

The Antimicrobial Dilemma in Animal Production

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The Bacteria:

Salmonella are ubiquitous in nature and have been recovered from nearly all vertebrates (Taylor and McCoy 1969). Over 2,400 species have been identified. More cases of meat and poultry food borne disease are attributed to *Salmonella* or *Campylobacter* than any other agent (USDA: APHIS 1990). In a study of food borne disease from 1977 to 1984, Bryan (1988) observed that pork was responsible for 11 % of the *Salmonella* outbreaks attributed to meat. Lammerding et al. (1988) recovered *Salmonella* from a number of different animal carcasses at slaughter. While the impact on the consumer is manifested after consumption of contaminated foods, the problem begins with infected animals on the farm.

Salmonellosis is also a major animal disease problem costing millions of dollars of lost income to the animal industries. Animals that harbor a bacterium, which they may or may not shed into the environment, are called carriers. Carrier animals are an important component in the epidemiology of *Salmonella* and all animal species have been implicated as carriers. Carriers can shed both long-term and short-term. Information regarding development of a carrier state is limited and confined to experimental work or retrospective field observations. In swine, Fedorka-Cray et al. (1994) demonstrated that pigs free of *Salmonella* can become infected with and shed *S. typhimurium* within 2 days after exposure to infected animals. However, long term carriage was not studied. Wood et al. (1989) demonstrated that *S. typhimurium* can persist in low numbers in swine to slaughter weight. Gray et al. (1995, 1996a) recently determined that development of the carrier state in swine following challenge with *S. choleraesuis* is dose dependent. Persistence is observed through 15 weeks post-exposure. However, he also demonstrated that following natural exposure to an infected population, the number of pigs developing carrier status was relatively low (Gray et al. 1996b) suggesting differences between experimental and natural exposure.

The shift in public opinion regarding food borne illness stimulated a public policy change. Ironically, while surveillance statistics have long documented that microbial contamination is indeed a far greater problem in food than residues (Bean and Griffin 1990), little applied research has been directed toward identifying cost-effective strategies for reducing microbial contamination. Public health authorities historically focused on the final preparation stage, arguing that adequate handling and cooling would negate any risk associated with contamination. Recognition of the shared responsibility along the entire farm to table continuum has stimulated research on the application of prevention and control methodologies along the entire food chain.

Salmonella and *Campylobacter* species have been identified as the top two etiologic agents responsible for a majority of food borne illness. Determination of their exact role in transmission of resistance attributes is currently being studied. Assessment of the role of any one particular species within each genera is confounded by the sheer number of serotypes with each genera (especially with *Salmonella*), the ability to recover and ascertain the number of different serotypes within a particular sample, and the ability to differentiate between unique isolates within the same serotype. Additional variables include the origin of the isolate (IE from a clinical versus nonclinical submission) and the treatment history of the animal. Control at the farm may in some cases provide the most cost-effective means of eliminating the pathogens or problems of animal origin. However, as control

measures are implemented, use of antimicrobials, and the subsequent development of resistance, may confound inroads being made on pathogen control.

The Problem:

Antimicrobial use in both human and animal populations has increased. As a result of this increase, the development of resistant bacteria has emerged as a global problem (ASM, 1995; OTA, 1995). This is due in part to the availability of the antimicrobials and the efficacy they impart in control of certain infectious diseases. However, use, and in particular misuse, of antimicrobials can lead to the development of resistance to the antimicrobials. Antimicrobial resistance (AR) can either diminish effectiveness or render an antimicrobial ineffective as a therapeutic. Although use may result in a portion of bacteria that are resistant, the exact fate of this population in terms of persistence and transmission has been difficult to determine. In addition to AR occurring in nosocomial bacteria (which are most often limited to hospitals), commensal and zoonotic bacterial populations are also developing resistance to antimicrobials. Commensal bacteria can serve as vectors for transfer of resistance genes to zoonotic pathogens. Although use of antimicrobials is contraindicated in most cases of food borne illness, immune status, resulting septicemia, and other factors may warrant their use and resistance to treatment can result in increased morbidity and mortality.

Use patterns in veterinary medicine (therapeutic versus subtherapeutic use) can complicate the issue of emerging AR. In the animal population, antimicrobials are used both therapeutically and non-therapeutically. Therapeutic use is thought to play a minor role in the development of resistance. However, treatment of entire flocks or herds may alter resistance outcome. Conversely, use of antimicrobials in large numbers of animals in low concentrations over long periods of time for prevention of disease and/or performance enhancement is thought to significantly increase resistance. Since use of antimicrobial drugs can result in the selection of bacterial populations that may become resistant, particularly when the antimicrobial is over prescribed or improperly used, any use must be carefully monitored. No consensus has been reached over the non-therapeutic use of antimicrobials in food animal production and debate focuses on the merits and consequences of both therapeutic and non-therapeutic use in animal production.

