The Role of Production Practices on the Development of Antibiotic Resistance

Alan G. Mathew
Associate Professor, Department of Animal Science, The University of Tennessee, Knoxville

Introduction

Since their discovery in the mid 20th century, antibiotics have remained in extensive use for human and animal therapies. Application of antibiotics in agriculture has allowed producers to increase production and lower costs (1), reduce the environmental impact of livestock operations (2), and reduce the prevalence of pathogens (3). While continuing to provide significant benefits, some concern has arisen that extensive use of antibiotics in livestock production has led to an increased prevalence of drug resistant bacteria (4, 5), possibly affecting their usefulness in human medicine. Although a number of agencies have sponsored research and conferences to address this issue, the common consensus remains that too little information is available from which to derive strategies for control (6, 7). In particular, very limited information is available regarding the prevalence of resistance to more recent antibiotics used in livestock production; and little or no information is available with regard to management strategies to decrease the occurrence of resistance. Some reports suggest that animal stressors can influence antibiotic resistance in bacteria associated with livestock (8, 9). These reports suggest that factors other than exposure to antibiotics may play a significant role in the development and/or prevalence of resistant organisms. We have conducted a number of studies to determine if production factors affect antibiotic resistance. Factors tested included animal stressors that might occur as a result of poor management, and antibiotic dosing schemes. These studies are described herein.

Methods

To determine if husbandry conditions affect antibiotic resistance in bacteria associated with swine, 18-day old weaned pigs with no history of antibiotic exposure were challenged intranasally with approximately 10^8 CFU of *Salmonella enterica* serovar Typhimurium (hereafter referred to as *Salmonella* Typhimurium). The challenge organism contained a nalidixic acid resistance marker for later isolation and identification. Pigs were blocked by litter, randomly assigned to one of eight treatments, and separated accordingly in identical isolation rooms equipped with commercially-manufactured swine pens and associated equipment. Treatments (Table 1) included one control group which was not exposed to antibiotics but managed under optimal conditions (10), and an additional control group treated with apramycin (Apr) in the feed (150 g/ton) beginning on Day 2 post challenge and managed under optimal conditions. Additional management treatments were applied beginning 5 days post challenge and included feeding Apr (beginning on Day 2) plus either crowding, cold stress, heat stress, intermingling with untreated pigs, poor sanitation or addition of oxytetracycline. Strict biosecurity was maintained between rooms to avoid transfer of bacteria between treatment groups. Fecal samples were obtained from pigs prior to inoculation with the challenge organism (Day 0) and on post challenge days 2, 7, 14, 28, 64, 148 (prior to shipping), and 149 (post-shipping) for the recovery of the test isolates. *Escherichia coli* were isolated via culture on lactose MacConkey agar (DIFCO, Detroit, MI) and suspected *E. coli* colonies were further cultured on Trypticase Soy Agar (DIFCO) plates containing 5% defibrinated sheep blood for selection of non-hemolytic

species prior to confirmation using API20E test strips (Vitek bioMerieux). The salmonella challenge organism was recovered according to standard procedures (11). Briefly, samples were cultured in Mueller-Hinton II cation adjusted broth (Becton Dickenson), followed by enrichment in tetrathionate (DIFCO). Aliquots of the enrichment culture were then transferred to XLT4 agar (DIFCO) containing 50 µg/mL nalidixic acid. Presumptive salmonella colonies were confirmed using triple sugar iron (DIFCO) and lysine iron agar (DIFCO). All isolates were tested for sensitivity to apramycin sulfate, ceftiofur sodium, oxytetracycline, and sodium sulfamethazine via a minimum inhibitory concentration (MIC) broth dilution method, according to National Committee for Clinical Laboratory Standards (12). Additionally, plasmid and chromosomal analyses were conducted via PCR using target-specific primers, arbitrarily primed PCR (AP PCR), DNA hybridization and pulse field gel electrophoresis (PFGE) to identify sources and prevalence of resistance genes. Numerical data were analyzed using Analysis of Variance (13), mixed model procedures to determine the effects of treatments and interactions of treatment by time. Percentage of resistant organisms and multiple resistance patterns were compared using the Proc Freq procedure of SAS (13).

