of animals (release) as well as an enumeration of the probable dose ingested by humans consuming contaminated foods (exposure). Consequence assessments are currently based entirely upon the relative importance of the antibiotic to human medicine, which fails to address the actual rates of human disease caused by these resistant pathogens.

Although a qualitative risk assessment is an important first step toward a science based risk policy (and is often the only possible assessment given the lack of research evaluating the effect of antimicrobial use in animals on human health), quantitative assessments may more accurately reflect the risk presented by antimicrobial use in animals. This would allow for assessment of not only whether animals will harbor resistant pathogens as a result of antibiotic use, but also the quantity of those bacteria they harbor and whether this is linked to the probability of food contamination.

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Additionally, quantitative measurement would estimate the dose of resistant bacteria humans are likely to consume in contaminated product. Finally, a better understanding of genetic diversity will shed light on what mechanisms are important for emergence and transmission of resistance (such as dominance of flora by resistant clones or transmission of plasmids between bacteria), which may lead to a better understanding on risk management strategies.

Third generation cephalosporins.

Third generation cephalosporins are broad spectrum beta-lactams with activity predominantly against Gram-negative bacteria. Bacterial resistance to third generation cephalosporins is predominantly mediated through production of beta-lactamases (which deactivate the beta-lactam) and decreased diffusion as a result of alteration of porins. Plasmid mediated AmpC beta-lactamases have been recently well reviewed.[30,31]

Initially, resistance to third generation cephalosporins were noticed in bacteria that harbored a chromosomally encoded beta-lacatamase (e.g. Citrobacter, Serratia and Pseudomonas) that was present prior to the introduction of penicillins.[31] *E coli* and *Shigella* harbor a chromosomally located AmpC ß-lactamase, but it is weakly expressed. Later, resistance emerged as a result of mutations in plasmid encoded TEM or SHV type Extended Spectrum beta-lactamases (ESBL), but these could be blocked by the use of beta-lactamase inhibitors (clavulanate, etc).

In the late 1980s, plasmids encoding AmpC-type beta-lactamases were recognized in bacteria that were previously considered Amp-. Over 20 plasmid-borne ampC genes have been identified. These plasmid mediated resistance genes conferred resistance to third generation cephalosporins and were not susceptible to commercially available beta-lactamase inhibitors. The plasmid located genes bear strong sequence homology to the chromosomally located AmpC beta-lacatmases, suggesting that there was a mobilization of this chromosomal allele to a plasmid.

This sequence homology between chromosomal and plasmid mediated AmpC beta-lacatmases requires that the location of the AmpC gene be identified as chromosome or plasmid. Although most of these resistant isolates have been cultured from human clinical cases, resistance in community acquired human infections and veterinary isolates has also been identified.[30] Plasmid-located AmpC beta-lacatmases have been identified in Africa, Europe, Asia, the Middle East, and the Americas.

The third generation cephalosporin resistant Salmonella identified and investigated to date in the U.S. have harbored the plasmid borne ampC gene CMY-2. Based on sequence phylogeny, is thought to have moved to a plasmid from the chromosomally encoded AmpC-beta-lactamase gene of *Citrobacter freundii*.[31]

Bacteria harboring plasmid mediated CMY-2 have been isolated from many domestic animal species.[4,18,27,32-34] Comparisons of plasmids between *E coli* and *Salmonella* harboring CMY-2 isolated from animals and humans suggests that common plasmids have been transferred between animal-associated *E coli* and *Salmonella* and that, since identical genes were identified in human and animal isolates on similar plasmids, that it may have been transmitted between humans and animals.[27]

Additionally, it appears that based on chromosomal DNA patterns of bacteria harboring plasmids that contain CMY-2, the dissemination of this resistance genetics is not attributable to spread of bacterial clones, but transfer of the plasmid genetics between bacteria.[18]

An important aspect of these CMY-2 harboring plasmids, particularly from the standpoint of understanding the association of ceftiofur use and emergence, is that genes conferring resistance to other classes of antimicrobials are also found on the same plasmid.[18,33] This is important to consider, as other antimicrobial exposures may co-select of third generation cephalosporin resistance and vice versa.

References available upon request to the Editor, Ragan Adams, radams@colostate.edu

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