

Multi-Drug Resistant *Salmonella*

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Introduction

Among foodborne pathogens, *Salmonella* present a significant public health hazard as *Salmonella* caused the greatest number of foodborne deaths in the United States and was among the top three most frequent causes of bacterial foodborne illness in the United States (Mead *et al.*, 1999). In 2002, *Salmonella* Typhimurium and *Salmonella* Newport accounted for 2 of the top 3 most common *Salmonella* serovars isolated from humans, and both of these serovars are commonly associated with multi-drug resistance patterns; 27% of *Salmonella* Typhimurium were resistant to ≥ 5 antimicrobial drugs and 22% of *Salmonella* Newport isolates were resistant to ≥ 8 antimicrobial drugs (CDC, 2004). Multi-drug resistance can present difficulties when treating severe *Salmonella* infections. Moreover, recent data indicate that some multi-drug-resistant *Salmonella* may be more virulent than pansusceptible variants.

Emergence of Antimicrobial Drug Resistance

To fully appreciate the complexities associated with the development of antimicrobial drug resistance in bacteria, several mechanisms associated with the development of resistance must be examined. It is commonly speculated that antimicrobial drug resistance in animal bacterial isolates occurs largely due to genetic mutations as a result of antibiotic use in animals. While this phenomenon can occur, it provides an incomplete and frequently incorrect picture of the development of bacterial antimicrobial drug resistance in animal agriculture. It is likely that exposure-related mutation only accounts for a small fraction of resistance emergence in bacterial populations in animals. It is important to note that resistant bacteria are almost always

present in the environment as well as in animals, and a variety of factors can allow the expansion of the resistant populations. Moreover, some bacteria are intrinsically (natural attribute of an organism) resistant to antimicrobial drugs. Acquired resistance generally develops either through mutation or acquisition of exogenous DNA; the latter being the most important

Acquired resistance occurs through acquisition of exogenous genetic material or through a genetic mutation in the DNA of the microorganism. Mutations in the DNA of a bacterial cell probably play a small role in the emergence of antimicrobial drug resistance. An example of this is the development of fluoroquinolone resistance following a point mutation in the *gyrA* gene of the bacterial DNA. The development of resistance is usually stepwise and causes a gradual decrease in susceptibility to the antimicrobial drug.

Acquired resistance through the acquisition of exogenous genetic material is more common than mutations. This can happen within or between species through the acquisition of mobile genetic elements. It is likely the most important mechanism for resistance dissemination. Plasmids play an important role as mobile genetic elements. They are exogenous, self-replicating, extra-chromosomal DNA that carry adaptation genes for bacteria and commonly carry resistance genes. Plasmids are transferred from one bacterial cell to another through conjugation and can occur across species. Transposons are another mobile genetic element that can also undergo conjugation and carry resistance genes between bacterial cells.

Integrations are also important in moving DNA from one bacterial cell to another. Integrations are scavengers of foreign DNA that can be present in the environment and they allow for rapid adaptation in hostile environments. Integrations can be inserted into plasmids, transposons, or into the chromosomal material and are commonly associated with the development of multi-drug resistance in bacterial cells. Once the gene cassettes from the integrations have been inserted into the bacterial DNA they can be stored for future needs and they can be transferred among bacterial cells.

Another means whereby there is an increase in multi-drug resistance (MDR) in populations is through genetic propagation. This occurs through clonal expansion or through horizontal transfer of genes among unrelated bacteria. During clonal expansion, some selective pressure (eg the presence of an antimicrobial drug) allows the resistant

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**Proceedings of the 59th American Meat Science Association
Reciprocal Meat Conference** (pp. 25-28)
June 18-21, 2006, Champaign-Urbana, Illinois
www.meatscience.org

population to expand while suppressing the susceptible population thus allowing the resistant population to become dominant. Horizontal transfer is similar to the concept of mobile genetic elements as discussed previously. However, in this case, a resistant population of bacterial cells can transfer resistance genes to another species of bacteria after the selective pressure has been applied thus allowing two populations of resistant organisms to arise.

When considering complexities of MDR in food-borne pathogens, one should consider the fact that genes can be genetically linked. Selective pressure may select for resistance of the antibiotic that is being used, but when resistance to this antibiotic develops additional resistance to other antimicrobial drugs also develops. The genes can also be linked to other factors such as resistance to acid, heavy metals, disinfectants, etc.

