RESEARCH REPORT
PORK SAFETY

Title: Characterizing the Origin and Transfer of Antibiotic Resistance in Swine Herds
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I. Abstract: Sows and pigs were used to characterize the origin, transfer and persistence of bacterial resistance in swine. Effects of previous sow exposure to antibiotics and subsequent use of antibiotics in their pigs on resistance of *Salmonella enterica* Typhimurium, *Enterococcus faecalis*, and *E. coli* were determined. Eight pregnant sows were divided into two groups, with four sows receiving oxytetracycline via the feed and 4 sows receiving no antibiotics. Fecal samples were obtained from the sows prior to antibiotic exposure, and at 1-week intervals until the pigs were weaned. Pigs were weaned at 21 days of age and challenged intranasally with a *Salmonella* Typhimurium isolate containing a nalidixic acid resistance marker. Pigs from each sow treatment group were then divided equally between a subtherapeutic antibiotic treatment regimen or exclusion of antibiotics. Pigs on the antibiotic treatment received apramycin at 150 g/ton of feed, beginning 7 days postweaning and lasting for 14 days, followed by oxytetracycline at 50 g/ton throughout the grow/finish period. At 81 days of age, each treatment group was further divided into high sanitation or low sanitation regimens. Fecal samples were obtained from the pigs while on the sows and at 2, 7, 14, 30, 60, 114 and 115 days postweaning. The *Salmonella* challenge organism, *E.coli* and *E. faecalis* were recovered from fecal samples and tested against both apramycin and oxytetracycline to determine the effects on resistance patterns, using a minimum inhibitory concentration (MIC) analysis. PCR and electroporation techniques were used to characterize genetic resistance elements and determine if resistance genes were located on bacterial chromosomes or plasmids. Treatments affected antibiotic resistance to a greater extent in *E.coli*, compared to *Salmonella* Typhimurium and *Enterococcus faecalis.* The greatest resistance to apramycin occurred in *E. coli* isolates from nursing pigs on sows that had earlier exposure to tetracyclines, and from pigs treated with apramycin during the postweaning period. Resistance to oxytetracycline was consistently high throughout the study in isolates from all pigs and sows, including those with no previous exposure to that drug; however, resistance was greater in isolates from nursing pigs derived from sows with previous antibiotic exposure. Genes responsible for apramycin resistance were found in approximately 90% of resistant isolates and their location was determined to be on bacterial plasmids.

II. Introduction: The use of antibiotics as growth promotants in swine feeds is widespread and has been well documented. Several investigations focusing on subtherapeutic use of antibiotics have shown an increase in the number of antibiotic-resistant and multiple resistant bacteria in the feces of swine following use of antibiotics. Strategies to reduce bacterial resistance through banning or strictly limiting agricultural use of antibiotics would likely be economically detrimental to pork producers because of the clear performance benefits that they provide. Therefore, research efforts have begun to seek strategies to minimize resistant populations of bacteria through a more strategic and prudent use of antibiotics, which will also allow a continuation of their proven benefits.

III. Objectives: The objective of this research was to characterize the origin, transfer, and persistence of bacterial antibiotic resistance across generations of swine, using a minimum inhibitory concentration (MIC) resistance analysis and DNA characterization. Effects of previous sow exposure to antibiotics, subsequent antibiotic use in their pigs, continual exposure to manure, and transport and intermingling of pigs on antibiotic resistance patterns and on genetic resistance elements of Salmonella Typhimurium, resident *E. coli* (gram negative organism), and *Enterococcus faecalis* (gram positive organism) were determined.