Table 1: Experiment 1 treatment applications and animal numbers.

Treatment	Description	Number of pigs
Control without apramycin (Control-1)	Optimal production conditions	6
Control with apramycin (Control-2)	Optimal conditions plus apramycin fed at 150 g/ton for 14 continuous days	6
Cold Stress with apramycin	6^{o} C reduction from recommended optimal temperature plus apramycin fed at 150 g/ton for 14 days	6
Heat Stress with apramycin	6^{o} C increase from recommended optimal temperature plus apramycin fed at 150 g/ton for 14 days	6
Oxytetracycline with apramycin	Apramycin fed at 150 g/ton for 14 days followed by oxytetracycline fed at 100g/ton for remainder of the study	6
Poor Sanitation with apramycin	Accumulation of manure in room plus apramycin fed at 150 g/ton for 14 days	6
Overcrowding with apramycin	A 40% reduction in floor space from optimal conditions plus apramycin fed at 150 g/ton for 14 days	10
Intermingling with apramycin	Apramycin fed at 150 g/ton in 6 pigs for 14 days then addition to the pen of 6 pigs not exposed to apramycin	12

To determine the effects of antibiotic regimens on resistance of bacteria, a similar challenge model was used. Weaned pigs were inoculated with the same salmonella challenge

organism, as above, and following the challenge, pig groups were housed separately in identical, biosecure SEW nursery rooms. Pigs were provided water and a phase diet, and randomly assigned to 6 antibiotic treatment regimens, as indicated in Table 2. Fecal samples were collected from each pig prior to application of antibiotic treatments (Day 3 following challenge with *Salmonella* Typhimurium), and on Days 6, 10, 13, 17, and 31 post challenge, and then twice per month until pigs reached market weight. Bacterial isolation and analysis was conducted as in the first experiment (above).

Table 2. Experiment 2 antibiotic dosing schemes and animal numbers[1].

Treatment	Dosing scheme	Number of Pigs
Control	No antibiotics	12
Label Use	Apramycin sulfate fed at 150 g/ton for 14 days	12
Gradient	Apramycin sulfate at 50 g/ton for 5 days, then100 g/ton for 5 days, then 150 g/ton for 4 days	12
Pulse	Apramycin sulfate at 150 g/ton for 3 days, then 3 days without antibiotics, then repeating this sequence throughout the 14-day period	12
Rotation with dissimilar antimicrobials	Apramycin sulfate at 150 g/ton for 5 days, then sodium sulfamethazine in the drinking water (118 mg/kg body weight) for 5 days, then carbadox at 50 g/ton for 4 days.	12
Rotation with similar antimicrobials	Apramycin sulfate at 150 g/ton for 5 days followed by gentamicin in the drinking water (25 mg/gallon) for 5 days, then neomycin sulfate in the drinking water, (22 mg/kg body weight) for 4 days.	12

[1]Drinking water formulations were based on best estimates of pig weight, NRC values for water intake, and manufacturers instructions for the in-line water medicator (Chemilizer, Model HN55, Largo, FL)

Results

In the first experiment, *E. coli* demonstrated the most pronounced effects of stressors on antibiotic resistance, as measured by MIC (Table 3). Isolates from pigs not exposed to antibiotics (Control-1) exhibited the lowest resistance throughout the study. Peak resistance was observed in isolates from pigs exposed to apramycin by Day 14 post challenge. Upon withdrawal of apramycin, MICs for isolates from pigs housed under optimal conditions (Control-2) returned to pretreatment levels; whereas some stressed groups maintained greater ($P < .05$) MICs through Day 28 post challenge. Isolates from cold stressed, crowded, and oxytetracycline-treated pigs demonstrated greater ($P < .05$) MICs up to Day 64 post challenge, before returning to pretreatment levels. Minimum inhibitory concentrations of *E. coli* following transportation were not significantly different from pre-transportation levels. High levels of resistance to oxytetracycline were exhibited by the majority of isolates throughout the study (data not shown).

