RESEARCH REPORT
PORK SAFETY

Title: Effect of Drug Combinations and Regimens on Antibiotic Resistance
NPB#98-208

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I. **Abstract:** In 2 replicate trials, 144 weaned pigs were used to test the effects of antibiotic dosing schemes on resistance in bacteria. Pigs were inoculated with the foodborne pathogen, *Salmonella typhimurium*, and the swine pathogen, K88 *E. coli* prior to being treated with feed- and water-based antibiotics. Treatments included maximum label use, rotation of similar and non-similar antibiotics, increasing gradient doses, and pulse doses of antibiotics for a period of 2 weeks following pathogen challenge. Fecal samples were obtained prior to initiation of treatments, and on various days during the treatment phase and throughout the grow-finish phase. The challenge organisms and non-pathogenic *E. coli* were recovered from fecal samples and tested against all antibiotics used in the study to determine effects on resistance patterns. Antibiotic resistance was affected to a greater extent in non-pathogenic *E. coli*, compared to *Salmonella typhimurium*. Greater ($P < .0001$) resistance occurred when similar antibiotics (apramycin, gentamicin, neomycin) were used in rotation compared to other treatments. Significant ($P < .05$) Time by Treatment interactions also occurred, with the greatest resistance occurring during or just following rotational treatment with similar antibiotics, compared to samples collected later and from other treatment groups. Pigs on the control and pulse dose treatments produced bacteria with lower resistance compared to other groups.

II. **Introduction:** Use of antibiotics remains commonplace in US livestock operations due to their therapeutic value and the enhanced performance of animals fed subtherapeutic concentrations. Increasingly, however, bacterial resistance has caused concern among health specialists and consumer groups. While numerous investigations have focused on emergence of drug resistance and a number of conferences have been assembled to address the risks of antibiotic use in the livestock industry, the common consensus is that too little information is available to determine the real risks and to develop strategies for control. In particular, very limited information is available regarding the incidence of resistance to more recent antibiotics used in swine production; and little or no information is available with regard to management strategies to decrease the occurrence of resistance. In order to promote increased consumption of pork, producers must demonstrate to consumers that a concerted effort is underway

to develop practices that limit risks to end users of our products. Because use of antibiotics in livestock production continues to provide clear production benefits, the most appropriate investigations should incorporate efforts to reduce resistance while maintaining efficient use of antibiotics. Thus research that includes production components as well as microbiological and biochemical components will better address current concerns over antibiotic use in livestock operations.

III. Objectives: The objective of this research was to compare the effects of different antimicrobial regimens, including label use, pulse dosing, increasing gradient, and rotation with similar and dissimilar antibiotics on resistance patterns in pathogenic and non-pathogenic *E. coli,* and *Salmonella typhimurium* in weaned pigs.

IV. Procedures: In 2 replicate trials, a total of 144, 18-day-old weaned pigs, verified to be salmonella free and with no history of antibiotic use, were challenged intranasally with 10^4 colony forming units of *S. typhimurium* derived from a confirmed case of swine salmonellosis. Additionally, pigs were orally inoculated with 10^5 colony-forming units of a hemolytic enterotoxigenic *E. coli* (K88). Both organisms contained a nalidixic acid resistance marker for subsequent isolation and confirmation. The *E. coli* isolate was used to indicate the efficacy of treatments against colonization by enteric pathogens, with this concentration simulating pathogen loads common to most swine farms. The salmonella isolate was used to indicate extraneous effects of antibiotics on foodborne pathogens.

Following the challenge, pig groups were housed separately in identical SEW nursery rooms with separate environmental and waste removal systems. Pigs were provided water and a phase diet, and randomly assigned to 6 treatments including: **1)** control, no antibiotic; **2)** apramycin (150g/ton of feed) for 14 days (maximum label duration); **3)** gradient application with apramycin at 50 g/ton of feed for 5 days, then 100 g/ton for 5 days then 150 g/ton for 4 days; **4)** pulse dosing with apramycin (150 g/ton of feed) for 3 days on, 3 days off, and repeating that sequence through 14 days; **5)** rotation with apramycin for 4 days, followed by sodium sulfamethazine in drinking water (118 mg/kg body weight) for 4 days, followed by carbadox (50 g/ton of feed) for 4 days (unrelated drug families); and **6)** rotation with apramycin in the feed, followed by gentamicin in drinking water (25 mg/gallon), followed by neomycin sulfate (22 mg/kg body weight) in the drinking water (3 related aminoglycoside antibiotics). Treatments 5, and 6 were used to determine effects of antibiotic regimens on resistance within and across antibiotic types. Fecal samples were collected from each pig prior to application of antibiotic treatments (day 3 following challenge with salmonella and *E. coli*), 3, 7, 10, 14, and 28 days following the application of antibiotic treatments, then twice per month until pigs reached market weight.

Fecal samples were serially diluted in phosphate buffered saline and aliquots were cultured on appropriate selective media containing nalidixic acid for enumeration of salmonella and *E. coli* (including the challenge strain and non-pathogenic strains). Colonies were confirmed to species by biochemical analysis. From each sample, a maximum of 4 *Salmonella typhimurium* and 4 *E. coli* colonies of each type were randomly selected, and each isolate was tested for resistance to the test antibiotics using a minimum inhibitory concentration (MIC) analysis, according to standard procedures outlined by the National Committee for Clinical Laboratory Standards (NCCLS). Data were

analyzed using general linear model (GLM) procedures for repeated measures.