Additionally, while transmission of resistant bacteria from animals to humans occurs, it has been difficult to assess the extent to which this occurs and the impact transmission has on actual dissemination of resistant populations. Despite growing concerns, information regarding the development and spread of AR in food borne and commensal bacteria is limited, and its impact on human health is poorly defined. Our goal is to identify and determine the etiologic fraction that may be attributed to the use of antimicrobials in animal production that impacts human health. This program leverages the information obtained through our participation in the National Antimicrobial Resistance Monitoring System-Enteric Bacteria (NARMS).

NARMS:

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 antimicrobials; 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.

To track emerging resistance, NARMS was established for both human and veterinary pathogens (CDC, 1996; Tollefson, 1996). *Salmonella* was chosen as the sentinel organism. *Campylobacter* (starting in 1998 for veterinary isolates) and *E. coli* 0157:H7 (when available) are also tested. Isolates are selected from research studies conducted by the USDA or collaborators, from the National Animal Health Monitoring System studies, from diagnostic sources (such as the National Veterinary Services Laboratories and Sentinel Sites which are Veterinary Diagnostic Laboratories), and from raw product collected from federally inspected slaughter and processing establishments.

Testing is done using a semi-automated system (Sensitre™, TREK™ Diagnostics, Inc., Westlake Ohio) at the USDA-ARS-RRC facility in Athens, GA (NARMS, 1997, 1998). Plates are custom made with 17 antimicrobials in an MIC format. This system is also used for the *E. coli* isolates. *Campylobacter* testing is done using the E-test (AB BIODISK). Testing for the human NARMS isolates is conducted at the CDC in Atlanta, GA using the same testing methodologies and antimicrobials as those used for the veterinary isolates.

A description of the panel of antimicrobials and their concentrations is shown in Table 1a and b. National Committee for Clinical Laboratory Standards (NCCLS) guidelines (1997) were followed throughout the testing procedure. Quality control strains *E. coli* ATCC 25922 and *S. typhimurium* ST129 (in-house strain) were used. Microtiter plates are read as per manufacturer's instructions. Isolates are classified as susceptible, intermediate, or resistant based on (NCCLS) established breakpoints used in human medicine with the exception of Cephalothin, Kanamycin, Streptomycin, Sulfamethoxazole, and Trimethoprim/Sulfamethoxazole (NCCLS, 1998). Currently, comparable veterinary breakpoints are not available.

Results and Discussion

Tables 2 and 3 indicate resistance over a three-year time frame. These isolates represented a broad range of species and came from diagnostic cases (Table 2) or from federally inspected establishments (Table 3). Diagnostic isolates were randomly collected from isolates submitted from throughout the country to the National Veterinary Services Laboratories, Ames, IA. The majority of diagnostic isolates were submitted as a result of a primary or secondary salmonella associated clinical illness in the host, although salmonellosis might not have been the primary etiologic agent in all cases. The salmonella slaughter samples were typically collected as part of the HACCP implementation testing.

Results indicated a higher level of resistance to antimicrobials among diagnostic isolates than for slaughter isolates from swine. This should be expected since diagnostic isolates generally represent isolates that have been problematic to treat. Many of these isolates would be submitted for serotyping because of a clinical episode that has led to a diagnostic effort. Since these diagnostic efforts are expensive, it is likely that something about these isolates stimulated the producer and the veterinarian to go to greater lengths than usual to determine the etiologic agent in a particular case. In addition, since these were from clinical cases, it is likely that many of these organisms were exposed to antimicrobial therapy. Therefore it should not be surprising that there was more resistance among diagnostic isolates than was seen for slaughter isolates.

The increase in resistance among diagnostic isolates requires further investigation and indicates a continued need for education among practitioners. Although the final outcome of diagnostic isolates with respect to entry into the food chain is unknown, these data suggest that there is not a predominant movement of antimicrobial resistant diagnostic isolates persisting through slaughter. Reports from the USDA-FSIS HACCP program suggest a reduction in prevalence of *Salmonella* (regardless of antimicrobial resistance status) since implementation of the program (8.7% of carcasses positive pre-HACCP v 6.5% post-HACCP implementation) (USDA, 1999). The decrease in percent antimicrobial resistance between years corresponds to this report and suggests that HACCP may be one tool for management of the prevalence of *Salmonella* which may, in turn, impact overall prevalence rates of resistant organisms.

In addition to the increase in resistance to individual antimicrobials, multiple antimicrobial resistance is also on the rise (Threlfall et al., 1994). As organisms become resistant to more antimicrobials, it compounds the problem of therapy. For diagnostic isolates from 1997 - 1999 the percent total swine diagnostic isolates that were either susceptible to all or resistant to only 1 antimicrobial was 39.5%, 32% and 36.8%, respectively. For slaughter isolates from 1997 - 1999 the percent total swine slaughter isolates that were either susceptible to all or resistant to only 1 antimicrobial was 55.7%, 64.8% and 51.6%, respectively. Resistance was most commonly to Tetracycline. The remaining isolates for each group per year were resistant to 2 or more antimicrobials.