Table 3: Effects of animal stressors on sensitivity to apramycin by *E. coli* isolated from pigs[1]

	Days Post Challenge							
Treatment[2]	**0**	**2**	**7**	**14**	**28**	**64**	**148**	**149**
C-1	2.3	2.8	1.3	1.2	1.0	1.2	1.0	1.3
C-2	3.0	1.5	1.3	245.1*	1.3	1.2	1.8	1.6
Cold	4.8	1.7	17.3*	224.8*	76.8*	15.5*	1.1	1.4
Heat	2.4	2.1	1.5	101.6*	4.1	3.5	1.1	1.9
Crowd	2.0	2.5	1.2	25.4*	10.0*	11.6*	1.2	1.6
Sanit	3.6	4.0	5.7	194.0*	4.9	2.2	1.5	1.4
Oxy	3.2	4.4	1.9	90.5*	5.0*	8.5*	1.1	1.4
Int	2.2	1.8	1.8	138.2*	11.3*	2.6	1.3	1.4

[1]Data are Least Squares Means of minimum inhibitory concentrations (MIC) in µg/mL, with 64 isolates for each mean.

[2]C-1 = Control-1, optimal conditions, no apramycin; C-2 = Control-2, optimal conditions and apramycin exposure; Cold = room maintained at 6°C below optimal temperature; Heat = room maintained at 6°C above optimal temperature; Crowd = Overcrowding; Sanit = Poor Sanitation; Oxy =Treatment with oxytetracycline; Int = intermingled pigs.

*Treatments differ from Control-1 ($P < .05$) within day.

Table 4: Effects of animal stressors on sensitivity to apramycin by *Salmonella* Typhimurium isolated from pigs[1]

	Days Post Challenge							
Treatment[2]	**0**	**2**	**7**	**14**	**28**	**64**	**148**	**149**
C-1	1.3	1.9	1.4	1.5	1.6	2.0	-	-
C-2	3.8	2.2	1.2	1.7	1.8	2.6	-	-
Cold	1.8	2.2	1.9	1.5	1.6	-	-	-
Heat	-	2.1	1.9	1.4	1.3	-	-	-
Crowd	2.4	2.0	1.7	1.2	1.8	2.0	-	-
Sanit	2.7	2.0	1.3	1.5	1.4	-	-	-
Oxy	4.6	2.5	1.4	1.8	1.1	-	-	-
Int	1.8	1.9	1.3	1.6	1.5	-	-	-

[1]Data are Least Squares Means of minimum inhibitory concentrations (MIC) in µg/mL, with a maximum of 32 isolates for each mean.

[2]C-1 = Control-1, optimal conditions, no apramycin, C-2 = Control-2, optimal conditions and apramycin exposure, Cold = room maintained at 6°C below optimal temperature, Heat = room maintained at 6°C above optimal temperature, Crowd = Overcrowding, Sanit = Poor Sanitation, Oxy =Treatment with oxytetracycline, Int = intermingled pigs.

- Indicates no isolates recovered.

Salmonella Typhimurium were recovered from pigs through Day 64 post challenge, with shedding of that organism generally declining after Day 28 post challenge. Throughout the study salmonella isolates remained susceptible to apramycin (data not shown). Minimum inhibitory concentrations for oxytetracycline in salmonella isolated from pigs administered that antibiotic

were greater (P < .05) through Day 28 post challenge compared to isolates from other groups (data not shown).

DNA analysis indicated that apramycin resistance in *E. coli* was associated with several distinct plasmid profiles. Additionally, resistance was associated with the known apramycin resistance gene aac(3)-IV, found to be located on the chromosome in *E. coli* isolated in this study. Apramycin resistance was observed to occur across a variety of *E. coli* isotypes, as determined by PGFE analysis (data not shown).

In the second experiment, *E. coli* from the Control group (no antibiotics) demonstrated the lowest resistance (Table 5). Label use of apramycin and a drug rotation that included the similar antibiotics, apramycin, neomycin and gentamicin, produced the greatest resistance to apramycin. In contrast, MICs in *E. coli* from the pulse-dosed group were not different in isolates from the Control group, which did not receive antibiotics. As in the first experiment, MICs of the Salmonella challenge organism were not affected by treatments (data not shown).