V. Results: Recovery of K88 *E. coli* was very low in this study, and thus an effective statistical analysis was not possible. The apparent lack of infection by K88 *E. coli* may have been due to competition from the challenge strain of Salmonella, which successfully colonized all pigs in large numbers. Results are thus reported for non-pathogenic *E. coli* and *Salmonella typhimurium*. Significant ($P < .0001$) treatment effects were noted for resistance to apramycin (Table 1 and Figures 1a and 1b), gentamicin (Table 2 and Figures 2a and 2b), neomycin (Table 3 and Figures 3a and 3b), and sulfamethazine (Table 4 and Figures 4a and 4b) in *E. coli.* The control group (no antibiotics) produced *E. coli* with the lowest resistance (greatest sensitivity) for all antibiotics tested. Rotational use of similar antibiotics and label use produced the greatest resistance to apramycin, neomycin, and gentamicin, while rotational use of both similar and dissimilar antibiotics produced the greatest resistance to sulfamethazine. It should be noted that the minimum inhibitory concentrations for sulfonamides are inherently greater for the test bacteria than for other antibiotics used in this study, thus while numerically high compared to those of the apramycin, gentamicin, and neomycin, these MIC's do not actually indicate greater clinical resistance to sulfonamides. Significant Time X Treatment interactions were also noted (Tables 1 through 4), with the greatest resistance generally occurring during or just following antibiotic treatment.

Main effects of treatments were not observed for antibiotic resistance in salmonella, however Treatment X Time interactions ($P < .05$) were noted for gentamicin, neomycin, and sulfamethazine (Tables 5 through 8 and Figures 5a and 5b through 8a and 8b). Generally, resistance to all antibiotics remained low in salmonella throughout the study, in contrast to an earlier study by our group. We suspect that this may have been due to housing the animals in a new facility, where no pigs had been previously housed, compared to the earlier study, which took place in a finishing unit built in the early 1970's and used frequently thereafter. In that study, salmonella quickly acquired resistance when pigs were treated with antibiotics, thus possibly indicating that resistance elements from salmonella were pre-existing in the building. It thus appears that *E. coli* may have a greater ability to mutate to resistance de novo, compared to salmonella. Such information could be very important, since it may indicate that non-pathogenic bacteria may initially generate resistance elements, which might be passed to and accumulated in pathogens over a more protracted period. It should also be noted that in this study, resistance to antibiotics decreased following withdrawal of antibiotic treatments, such that no differences occurred in resistance patterns compared to control, following day 31 post challenge (14 days following withdrawal of antibiotic treatments). Other studies, including some conducted by our group, indicate that use of different antibiotics, other housing and management conditions, or longer term use of antibiotics produce more long-term resistance patterns in bacteria associated with swine.

Please note:
The following tables and figures depict data through the first 6 sampling periods, which includes a baseline sample obtained prior to initiation of treatments (Day 3 post challenge). While data derived throughout the grow/finish phases were analyzed, later periods showed no significant differences across treatments or over time, compared with Day 31 post challenge.

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Table 1. Resistance to Apramycin by *E. coli* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	4.3	3.5	2.6	3.5	5.2	5.9
6	3.9	4.2	38.8	3.5	4.3	41.1
10	3.5	200.5	44.1	3.5	3.6	55.5
13	3.1	182.3	13.8	68.5	4.0	49.0
17	2.3	140.9	14.0	109.9	7.0	49.5
31	2.6	7.6	3.8	2.8	3.7	6.6

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .0001
Trt x time Effect P < .0001