The emergence of a multi-drug resistant strain of *Salmonella typhimurium* DT104 has caused a great deal of concern in the U.S. and abroad (Threlfall et al., 1994). One hallmark of this organism is a pattern of resistance that includes Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides, and Tetracycline (ACSSuT). DT104 has been isolated from swine (NARMS, 1997, 1998).

In an effort to determine the impact of antimicrobial use on the farm, a study was undertaken to determine the effect different antimicrobial use patterns have on the development/transfer/persistence of resistance on the farm. Three farms were selected based on their antimicrobial use: Farm A uses antimicrobials both therapeutically and subtherapeutically. For subtherapeutic medications, Aureomycin is used in the lactation feed, Mecadox is used in the nursery and Tylan 10 is used in the grower (40g/ton) and finisher (10g/ton). Farm B has routinely used antimicrobials in production for therapeutic purposes. However, following commencement of the study, Tylan 10 was used for five days or less as pigs entered the nursery to reduce the incidence of scours. Other subtherapeutic uses were recorded at varying times. Farm C has no routine antimicrobial use.

Farms were sampled once every three months. Approximately 150 samples (30/group) were collected from farrowing, gestating, suckling, nursery and finishing pigs. Samples were transported overnight to the lab and cultured (Gray et al., 1996a, b). A herd history was obtained from each farm.

Five visits have been made to Farm A and a total of 750 samples have been cultured for *Salmonella*. Seventy-five (10%) of the samples were positive for *Salmonella* with the order of frequency of recovery as follows: nursery (23.3%), farrowing (10.7%), gestation (7.3%), suckling (7.3%) and finishing (1.3%). Six serogroups have been identified with serogroup B identified most often (76%). Fifteen serotypes have been identified, an untypable 4,12:1-monophasic has been identified most often (62.7%) followed by *S. infantis* (9.3%) and *S. typhimurium* (4%). From three of the five visits, samples were cultured for *Campylobacter* and 129/450 (28.7%) of the samples were positive. *Campylobacter* was most often recovered from nursery (38.9%) followed by suckling (30.0%), gestation (26.7%), finishing (24.4%) and farrowing (23.3%) pigs.

For Farm B four visits have been completed and a total of 600 samples have been cultured for *Salmonella*. Thirty-eight (6.3%) of samples were positive for *Salmonella* with the order of frequency of recovery as follows: farrowing (17.5%), gestation (10%), suckling (2.5%) and nursery (1.7%). No *Salmonella* was recovered from finishing pigs. Only two serogroups (B [76.3%] and E [23.7%]) and three serotypes *S. derby* (76.3%), *S. london* (21.1%) and *S. anatum* (2.6%) were identified. Of the 600 samples, 450 were cultured for *Campylobacter*. Fifty-five were positive for *Campylobacter* (12.2%) which was recovered most often from suckling (18.9%), farrowing (12.2%), nursery (11.1%), finishing (10%), and gestation (8.9%) pigs.

For Farm C five visits have been completed and a total of 509 samples have been cultured for *Salmonella*. Eight (1.6%) of samples were positive for *Salmonella* with the order of frequency of recovery as follows: gestation (4.7%), farrowing (2.9%) and nursery (0.6%). No *Salmonella* was recovered from suckling or finishing pigs. Four serogroups were identified with serogroups D and C2 occurring most often (37.5% each). Of the five serotypes identified, *S. newport* was the most common serotype (n=3; 37.5%). Of the 509 samples, 405 were cultured for *Campylobacter*. One hundred-eight (26.7%) were positive for *Campylobacter* which was recovered

most often from the nursery (48%), followed by suckling (25%), farrowing (21.4%), gestating (18.2%), and finishing (12.4%), pigs.

Antibiograms for both *Salmonella* and *Campylobacter* and culture of *E.coli* and *Enterococci* are in progress.

It is interesting to note that the *Salmonella* and *Campylobacter* prevalence between farms is not consistent and that the highest *Salmonella* prevalence occurs on Farm A which uses antimicrobials both therapeutically and subtherapeutically. Conversely, the lowest prevalence of both *Salmonella* and *Campylobacter* occurs on Farm B which used minimal antimicrobials. However, the lowest *Salmonella* prevalence, but highest *Campylobacter* prevalence, occurred on Farm C which has not used antimicrobials in the recent past.

Although *Salmonella* was isolated on each farm, only *S. anatum* was common among all three farms suggesting that there is geographic grouping of isolates which may be influenced by production type, presence of other livestock in the area, and other factors. Further characterization of the isolates will aid in determining the effect antimicrobials have in swine production.