Table 5. Effects of antibiotic dosing regimens on sensitivity to apramycin by *E. coli* from pigs[1]

Antibiotic Treatment[2]	**Days Post Challenge**					
	3	**6**	**10**	**13**	**17**	**31**
Control	4.3	3.9	3.5	3.1	2.3	2.6
Rotation S	3.5	4.2	200.5*	182.3*	140.9*	7.6
Rotation D	2.6	38.8*	44.1*	13.8	14	3.8
Gradient	3.5	3.5	3.5	68.5*	109.9*	2.8
Pulse	5.2	4.3	3.6	4.0	7.0	3.7
Label	5.9	41.1*	55.5*	49.0*	49.5*	6.6

[1]Data are Least Squares Means of minimum inhibitory concentrations (MIC) in µg/mL, with 64 isolates for each mean.

[2]Control = no antibiotic treatment; Rotation S = rotation with similar antibiotics; Rotation D = rotation with dissimilar antibiotics; Gradient = increasing antibiotic treatment over time; Pulse = pulse dosing; Label = maximum label use.

***** Treatments differ from Control (P < .05) within day.

Discussion

Our studies indicate that husbandry practices and antibiotic dosing regimens affect the prevalence and persistence of antibiotic resistant bacteria in pigs. It is not clear as yet how stressors when applied to animals might affect the resistance patterns of the intestinal and/or fecal microflora. Langlois, et al (8). speculated that temporary selective shedding of resistant isotypes by swine may occur during stress, such as that caused by transportation. However, the increase in resistance that we observed in our studies was not transient but rather appeared to occur over a longer period of time. It is possible that changes in feed intake, gut physiology, and/or motility as a result of stress may affect the GI environment, which then could impact upon intestinal bacteria. Earlier work by our group has shown that weaning in pigs results in physiochemical effects in the gut, including changes in pH and fermentation acid concentrations, which appear to impact the microflora (14). Such gut environment changes may cause an increased ability of bacteria to transfer and/or acquire resistance genes, or allow resistant subtypes, which may naturally occur in low numbers, to gain an advantage and increase in number. Changes in the gut environment may also promote increased cellular efflux mechanisms

in bacteria causing exclusion of the antibiotic from the cell (15). Additionally, increased resistance may be associated with stress-induced genes in the bacteria, such as those that code for shock proteins (16). Such genes may be linked to resistance factors. As such, selecting for stress-resistant isolates may indirectly select for antibiotic resistant organisms.

Our data also indicate that rotation with similar antibiotics produces the greatest resistance in *E. coli.* Isolates from pigs subjected to apramycin, gentamicin, and neomycin, were commonly resistant to all three aminoglycoside drugs following application of that treatment. The rotation with unlike antibiotics produced less resistance in recovered *E. coli*. Importantly, we noted that pulse dosing with apramycin produced little, if any resistance, with MICs of bacteria not differing from *E. coli* obtained from pigs without exposure to antibiotics. If performance and health of pigs is found to be similar between label use and pulse dosing, the latter may provide a cheaper alternative, while at the same time lessening the risk of antibiotic resistance in the intestinal bacteria.

In these experiments, we noted that the *Salmonella* Typhimurium challenge organism was less affected by treatment with antibiotics and/or stressors, compared to *E. coli*. The lack of response in salmonella may be due to the invasive nature of that organism, which may help that bacterium escape exposure to antibiotics and gut changes by internalization into intestinal enterocytes and other host cells. However, it should also be considered that our recovery methods in these studies were strictly selective for the challenge strain of *Salmonella* Typhimurium, which was known to be initially sensitive to the test antibiotics. This is in contrast to *E. coli* recovered in these studies, for which a variety of isotypes were isolated. Some of those isotypes may have been resistant prior to the study, and may have then proliferated or disseminated resistance factors upon exposure to antibiotics or stressors.

In total, these data indicate that poor husbandry practices and specific drug regimens can exacerbate problems associated with antibiotic resistance in bacteria associated with livestock, particularly in naturally-occurring *E. coli*. Because this species may be a key enteric group involved in the transfer of genetic resistance genes to other bacteria, which may include foodborne pathogens, husbandry practices that reduce the prevalence of antibiotic resistant isotypes should be considered. Such practices should also be consistent with optimal production, and thus should provide a monetary incentive, while limiting the presence of antibiotic resistant organisms.