Effect of Antibiotic Treatment on Apramycin Resistance in *E. coli* from Pigs

Figures 1a and 1b

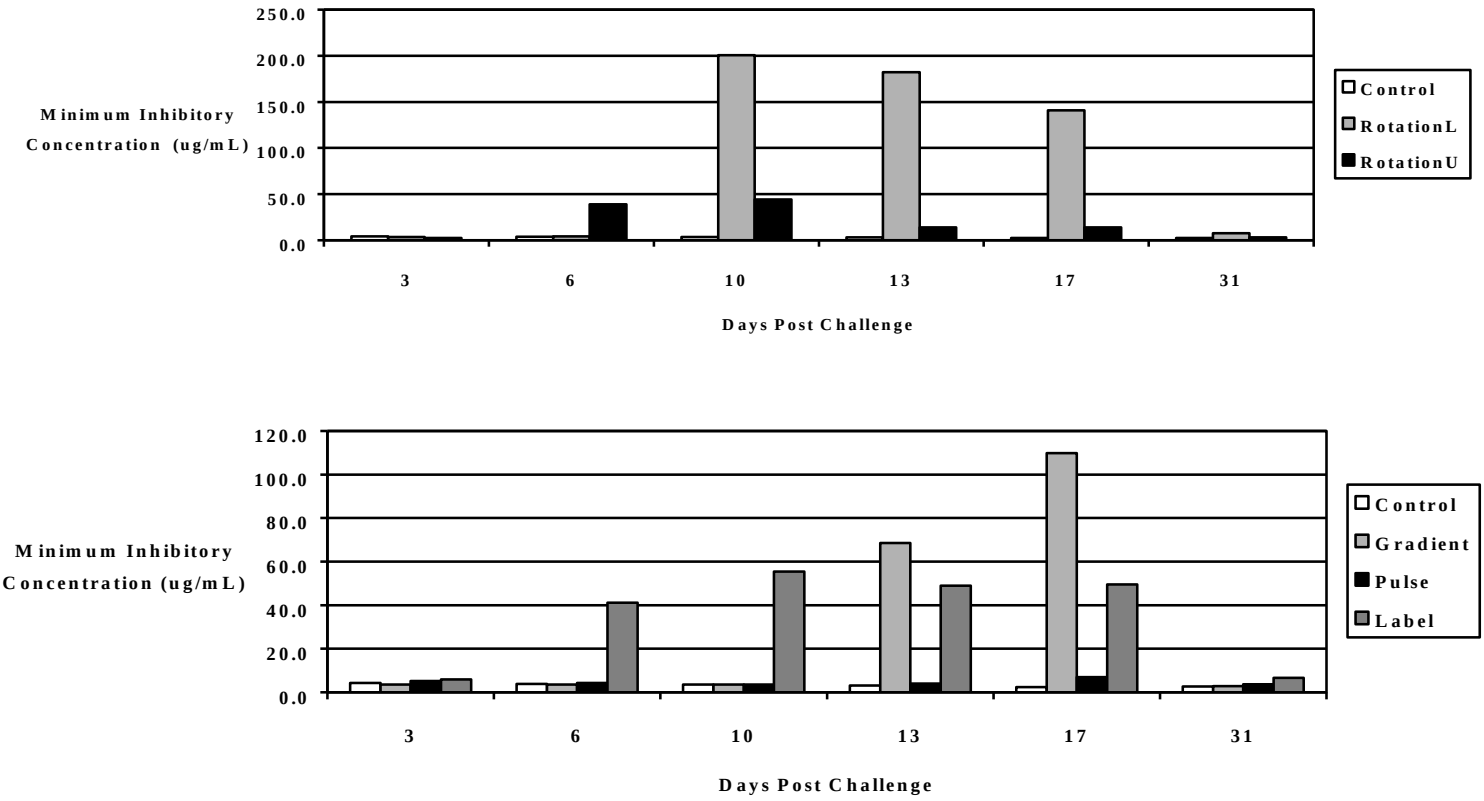

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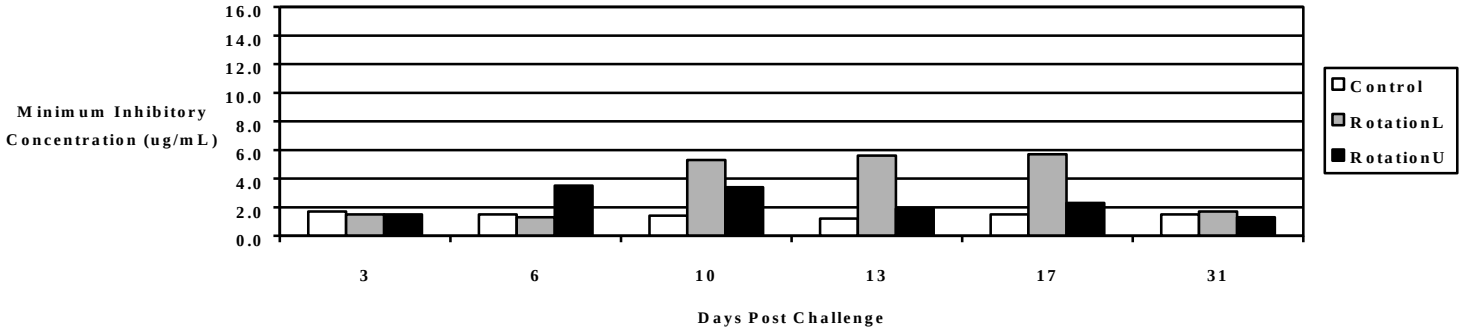
Table 2. Resistance to Gentamicin by *E. coli* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	1.7	1.5	1.5	1.4	1.5	1.5
6	1.5	1.3	3.5	1.4	1.5	4.2
10	1.4	5.3	3.4	1.8	1.5	5.0
13	1.2	5.6	1.9	3.6	1.5	3.8
17	1.5	5.7	2.3	3.7	1.9	3.9
31	1.5	1.7	1.3	1.4	1.5	1.7

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .0001
Trt x time Effect P < .0001