Results from the NARMS and research programs will facilitate the identification of resistance in humans and animals as it arises, provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and *Campylobacter* from human and animal populations, provide timely information to veterinarians and physicians, prolong the lifespan of approved drugs by promoting the prudent and judicious use of antimicrobials, and identify areas for more detailed investigation.

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Table 1. Antimicrobials - *Salmonella*

Antimicrobial	Antimicrobial Concentrations (ug/ml)*	Breakpoint		
		(S)	(I)	(R)
Amikacin	4 - 32	≤16	32	≥64
Amoxicillin/Clavulanic Acid	0.5/0.25 - 32/16	≤8	16	≥32
Ampicillin	2 - 64	≤8	16	≥32
Apramycin**	2 - 32	≤8	16	≥32
Ceftiofur**	0.5 - 16	≤2	4	≥8
Ceftriaxone	0.25 - 64	≤8	32	≥64
Cephalothin	1 - 32	≤8	16	≥32
Chloramphenicol	4 - 32	≤8	16	≥32
Ciprofloxacin	0.015 - 4	≤1	2	≥4
Gentamicin	0.25 - 16	≤4	8	≥16
Kanamycin	16 - 64	≤16	32	≥64
Nalidixic Acid	4 - 256	≤16		≥32
Streptomycin**	32 - 256	≤32		≥ 64
Sulfamethoxazole	128 - 512	≤256		≥512
Tetracycline	4 - 64	≤4	8	≥16
Ticarcillin	2 - 128	≤16	32	≥ 128
Trimethoprim/Sulfamethoxazole	.012/2.4 - 4/76	≤2/38		≥4/76

*ranges may be outside of the breakpoint for some years, refer to the annual NARMS reports for individual years

**breakpoints based on those used for human isolate testing

TABLE 1b - Antimicrobials - *Campylobacter*

Antimicrobial	Antimicrobial Concentrations (ug/ml)*	Breakpoint		
		(S)	(I)	(R)
Azithromycin	0.016 - 256	≤0.025	0.5 - 1	≥2
Chloramphenicol	0.125 - 256	≤8	16	≥32
Ciprofloxacin	0.016 - 32	≤1	2	≥4
Clindamycin	0.032 - 256	≤0.5	1 - 2	≥4
Gentamicin	0.025 - 16	≤4	8	≥16
Erythromycin	0.047 - 256	≤0.5	1 - 4	≥8
Nalidixic Acid	0.047 - 256	≤16		≥32
Streptomycin	32 - 256	≤32		≥64
Tetracycline	0.023 - 32	≤4	8	≥16

Table 2. Percent total resistance for Swine Diagnostic *Salmonella* isolates in NARMS by year

Antimicrobial	Year		
	1997 n=195	1998 n=256	1999 n=223
Amikacin	0	0	0
Amoxicillin/Clavulanic Acid	0.5	3.1	2.7
Ampicillin	23.6	29.3	37.7
Apramycin*	3.6	8.3	6.3
Ceftiofur*	0	2.7	3.1
Ceftriaxone	0	1.6	0.4
Cephalothin	0.5	3.5	3.6
Chloramphenicol	15.4	16.0	20.6
Ciprofloxacin	0	0	0
Gentamicin	4.6	8.6	8.5
Kanamycin	19.0	23.8	16.6
Nalidixic Acid	0	0	0.4
Streptomycin*	38.5	58.6	49.3
Sulfamethoxazole	44.1	59.4	52.9
Tetracycline	75.4	72.3	54.3
Ticarcillin	23.6	28.5	ND**
Trimethoprim/ Sulfamethoxazole	13.3	5.1	4.9

*breakpoints based on those used for human isolate testing

**ND=not done

Table 3. Percent total resistance for Swine Slaughter *Salmonella* isolates in NARMS by year

Antimicrobial	Year		
	1997 n=113	1998 n=798	1999 n=876
Amikacin	0	0	0.1
Amoxicillin/Clavulanic Acid	0	0.4	1.0
Ampicillin	16.8	13.3	10.8
Apramycin*	2.7	1.4	1.8
Ceftiofur*	0.9	0.1	1.9
Ceftriaxone	0	0	0
Cephalothin	0.9	0.1	0.8
Chloramphenicol	11.5	8.8	8.0
Ciprofloxacin	0	0	0
Gentamicin	1.8	1.0	1.1
Kanamycin	12.4	7.3	6.7
Nalidixic Acid	0	0	0
Streptomycin*	27.4	29.7	29.3
Sulfamethoxazole	33.6	29.3	30.7
Tetracycline	51.3	47.9	48.4
Ticarcillin	16.8	13.3	ND**
Trimethoprim/ Sulfamethoxazole	1.8	0.5	1.1

*breakpoints based on those used for human isolate testing

**ND=not done