

# Development of Resistance in *Salmonella* Isolates of Veterinary Origin

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## Introduction

The emergence of resistance to antimicrobics has compromised control of many bacterial pathogens. Recently, resistance has been observed in bacteria known to cause plague and well as *Staphylococcus aureus*, a common pathogen of wound and blood infection. Additionally, multiple resistance has also emerged among many bacterial strains including *Salmonella* species. A penta-resistant strain (*Salmonella typhimurium* DT104) in which the resistance genes have been chromosomally integrated is proving to be particularly problematic resulting in increased morbidity and mortality in both animals and humans<sup>1-5</sup>. The main reservoir appears to be cattle although it has been recovered from a variety of animal species<sup>1,3</sup>. The development of antimicrobial resistance has emerged as a global problem. Expert scientific groups such as the Institute of Medicine, the American Society for Microbiology and the World Health Organization expressed apprehension about the national and global increase in antibiotic resistance and the complex issues surrounding the increase in the community and institutional settings<sup>6-8</sup>.

The development of resistant human pathogenic bacteria results from direct use of antimicrobial agents in humans and acquisition of resistant organisms or resistance factors from animal and environmental bacteria<sup>9</sup>. Recovery of antibiotic resistant bacteria occurs more often in urban than rural settings implicating contaminated food products as the likely vehicle rather than the actual animals<sup>10</sup>. Although food borne illness transmitted through foods contaminated by infected human food handlers must be considered along with animal sources<sup>11</sup>, this mode of transmission has historically been of less importance. Person-to-person spread of food borne pathogens is also possible<sup>11</sup>, although food borne outbreaks are generally contained with few or no secondary cases except in institutions; transmission by this route is more common with *E. coli* and *Shigella* infections. Interestingly, the resistance patterns of intestinal flora from meat eaters does not differ when compared to vegetarians<sup>12</sup>.

The intestinal flora of animals that have been treated or prophylaxed with antimicrobial agents can serve as a reservoir of resistance factors<sup>13</sup>. Use of antibiotics in animals can result in a human health hazard in a number of ways: if antibiotic-resistant bacteria pathogenic to humans are selected and food is contaminated during slaughter or food preparation, the bacteria may cause an infection that requires treatment and therapy is compromised; if antibiotic resistant bacteria pathogenic to humans are selected in the animal and food is contaminated, the bacteria may transfer the resistance to the other bacteria in the human gut; or if antibiotics remain as residues in animal products, the residues may allow the selection of antibiotic-resistant bacteria in the consumer<sup>13</sup>.

### **History of Antimicrobial Susceptibility Monitoring in the US**

Because of the public health concerns associated with the approval of fluoroquinolones for use in food-producing animals, an antimicrobial resistance monitoring program was proposed by the Food and Drug Administration Center for Veterinary Medicine (FDA) as a post-marketing activity to help ensure the continued safety and effectiveness of the fluoroquinolones. In early 1995 the USDA began a study to assess the degree of resistance among *Salmonella* isolates of veterinary origin. In late 1995, CDC, the USDA, and the FDA established the National Antimicrobial Susceptibility Monitoring System to prospectively monitor changes in antimicrobial susceptibilities of zoonotic pathogens from human and animal clinical specimens, from healthy farm animals, and from carcasses of food-producing animals at slaughter<sup>14,15</sup>. Non-typhoid *Salmonella* was selected as the sentinel organism because it is an important food borne pathogen, it is ubiquitous in nature, it is reportable for CDC, and both human and veterinary isolates are available.

Veterinary testing is conducted at USDA's Agricultural Research Service facility in Athens, GA. Isolates are selected from testing conducted by the USDA or collaborators, from the National Animal Health Monitoring System studies, from clinical sources, especially from the National Veterinary Services Laboratories, and from slaughter samples. Testing is done using a semi-automated system (Sensititre™ Accumed, Westlake' Ohio) for the testing of isolates. All isolates are maintained at -70 °C to serve as a bank for future use. A description of the antimicrobics and their concentrations is shown in Table 1. Because the systems evolved independently before merging, for FY95 and FY96 the USDA used plates with breakpoint configurations while the

CDC used an MIC format. In 1997, both systems are using a MIC format with the same antimicrobics to compliment both the veterinary and human studies.