Effect of Antibiotic Treatment on Gentamicin Resistance in *E. coli* from Pigs

Figures 2a and 2b



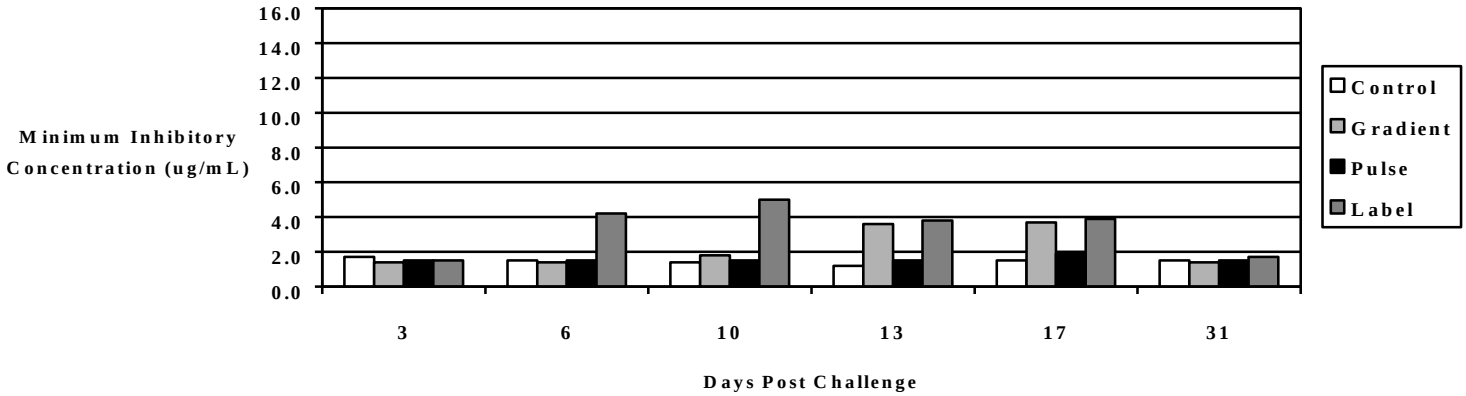


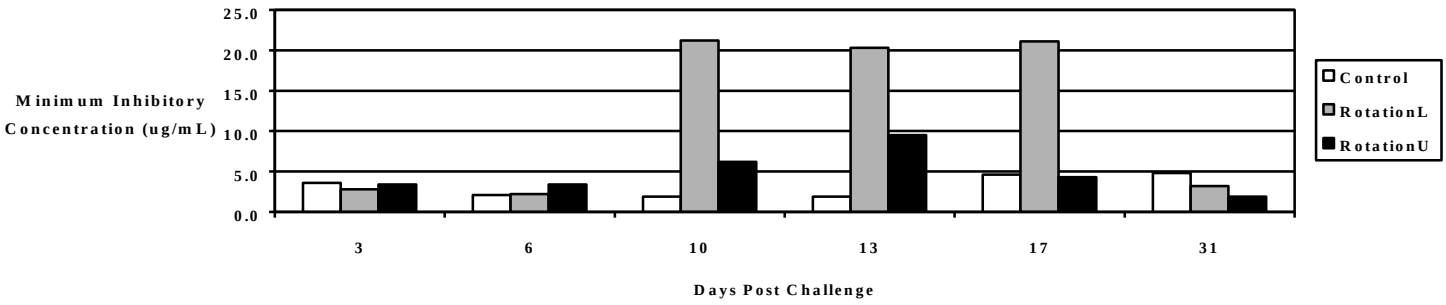
Table 3. Resistance to Neomycin by *E. coli* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	3.6	2.8	3.4	2.7	3.8	3.4
6	2.1	2.2	3.4	2.9	4.0	8.3
10	1.9	21.2	6.2	4.9	3.4	9.0
13	1.9	20.3	9.5	5.0	2.1	7.1
17	4.6	21.1	4.3	7.1	3.0	6.6
31	4.8	3.2	1.9	7.0	2.1	2.9

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .0001
Trt x time Effect P < .0001