References:

1. Stahly TS, Cromwell GL, Monegue HJ. Effects of dietary inclusion of copper and(or) antibiotics on the performance of weanling pigs. *J Anim Sci*. 1980; 51:1347-1351
2. Roth FX, Kirchgessner M. Influence of avilamycin and tylosin on retention and excretion of nitrogen in finishing pigs. *J Anim Physiol Anim Nutr* 1993; 69:245-250
3. Kyriakis SC, Sarris K, Kritas SK, Tsinas AC, Giannakopoulos C. Effect of salinomycin in the control of *Clostridium perfringens* type C infections in suckling pigs. *Vet Rec* 1996; 138:281-283
4. Langlois BE, Dawson KA, Staly TS, Cromwell GL. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. *J Anim Sci* 1984; 58:666-667
5. Ebner PD, Mathew AG. Effects of antibiotic regimens on fecal shedding patterns of pigs infected with *Salmonella typhimurium*. *J Food Protect.* 2000; 63:709-714

6. The Use of Drugs in Food Animals: Benefits and Risks. 1998. National Research Council Institute of Medicine. National Academy Press, Washington, D.C.
7. American Society for Microbiology. 1993. Report of the ASM Task Force on antibiotic resistance. Antimicrob. Agents Chemother. Suppl: 1-23.
8. Langlois BE, Dawson KA. Antimicrobial resistance of gram-negative enteric bacteria from pigs in a nonantimicrobial-exposed herd before and after transportation. *J Food Protect.* 1999; 62:797-799
9. Corrier DE, Purdy CW, DeLoach JR. Effects of marketing stress on fecal excretion of *Salmonella* spp in feeder calves. *Amer J Vet Res.* 1990; 51: 866-869.
10. *Pork Industry Handbook.* State Cooperative Extension Service, National Pork Producers Council, and USDA. 1994. Media Distribution Center, Lafayette, IN.
11. *Bacteriological Analytical Manual/Food and Drug Administration*. 8th ed. 1998. Association of Official Analytical Chemists International. Gaithersburg, MD.
12. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 1997. National Committee for Laboratory Standards, Villanova, Pennsylvania.
13. SAS. *SAS/STAT Users Guide* (Version 6). 1990. SAS Institute Incorporated, Cary, North Carolina
14. Mathew AG, Franklin MA, Upchurch WG, Chattin SE. Effect of weaning on ileal short-chain fatty acid concentrations in pigs. *Nutr Res.* 1996; 16:1689-1698.
15. Dzwokai M, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. Genes acrA and acrB encode a stess-induced efflux system of *E. coli*. *Mol Microbiol.* 1995;16:45-55.
16. Audia JP, Webb CD, Foster JW. Breaking through the acid barrier: An orchestrated response to proton stress by enteric bacteria. *Int J Med Microbiol.* 2001; 291:97-106.

Biography

Alan G. Mathew, Ph.D.

Alan Mathew is a former native of Indiana where he was raised on a swine and grain farm. He received a BS in Biology in 1976 and an MS in Animal Science in 1978 from Purdue University and then returned home to farm, establishing a 200-sow farrow to finish and 700-acre cash grain operation, of which he is still part owner. In 1991 he returned to Purdue to earn his PhD in Animal Science and then took a position as a Postdoctoral Research Associate in the Department of Biology at that same institution, where he conducted research on molecular aspects of bacterial pathogens. He accepted his current faculty position with the University of Tennessee in 1993. He currently teaches courses in the Department of Animal Science and the College of Veterinary Medicine, and serves as the Chair of the Institutional Animal Care and Use Committee. He conducts research in animal nutrition, gastrointestinal pathogens, antibiotic resistance, and pre-harvest food safety associated with livestock production. During his time at The University of Tennessee, he has garnered more than $1.7 million in grants and contracts, authored more than 40 publications, including 6 book chapters, given numerous national and international invited presentations, and has received a number of esteemed awards, in recognition of outstanding research and teaching efforts.

BACK TO TABLE OF CONTENTS