### Goals and Objectives

The goals and objectives of the monitoring program are to 1) provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms from the human and animal populations; 2) facilitate the identification of resistance in humans and animals as it arises; 3) provide timely information to veterinarians and physicians; 4) prolong the life span of approved drugs to promote prudent and judicious use of antimicrobics; and 5) identify areas for more detailed investigation. Information resulting from the monitoring program and follow-up outbreak investigations will be distributed to veterinarians, physicians, and food animal producer groups in a timely manner. Use of the information will be targeted to redirecting drug use so as to diminish the development and spread of resistance over the short term with directives involving long-term use developed in collaboration with the appropriate professional practitioner groups. Outbreak investigations and field studies will be initiated as a result of major shifts or changes in resistance patterns in either animal or human isolates.

### Outcome

As part of the 1995 baseline study for the monitoring system 1,041 *Salmonella* isolates of veterinary origin were tested against 16 antimicrobics using a Sensititre™ custom designed microtiter plate. Breakpoint concentrations were used for all antimicrobics. Isolates were obtained from cattle, swine, chickens, turkeys, swine feed, ground product, exotics, dogs, and cats from both clinical and non-clinical isolations. All isolates were susceptible to amikacin, cefotaxime, and ciprofloxacin. Approximately 34% and 28% of the isolates were resistant to tetracycline and sulfamethoxazole, respectively while 13% of the isolates were resistant to both ampicillin and ticarcillin. The following percent resistance was observed for all other antimicrobics - amoxicillin/clavulanic acid (1%), apramycin (1%), ceftiofur (<1%), cephalothin (2%), gentamicin (4%), neomycin (8%), piperacillin (7%), ticarcillin/clavulanic acid (4%), and trimethoprim/sulfa (1%).

As part of the 1996 study for the monitoring system 1,921 *Salmonella* isolates of veterinary origin were also tested against 16 antimicrobics. Breakpoint concentrations were used for all antimicrobics. Isolates were obtained from cattle, swine, chickens, turkeys, swine feed, ground product, exotics, dogs, and cats from both clinical and non-clinical isolations. All isolates were susceptible to amikacin and ciprofloxacin. Approximately 47% and 35% of the isolates were resistant to tetracycline and sulfamethoxazole, respectively, while 19% of the isolates were resistant to both ampicillin and ticarcillin. The following percent resistance was observed for all other antimicrobics - amoxicillin/clavulanic acid (3%), apramycin (2%), cefotaxime (1%), ceftiofur (2%), cephalothin (3%), gentamicin (8%), neomycin (14%), piperacillin (13%), ticarcillin/clavulanic acid (9%), and trimethoprim/sulfa (4%). Comparison with the baseline 1995 data indicates an increase in resistance among all antimicrobics except amikacin and ciprofloxacin. Ninety-seven serotypes were identified within the 1,921 isolates. Of the 97 serotypes tested, 51% are susceptible to all antimicrobics while 7% each were resistant to only one or two antimicrobics. Of 137 (for 1995) and 427 (for 1996) *S. typhimurium* (including copenhagen) isolates tested, 14 and 45, respectively, (1.3% and 2.3% of the total number of isolates) were resistant to the five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline; ACSSuT) to which *S. typhimurium* DT104 is commonly resistant.

As part of the 1997 study 1,812 *Salmonella* isolates of veterinary origin have been tested to date against 17 antimicrobics using a Sensititre™ custom designed microtiter plate (additional samples will be tested through January 1998). Minimal inhibitory concentrations (MICs) were used for all antimicrobics. Isolates were obtained from cattle, swine, chickens, turkeys, carcass rinses/washes, swine and cattle feeds, exotics; dogs, and cats. These isolates were from both clinically ill and non-clinical animals. Overall, all isolates were susceptible to amikacin and ciprofloxacin. The following percent sensitivity was observed for all other antimicrobics - amoxicillin/clavulanic acid (94%), ampicillin (91%), apramycin (98%), ceftiofur (>99%), ceftriaxone (>99%), cephalothin (97%), chloramphenicol (96%), gentamicin (95%), kanamycin (92%), naladixic acid (>99%), streptomycin (86%), sulfamethoxazole (88%), tetracycline (76%), ticarcillin (91%), and trimethoprim/sulfa (98%). More resistance was observed for cat and turkey isolates as compared to all other species. Analysis of the MIC values for ciprofloxacin and naladixic acid indicate that >99% of the isolates for both antimicrobics had MIC values less than or equal to 0.03 and 8, respectively. Eighty-six different serotypes were identified and 41