Effect of Antibiotic Treatment on Neomycin Resistance in *E. coli* from Pigs

Figures 3a and 3b



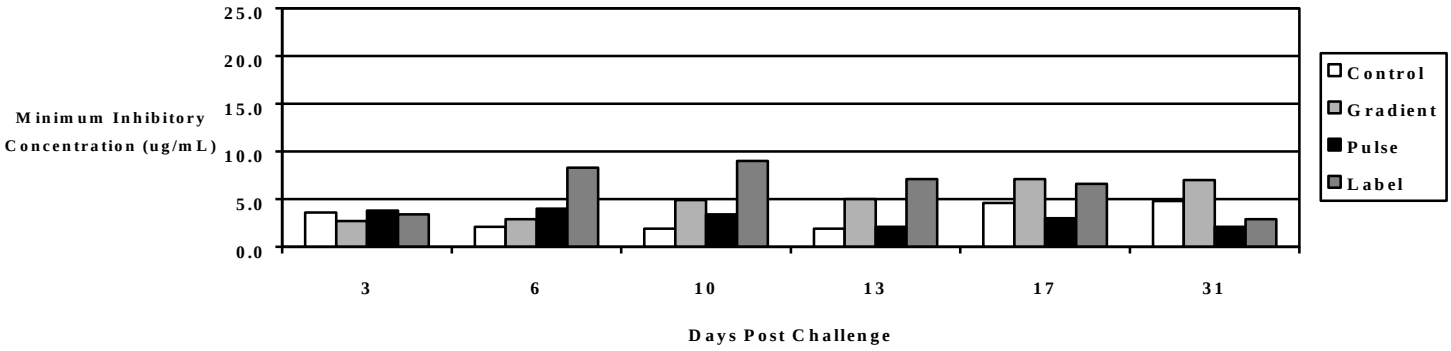


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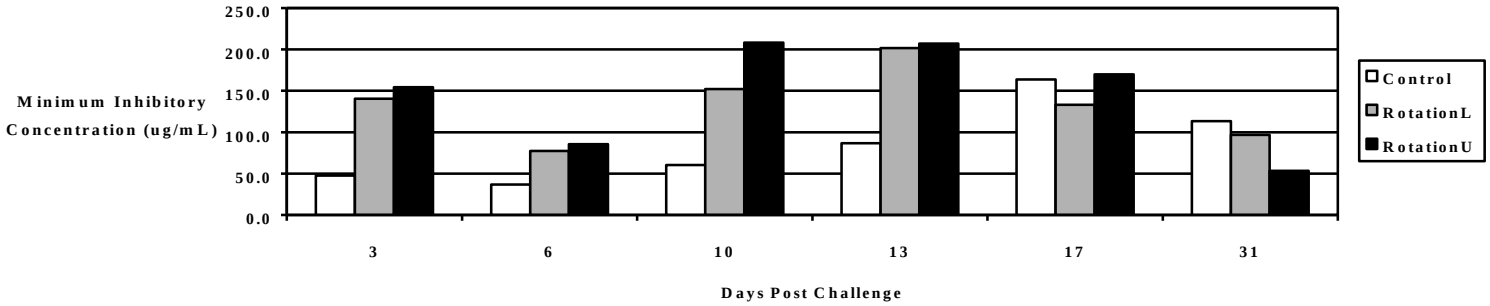
Table 4. Resistance to Sulfamethazine by *E. coli* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	47.6	140.6	154.6	73.5	151.2	79.2
6	36.7	77.5	85.4	47.9	107.6	66.9
10	60.4	152.2	208.1	87.4	59.8	87.6
13	86.6	201.7	207.1	122.9	54.6	119.4
17	163.6	133.0	169.7	91.5	89.3	110.0
31	113.4	96.6	53.5	117.8	41.1	102.4

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .0001
Trt x time Effect P < .0001

Effect of Antibiotic Treatment on Sulfamethazine Resistance in *E. coli* from Pigs

Figures 4a and 4b



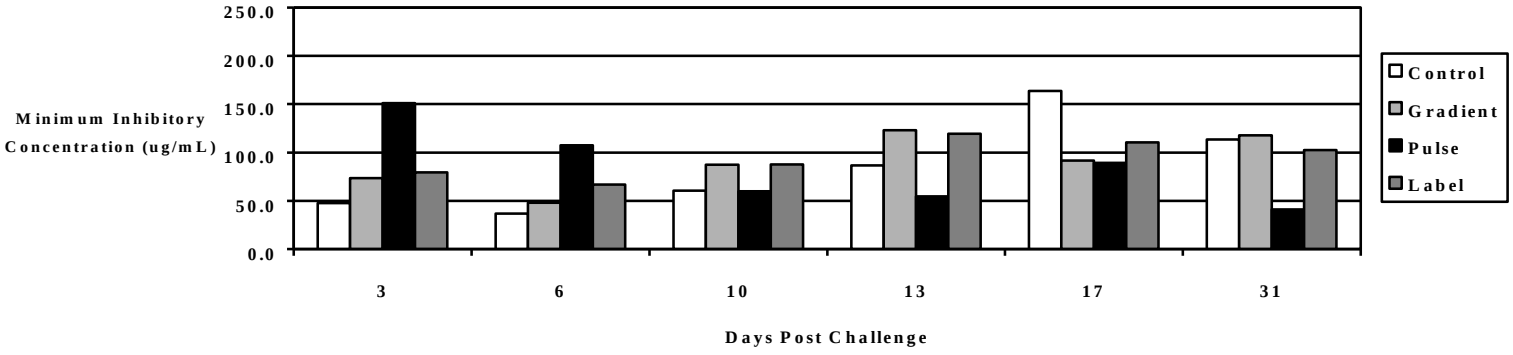


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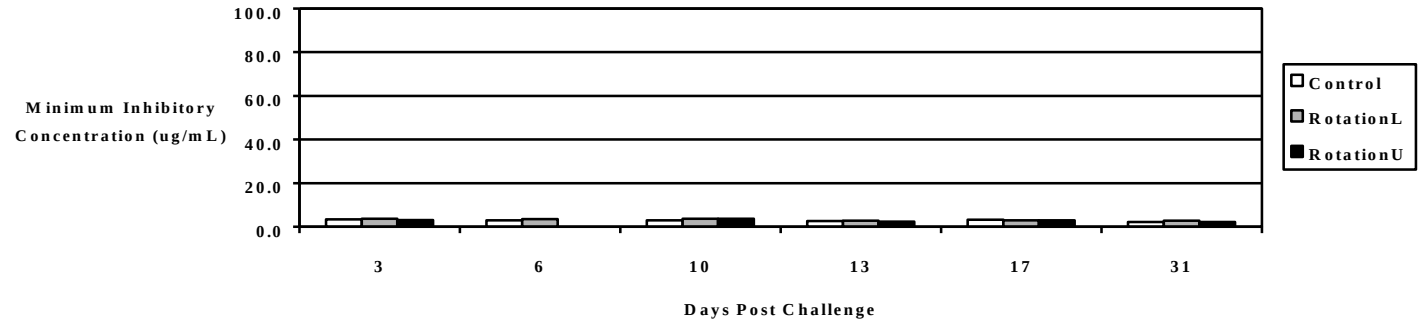
Table 5. Resistance to Apramycin by *Salmonella typhimurium* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	3.3	3.7	3.1	3.0	3.2	3.2
6	2.9	3.5	0.0	2.7	2.9	3.0
10	3.0	3.7	3.7	3.9	3.3	3.2
13	2.7	2.8	2.3	2.5	2.0	0.0
17	3.2	2.9	2.9	2.9	2.1	2.6
31	2.2	2.8	2.2	2.8	2.9	2.0