Salmonella and Multi-Drug Resistance

The development of multi-drug resistance in *Salmonella* is of great concern as *Salmonella* is a pathogen in humans and livestock and has presented a challenge in treating severe *Salmonella* infections. Previously, concern for multi-drug resistance in *Salmonella* focused on *Salmonella* Typhimurium definitive phage type (DT) 104. Multi-drug resistant *Salmonella* Typhimurium DT104 is associated with a resistance pattern to: ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (CDC, 2004; Ziprin and Hume, 2001). This pattern of resistance is also referred to as resistance type or R-type ACSSuT. *Salmonella* Typhimurium DT104 emerged in the United States in the early 1980s (Glynn *et al.*, 1998). Between 1984 - 1985, 1% of *Salmonella* Typhimurium isolates were DT104 and displayed an ACSSuT resistance pattern and the prevalence rose steadily to 69% by 1996; however, only 13 of 103 isolates with resistance pattern ACSSuT were phage-typed in 1996 (Glynn *et al.*, 1998). The antimicrobial resistance genes in *Salmonella* Typhimurium DT104 have been incorporated into the chromosome on a large integron complex which decreases the ability to lose the resistance factors, and resulted in clonal dissemination/expansion of *Salmonella* Typhimurium DT104 (Threlfall, 1994). Also important to note is the fact that no associations were ever made between DT104 outbreaks and antimicrobial drug use (Khakhria, 1983). It is possible that DT 104 has been replaced by other strain types. Additionally, in Norway, there are very strict controls governing on-farm antimicrobial drug use and DT104 is widely disseminated in this country.

In 2002, *Salmonella* Typhimurium was the most common serovar isolated from humans, and 27% of these isolates were resistant to ≥ 5 antimicrobial drugs (CDC, 2004). Also in 2002, *Salmonella* Newport was the third most common serovar isolated from humans, and 22% of these isolates were resistant to ≥ 8 antimicrobial drugs which is referred to as MDR-AMP C (CDC, 2004). There appears to be an emergence of *Salmonella* Newport MDR-Amp C in the US. The resistance in this organism is mediated by genetic determi-

nants located on both plasmids and chromosome. This pathogen has been associated with outbreaks in ground beef and is commonly recovered from human cases of salmonellosis.

Multi-drug resistance in *Salmonella* Typhimurium can be conferred via plasmids (Vahaboglu *et al.*, 1996) and other genetic elements as previously described. Furthermore, Tosini *et al.* (1998) characterized 3 different integrons (In-t1, In-t2 and In-t3) present on two types of conjugative plasmids (IncFI and IncL/M) that encoded resistance for a variety of antimicrobial drugs in *Salmonella* Typhimurium. Interestingly, Carattoli *et al.* (2001) examined five strains of *Salmonella* Typhimurium isolated in the 1970s and one strain isolated in 1997 and found that the plasmids present in the 1970s isolates conferred a similar pattern of resistance to the 1997 isolate; 3 of the 1970s plasmids having an almost identical pattern of resistance when compared to the 1997 isolate. In the same study, it was observed that the IncFI plasmids from 3 of the 1970s strains and the 1997 strain contained a conserved integron (In-t2); the 1997 isolate contained a second integron (In-t1) in the IncFI plasmid. Hence, the components that confer multi-drug resistance appeared to have been present in *Salmonella* Typhimurium for at least 30 years, but these strains did not increase in prevalence immediately. Furthermore, as would be expected in any biological system, evolution continues to occur and more mobile genetic elements are being introduced to the *Salmonella* Typhimurium genome over time which may confer additional resistance genes. Ribot *et al.* (2002) examined strains of *Salmonella* Typhimurium DT104 isolated from human infections in 1985, 1990 and 1995 and found that all strains contained very similar integrons that were responsible for the multi-drug resistant phenotype. Therefore, the chromosomally-incorporated genetic elements have remained highly stable over time.

Antimicrobial Use in Cattle

The use of antimicrobial drugs in the cattle industry is common. Subtherapeutic doses are used to prevent liver abscesses and improve feed efficiency (NAHMS, 1999). Additionally, therapeutic doses are commonly given upon arrival to prevent infectious diseases (Morck, 1993; Singer, 2003) or are administered during the feedlot period to treat infectious diseases (NAHMS p I and II, 1999). In theory, the use of antimicrobial drugs in the feedlot environment could potentially select for resistant bacteria that could be transferred to humans through the food supply. However, the presence of multi-drug resistant microorganisms, especially *Salmonella*, is unlikely to be linked back to one specific practice or event. Multi-drug resistant *Salmonella* are present in both animals and humans, but the understanding of the link between the two is not currently well understood.