IV. Procedures: Eight pregnant sows were divided equally into two groups and separated into identical, biosecure farrowing rooms. One group of sows with previous exposure to antibiotics (tetracyclines) received subtherapeutic concentrations of oxytetracycline (10mg/lb body weight) via the feed for two weeks prior to farrowing, whereas the other sow group, without previous antibiotic exposure received no antibiotics. Upon farrowing, antibiotic use was discontinued and all sows and pigs were maintained with normal production procedures. Fecal samples were obtained from the sows prior to antibiotic exposure, and at 1-week intervals until the pigs were weaned. Pigs were weaned at 21 days, grouped by sow treatment and moved to identical segregated early weaning nursery rooms with separate environmental and waste removal systems. Each sow treatment group was represented by four nursery rooms. At two days postweaning, all pigs were challenged intranasally with a 10^7 colony forming units of *Salmonella* Typhimurium. This isolate contains a naladixic acid resistance marker to assure subsequent isolation and identification. Beginning 7 days postweaning, two pig groups from each sow treatment received apramycin in the feed (150g/ton) for 14 days, followed by oxytetracycline in the feed (50 g/ton) for the remainder of the experiment; whereas antibiotics were excluded from the feed of the other pig groups. At 60 days postweaning, pig rooms were further assigned to either a high sanitation (daily room cleaning) or low sanitation (no cleaning and allowing manure to accumulate) regimen such that each of the above 4 treatments was represented in each sanitation treatment. At the end of the experiment, three pigs from each treatment group (n = 24) were transported to a different facility approximately one hour from the original site and intermingled to simulate transport and holding prior to slaughter. All other pigs (n=32) remained in the original isolation facility through the final sampling period.

Fecal samples were obtained from pigs whenever the sows were sampled postfarrowing, and at 2 days following weaning (just prior to *Salmonella* challenge), 7 days postweaning (prior to assignment to antibiotic treatments), and 14, 30, 60, 114 (prior to transport of pigs), and 115 (following transport of pigs) days postweaning. The challenge organism, non-pathogenic *E. coli* (gram negative sentinel organism) and *Enterococcus faecalis* (gram positive sentinel organism) were isolated on the appropriate selective media for resistance determinations. From each sample, a maximum of four *Salmonella* Typhimurium, four *E. coli* and four *Enterococcus faecalis* colonies were randomly selected and each isolate was tested for resistance using a minimum inhibitory concentration (MIC) analysis, according to standard procedures outlined by

the National Committee for Clinical Laboratory Standards (NCCLS).

DNA was isolated from apramycin-resistant bacteria and PCR was used to detect the presence of the aac(3)-IV gene, which codes for resistance to apramycin. Plasmid DNA isolated from resistant *E. coli* derived from test pigs was electroporated into a sensitive strain of *E. coli* (JM109). A resistance analysis was conducted on recipient cells and a plasmid profile was conducted to determine if the resistance gene was associated with plasmids.

V. Results: Significant effects ($P < .05$) of previous sow exposure to antibiotics were noted for resistance to apramycin and tetracycline in pig *E. coli* (Tables 1 and 2). Isolates from pigs derived from sows that had previous antibiotic exposure had greater initial resistance to apramycin and oxytetracycline during the nursing period compared to other groups. Additionally isolates receiving apramycin had greater resistance to that drug by the end of the apramycin treatment period, regardless of sow treatment. Resistance to oxytetracycline remained high in all treatment groups throughout the study. Sanitation did not appear to produce an interaction with main effects of previous antibiotic exposure for either apramycin or oxytetracycline (Tables 3 and 4). No consistent treatment effects or interactions were observed for *E. faecalis* (Tables 5 and 6). Resistance to both antibiotics remained high for this species throughout the study. There was low recovery of the salmonella challenge organism beyond two weeks post challenge. No treatment effects or interactions were observed for either apramycin or oxytetracycline resistance (Tables 9 and 10) and resistance remained low in *Salmonella* from all of the treatment groups throughout the period that the organism was recovered.

Genetic analysis indicated that approximately 90% (101 out of 111) of resistant *E. coli* contained a sequence for the aac(3)-IV gene, which is known to code for apramycin resistance (Figure 1). DNA profiles revealed that a large plasmid was consistently present in resistant isolates from test pigs (Figure 2). After transforming the plasmid via electroporation into a sensitive *E. coli* control strain, JM109, DNA was isolated from recipient cells and a plasmid profile revealed the presence of the suspected resistance plasmid (Figure 3). Subsequent resistance analysis and PCR analysis confirmed that the aac(3)-IV gene was transferred with the plasmid into the recipient strain. This finding indicates that the resistance gene is present on plasmids and that this gene was common among resistant *E. coli*, sometimes occurring in resistant isolates from pigs without previous exposure to apramycin. A single apramycin-resistant *S*. Typhimurium was isolated from test pigs and the above PCR procedure was used to test for the presence of the aac(3)-IV gene. No evidence of that gene was not found in that isolate. Therefore, no indication of transfer of apramycin resistance between *E. coli* and *Salmonella* was noted in this study.