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .7093
Trt x Time Effect P < .5205

Effect of Antibiotic Treatment on Apramycin Resistance in *Salmonella typhimurium* from Pigs

Figures 5a and 5b



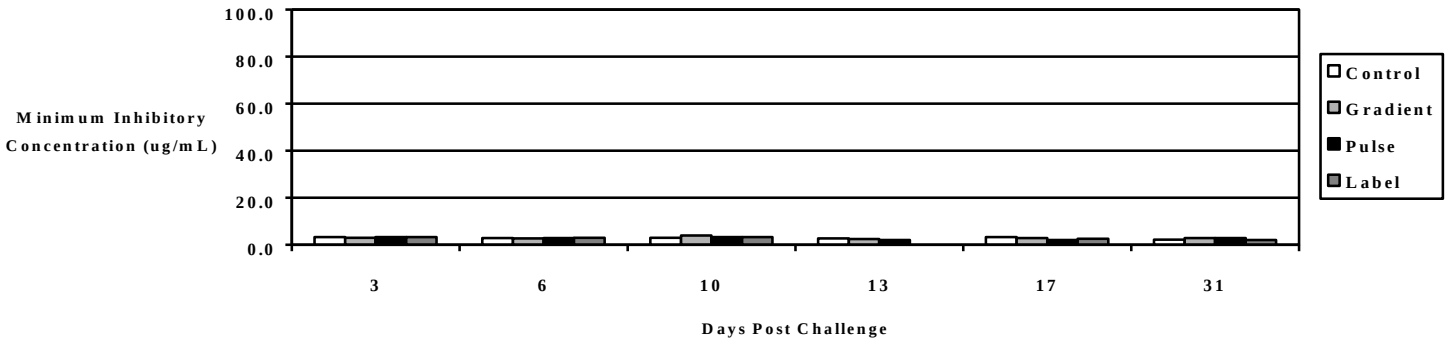


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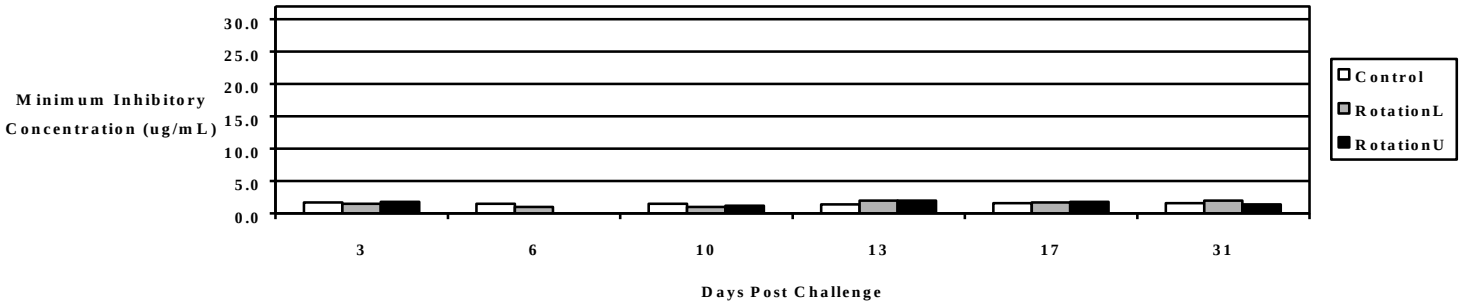
Table 6. Resistance to Gentamicin by *Salmonella typhimurium* Isolated from pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	1.7	1.5	1.8	1.8	1.5	1.6
6	1.5	1.0	0.0	1.6	1.4	1.4
10	1.5	1.0	1.2	1.2	1.3	1.2
13	1.4	2.0	2.0	1.5	2.0	0.0
17	1.6	1.7	1.8	1.5	2.0	2.0
31	1.6	2.0	1.4	1.7	1.7	2.0

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .90
Trt x time Effect P < .02

Effect of Antibiotic Treatment on Gentamicin Resistance in *Salmonella typhimurium* from Pigs

