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Ecology of antibacterial resistance

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Development of resistance to antimicrobials has been well-documented for some bacteria (Levy 1997; Salyers and Amabile-Cuevas 1997). Of particular concern is resistance to antibacterial drugs of clinical importance. Amidst worries that the ability to treat bacterial infections might soon return to the "pre-antibiotic era," researchers are now seeking alternative therapies and prevention measures, as well as developing new classes of antimicrobials for use in human and animal medicine. To help solve the complex problem of reducing resistance, it is useful to identify activities that are major contributors to the emergence of resistance. The alteration or elimination of these activities could then slow the loss of antibiotic efficacy.

Antibiotic use is the major selection pressure influencing changes in antibiotic resistance. It must be emphasized, however, that resistant bacteria can also be resistant for reasons entirely independent of the use of antibiotics. Bacterial pathogens that are typically considered "food-borne" can be introduced onto agricultural premises from human sources, such as human wastewater plants (Kinde et al. 1996). The discharge of wastewater from animal agricultural facilities (Aminov et al. 2002; Chee-Sanford et al. 2001), human sewage treatment plants (Kinde et al. 1997; Kinde et al. 1996), hospitals (Guardabassi et al. 1998; Reinthaler et al. 2003) and pharmaceutical plants (Guardabassi et al. 1998) has been associated with increased levels of zoonotic pathogens as well as increasingly resistant and virulent organisms. Antibiotics are often discharged from these sites. Once in the environment these antibiotics can act as a selection pressure, further influencing the acquisition of resistance genes (Kummerer 2003; Kummerer and Henninger 2003). All of these possibilities must be considered in order to identify the causes of resistance and to subsequently estimate the amount of risk attributable to animal antibiotic use.

Another issue that must be considered in relation to antibiotic resistance is the presence of genetic linkages. Genes that confer resistance to antimicrobial agents are often linked genetically within the bacterial cell (Summers 2002). In addition antimicrobial resistance genes can be linked to genes that influence the organism's fitness but that have nothing to do with antibiotic resistance. A factor that selects for any one of these genes might be selecting for all genes that are linked to the gene under pressure. Consequently there can be causes for observed resistance levels that might have no relation to primary antibiotic use.

Consider a hypothetical scenario of a genetic linkage between two genes which confer resistance to two unrelated antibiotics. When one antibiotic is used, the antibiotic resistance genes to both antibiotics are under a positive selection pressure because they are genetically linked within the bacterium. Consequently, the linkage will result in an increase in the levels of resistance to both antibiotics, whether or not both are being used. In addition, the linkage between the two genes will make it difficult to quantify the relationship between antibiotic use and antibiotic resistance.

Many examples of this effect have been described. As some countries have reduced the antibiotics that are used in animal agriculture, specific resistances have persisted in the apparent absence of primary selection pressure (Aarestrup et al. 2001; Heuer et al. 2002; Sorum et al. 2006). Upon a more thorough genetic analysis of these isolates, (Borgen et al. 2002; Aarestrup et al. 2001) linkages to other antibiotics that are still in use can partially explain this persistence. Commonly used disinfectants can also help explain certain patterns of antimicrobial resistance. For example, quaternary ammonium compounds (QAC) are used in animal production, and resistance can develop to these disinfectants (Sander et al. 2002; Sidhu et al. 2002b). There are now many examples of QAC resistance genes that have a genetic linkage with antimicrobial resistance genes (Sidhu et al. 2002a; Sidhu et al. 2001). The location of the QAC resistance gene is often on a plasmid thus enabling an increased potential for horizontal gene transfer among bacteria. In addition, a QAC resistance gene cassette has been found within the class I integron in various bacteria, including *Salmonella* spp. (Guerra et al. 2002). Consequently, the presence of genetic linkages allows antibiotics or disinfectants to select for antibiotic resistance in the absence of primary selection pressure.

With respect to antibiotic use in the swine industry, there is a linkage among resistance genes which might impact the future efficacy of several antibiotics. Tetracyclines, third generation cephalosporins, and florfenicol are all used in swine production. Florfenicol is a fluorinated analog of chloramphenicol that was approved by the Food and Drug

Administration (FDA) in 1996 for the treatment of bovine respiratory disease pathogens. It was recently approved for use in swine production in the U.S. Resistance to florfenicol in *E. coli* and *Salmonella* has primarily been related to a gene known as *flo*. This gene confers resistance to florfenicol and chloramphenicol and is typically located on large transferable plasmids (Meunier et al. 2003). The *flo* gene is often located near other resistance genes within *E. coli* and *Salmonella*. For example, on some plasmids, the *flo* gene can be found linked to *tetA*, a tetracycline resistance gene, and *cmy-2*, a gene conferring resistance to third-generation cephalosporins. Consequently, the use of any one of these three antibiotics has the potential to select for bacterial strains that are resistant to all three antibiotics. This might help explain why resistance to florfenicol can be found in swine production systems even though the antibiotic was only recently approved.

Concerns about antibiotic resistant bacteria infecting humans continue to grow. One way in which to reduce the overall level of resistance is to reduce the use of antibiotics, especially those that are important in human medicine. For this reason, florfenicol would appear to be an attractive option because it is not used in human medicine. In addition, chloramphenicol is no longer used in humans in many countries. Therefore, one might expect florfenicol use to pose little risk to human health. Unfortunately, a theme that will continue to become more and more common as we delve into bacterial genetics is the presence of multiple resistance genes that are linked within the bacterial cell. The use of antibiotics that appear to have no relevance in human medicine may still be selecting for resistances to antibiotics that are important in human medicine. Because the complete cessation of all antibiotics in animal production is not a viable option, the key is to continually monitor changes in antibiotic resistance over time, especially as the use of new compounds increases. Only through a rigorous monitoring program can we evaluate the potential impacts of the use of an antibiotic on resistances to other antibiotics and thus comprehend the animal and human health risks. In addition, such a monitoring program would help ensure that the most efficacious antibiotic is being used for each specific health problem.

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