Commonly used antimicrobial drugs in feedlot production in the US include monensin, an ionophore and tylosin, a macrolide. These drugs are fed through the entire feeding period with 96.5% (NAHMS P I) given ionophores and

42.3% given tylosin. These drugs are given to improve feed efficiency and to prevent liver abscesses in the animal, respectively. Other antimicrobials that are less commonly used, but also administered in the feed, are chlortetracycline, oxytetracycline and combinations of chlortetracycline and sulfamethazine (NAHMS P II). Virginiamycin, a streptogramin is also approved for long term subtherapeutic use but it is not commonly used in current production systems (Rogers, 1995).

Therapeutic drugs are commonly given in injectable forms and include tilmicosin, a macrolide, which is given cattle entering the feedyard to prevent bacterial diseases or bovine respiratory disease (BRD)(NAHMS P II, 1999). This practice is referred to as metaphylaxis (mass treatment) and approximately 10.4% of cattle entering feedyards are given this treatment (Vogel, 1998). Injectable drugs are also given to cattle when they are diagnosed with BRD. Nationwide, 14.4% (4 million) cattle require treatment for BRD (NAHMS P II, 1999). The most commonly given antimicrobial drugs for this illness are tilmicosin, florfenicol, enrofloxacin, oxytetracycline and ceftiofur.

Public Health Concerns

Antimicrobial drug resistance has emerged as one of the highest priorities for public health officials in developed countries. Resistance increases the cost of health care and the morbidity and mortality of diseases. In recent years, there has been an increase in resistance of various pathogens important to human health and it has been speculated, but unproven, that the resistance is linked back to antimicrobial drug use in livestock production. Because antimicrobial drugs are commonly used in animal systems, it is likely that this has some impact on the emergence of antimicrobial drug resistant populations. However, the extent to which this occurs and the final impact on human health is uncertain. There is a public health perception that this phenomenon has a serious and significant impact on human health. However, it is important separate statements based on sound data from those based on speculation and emotion.

Zhao *et al.* (2003) reported that *Salmonella* Newport MDR-AmpC was commonly identified in humans and in food animals indicating that this pathogen could be transmitted through the food chain from the animals to the humans. Fey *et al.* (2000) proposed that antibiotic resistant strains of *Salmonella* in the United States evolve primarily in livestock. This study was based on a single illness and a single animal observation. In a request for proposals from the CDC, they reported that antimicrobial resistance in *Salmonella* and other foodborne bacteria is largely a consequence of the use of antimicrobial agents in food-producing animals. The statements made by these studies are broad-sweeping, generally speculative, and at present not supported by the data. More evidence is needed to determine the impact of the use of antimicrobial drugs in animals on

the emergence of antimicrobial drug resistant pathogens in human health.

In regard to human health issues, vancomycin-resistant *Enterococcus* (VRE) in patients results in a 5-11 day increase in the duration of the illness, and \$11,000-\$27,000 increase in cost and a 5 times greater likelihood of mortality. Fluoroquinolone resistant *Salmonella* is associated with a 10 fold increase in mortality while *Salmonella* with the ACSSuT pattern is associated with a 5 fold increased risk of death. These bacterial infections which were once treatable with low cost antibiotics, are not treatable or are only treatable with expensive alternatives.

In addition to being less treatable, recent evidence indicates that some resistant variants of *Salmonella* are associated with a more severe illness (more virulent). It has been reported that there is a higher risk of death (Helms, 2002) and septicemia is more likely to result if a resistant variant of *Salmonella* is present (Varma, 2005). Additionally, hospitalization is more likely to occur if the *Salmonella* exhibits antimicrobial drug resistance (Varma, 2005).

Conclusions

There are several unknowns when dealing with MDR. We know that the acquisition, emergence, and maintenance of antibiotic drug-resistance is very complex and is not fully understood. Recently the World Health Organization reported that Through massive and increasing use of antimicrobials in human, animals, fish and in agriculture, a resistance problem has been created that is rapidly moving to the forefront of public health concerns. While the emergence of antimicrobial drug resistance is an important public health concern, many data still are needed to identify the actual impact of animal practices on human health.

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