Figures 6a and 6b



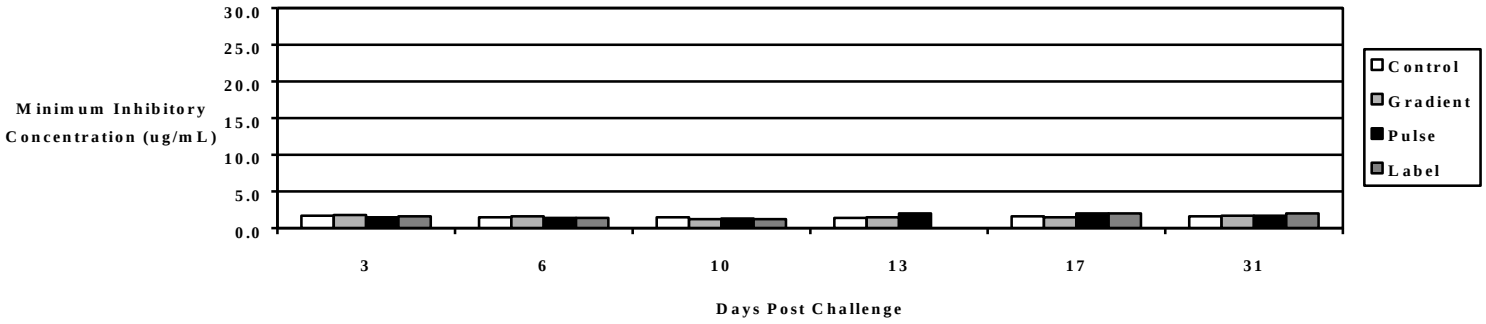


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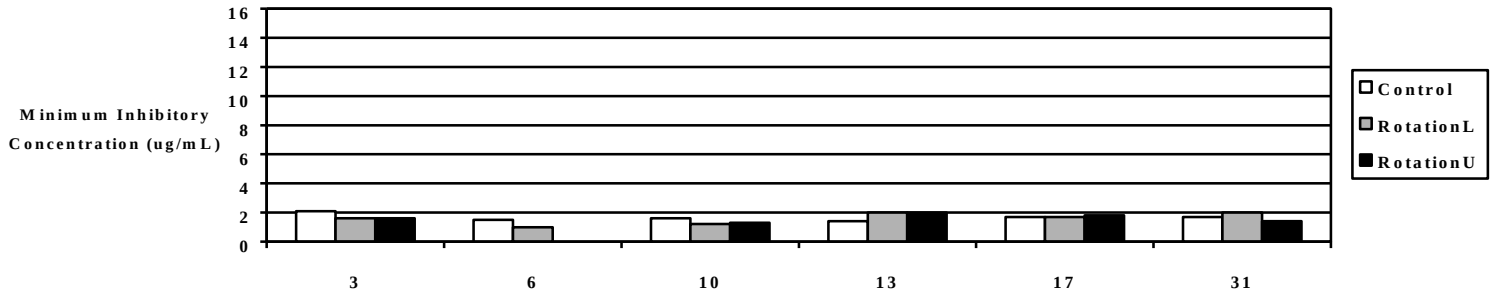
Table 7. Resistance to Neomycin by *Salmonella typhimurium* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	2.1	1.6	1.6	2.1	1.6	1.7
6	1.5	1.0	0.0	1.7	1.4	1.6
10	1.6	1.2	1.3	1.5	1.3	1.3
13	1.4	2.0	2.0	1.5	2.0	0.0
17	1.7	1.7	1.8	1.5	2.0	2.0
31	1.7	2.0	1.4	1.8	1.8	2.0

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .8899
Trt x time Effect P < .0498

Effect of Antibiotic Treatment on Neomycin Resistance in *Salmonella typhimurium* from Pigs

Figure 1a and 1b.