serotypes (48%) were susceptible to all antimicrobics. Multiple resistance was observed for the majority of the 45 other serotypes. Within the *S. typhimurium* (n=103) and *S. typhimurium* var. *copenhagen* (n=102) serotypes resistance was observed for 14 and 12 antimicrobics, respectively. Other serotypes with resistance to 9 or more antimicrobics include *S. agona*, *S. derby*, *S. hadar*, *S. heidelberg*, *S. krefield*, *S. ohio*, and *S. worthington*. Within the *S. typhimurium* and *S. typhimurium* var. *cop* serotypes, penta-resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (ACSSUT) typically associated with phage type DT104 has been observed for 29/205 isolates. The development of multiple resistance is particularly worrisome as clinical illness may be more severe and treatment (when indicated) may be compromised. With the limited availability of new drugs to combat pathogens, prudent and judicious use of antimicrobics is warranted.

#### DT104

In 1995 and 1996, 137/1,041 (13.2%) and 427/1,921 (22.2%) of the isolates were serotype *S. typhimurium* including var. *copenhagen*. Of these 14/1,041 (1.3%) and 45/1,921 (2.3%) for 1995 and 1996, respectively, were resistant to the five antimicrobials (ACSSuT) to which *S. typhimurium* DT104 is commonly resistant. Following phage typing 9/14 (64.3% of ACSSuT; 6.6% (9/137) of *S. typhimurium* isolates; or 0.9% (9/1,041) total isolates) and 10/45 (22.2% of ACSSuT; 2.3% (10/427) of *S. typhimurium* isolates; or 0.5% (10/1,921) total isolates) were identified as DT104 for 1995 and 1996, respectively. Isolates were recovered from swine, cattle, chicken and cats. Isolates for 1997 are being phage typed. These data confirm the emergence of DT104 in the U.S. and implicate multiple species as reservoirs. The identification of DT104 and the capability to investigate resistance patterns and trends identified through the Monitoring System are essential elements to facilitate timely and appropriate public health response activities.

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Table 1. Antimicrobial agents used in the National Antimicrobial Susceptibility Monitoring System and concentrations in ug/ml

Veterinary Isolates Breakpoint Plate	Veterinary and Human Isolates
Amikacin (16 - 32)	Amikacin (4 - 32)
Amoxicillin/Clavulanic Acid (8/4 - 16/8)	Amoxicillin/Clavulanic Acid (0.5/0.25 - 32/16)
Ampicillin (16 - 32)	Ampicillin (2 - 64)
Apramycin (8)	Apramycin (2 - 16)
Ceftiofur (2 - 4)	Ceftiofur (0.5 - 16)
Cepotaxime (8 - 32)	Ceftriaxone (0.25 - 16)
Cephalothin (8 - 16)	Cephalothin (1 - 32)
Ciprofloxacin (1 - 2)*	Chloramphenicol (4 - 32)
Gentamicin (4 - 8)	Ciprofloxacin (0.015 - 2)
Neomycin (8)	Gentamicin (0.25 - 16)
Piperacillin (16 - 64)	Kanamycin (16 - 64)
Sulfamethoxazole (256)	Nalidixic Acid (4 - 64)

Veterinary Isolates Breakpoint Plate	Veterinary and Human Isolates
Tetracycline (4 - 8)	Streptomycin (32 - 256)
Ticarcillin (16 - 64)	Sulfamethox. (128 - 512)
Ticarcillin/Clavulanic Acid (16/2 - 64/2)	Tetracycline (4-64)
Trimethoprim/Sulfamethox (2/38)	Ticarcillin (2 - 128)
	Trimethoprim/Sulfamethox. (0.12/2.4 - 4/76)

\*1996 Veterinary isolate testing included determination of minimum inhibitory concentrations to nalidixic acid (0.5 - 32) and ciprofloxacin (0.03 - 4)