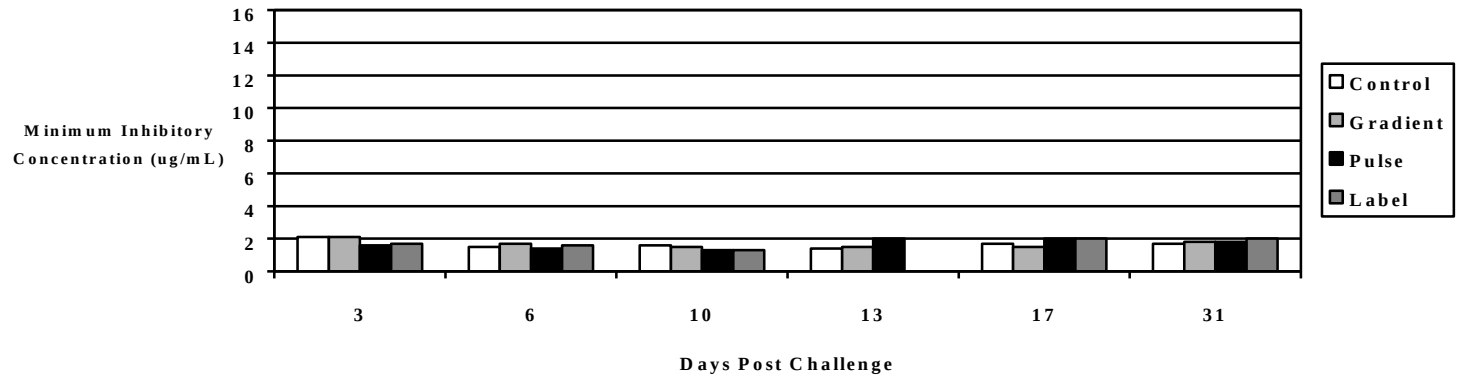


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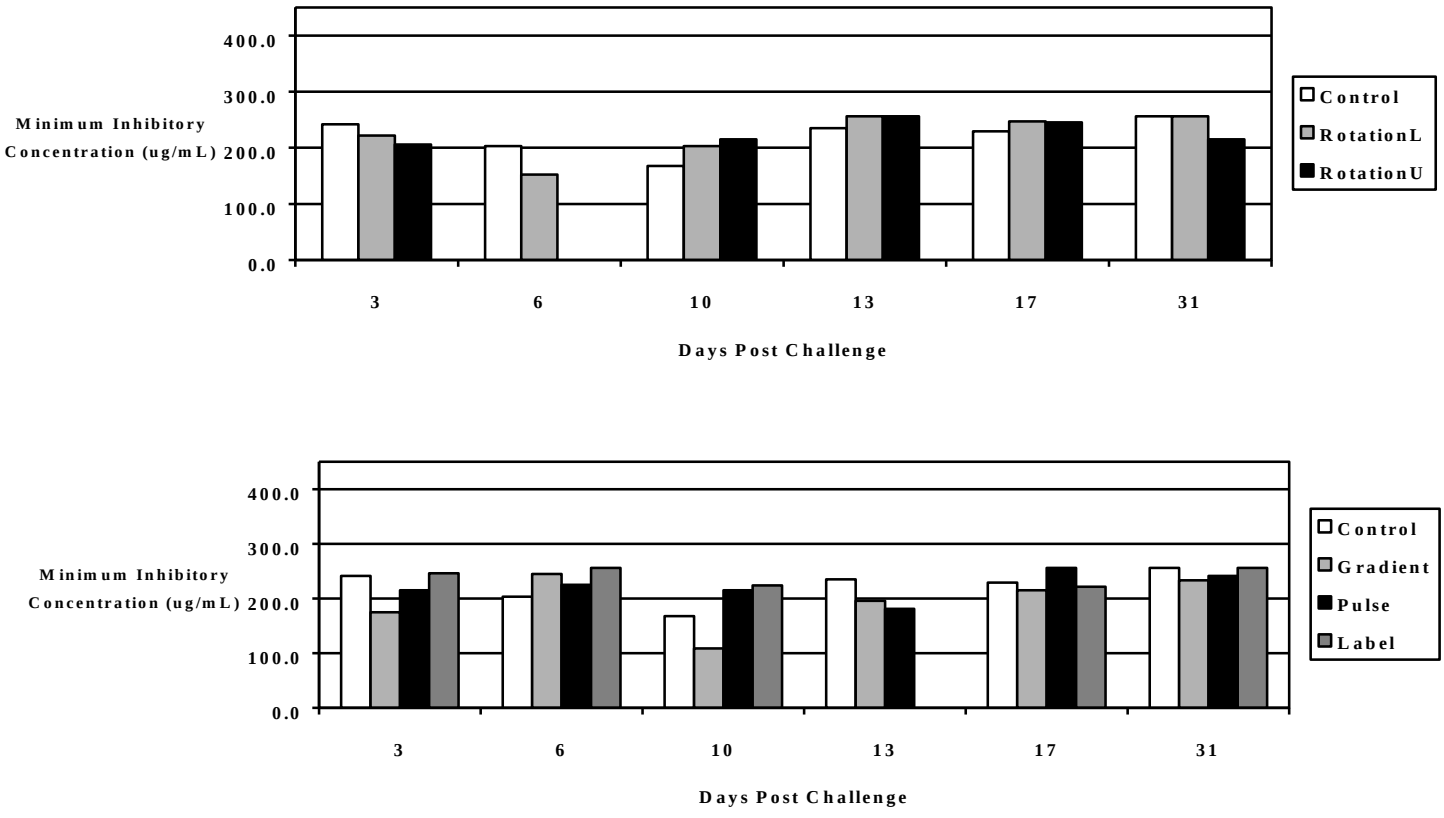
Table 8. Resistance to Sulfamethazine by *Salmonella typhimurium* Isolated from Pigs, verses Antibiotic Regimen Over Time*

	Antibiotic Regimen					
Days Post Challenge	Control	Rotation, Like Antibiotics	Rotation, Unlike Antibiotics	Gradient	Pulse	Label Use
3	241.6	222.1	206.0	174.8	215.2	246.0
6	203.1	152.2	0.0	245.1	224.8	256.0
10	167.9	203.1	215.2	108.5	215.2	223.7
13	234.7	256.0	256.0	195.5	181.0	0.0
17	229.2	247.2	245.1	215.2	256.0	221.5
31	256.0	256.0	215.2	233.4	241.6	256.0

***Data are minimum inhibitory concentrations (MIC) measured in micrograms per milliliter**
Trt Effect P < .0660
Trt x time Effect P < .0004

Effect of Antibiotic Treatment on Sulfamethazine Resistance in *Salmonella typhimurium* from Pigs

Figures 8a and 8b



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