These results indicate that apramycin and tetracycline resistance in *E. coli* can be affected by previous use of tetracyclines in sows. Additionally, subsequent use of antibiotics in pigs also affects resistance levels in *E. coli*. Pig room sanitation, and subsequent transport and intermingling of pigs did not produce interactions with previous antibiotic exposure of either sows or pigs. Resistance in other bacterial species, including *Salmonella* Typhimurium and *Enterococcus feacalis* was also not affected by previous antibiotic exposure in sows or pigs.

Table 1. Sensitivity to apramycin by *E. coli* isolated from pigs derived from sows with or without previous exposure to antibiotics*

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
7	9.1	39.0	10.9	8.5	3.12
14	46.0	46.0	4.8	4.6	3.28
21	5.5	27.6	7.2	4.4	3.06
23	4.0	5.2	2.9	4.6	1.14
28	6.7	6.2	5.5	10.6	2.17
35	7.8	5.3	3.2	2.8	2.05
51	227.9	19.6	209.4	2.5	9.93

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *E. coli* isolated from pigs prior to and following weaning, through 51 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

Table 2. Sensitivity to oxytetracycline by *E. coli* isolated from pigs derived from sows with or without previous antibiotic exposure*

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
7	123.6	125.8	64.5	43.8	4.67
14	123.9	129.6	87.4	69.1	4.55
21	111.3	127.9	21.3	39.3	5.27
23	413.1	712.5	367.1	210.9	10.89
28	622.0	684.4	485.7	289.2	13.72
35	844.2	653.2	498.0	892.4	17.41
51	326.4	308.3	335.5	386.2	10.31

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *E. coli* isolated from pigs prior to and following weaning, through 51 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

Table 3. Sensitivity to apramycin by *E. coli* isolated from pigs exposed to high or low room sanitation*

Days of age	SW-PW-HS	SW-P0-HS	SW-PW-LS	SW-P0-LS	S0-PW-HS	S0-P0-HS	S0-PW-LS	S0-P0-LS	SEM
81	6.2	3.5	2.3	4.5	3.5	3.4	4.2	3.6	1.37
135	3.6	3.0	3.3	2.9	3.1	5.6	9.6	33.0	3.30
136	5.8	2.5	3.0	2.3	3.1	3.9	49.0	2.7	4.66

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *E. coli* isolated from growing pigs.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics, HS= High sanitation, LS= Low sanitation. SEM = maximum standard error for Lsmeans within row.

Sensitivity to oxytetracycline by *E. coli* isolated from pigs exposed to high or low room sanitation*

Days of age	SW-PW-HS	SW-P0-HS	SW-PW-LS	SW-P0-LS	S0-PW-HS	S0-P0-HS	S0-PW-LS	S0-P0-LS	SEM
81	433.5	455.1	596.3	256.0	526.4	501.5	439.6	948.8	12.82
135	689.8	144.0	596.3	342.5	512.0	102	347.3	44.0	23.62
136	792.3	786.9	396.2	390.7	643.6	982.3	467.9	249.0	28.18

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *E. coli* isolated from growing pigs.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics, HS= High sanitation, LS= Low sanitation. SEM = maximum standard error for Lsmeans within row.

Table 5. Sensitivity to apramycin by *Enterococcus faecalis* isolated from pigs derived from sows with or without previous exposure to antibiotics*

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
7	128.6	60.4	278.5	404.4	6.19
14	150.6	130.8	313.4	309.3	3.68
21	411.1	260.4	500.6	375.5	5.59
23	200.3	138.2	302.8	257.9	3.91
28	316.5	326.1	398.6	289.5	5.65
35	174.0	129.1	474.1	505.6	6.93
51	--------	313.2	300.9	389.1	5.75

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Enterococcus faecalis* isolated from pigs prior to and following weaning, through 51 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

Table 6. Sensitivity to oxytetracycline by *Enterococcus faecalis* isolated from pigs derived from sows with and without previous antibiotic exposure*

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
7	55.9	42.2	61.4	42.6	2.87
14	60.1	78.1	76.9	61.7	2.50
21	93.1	90.7	64.8	73.3	2.97
23	52.1	81.7	103.5	88.7	3.30
28	202.0	133.7	170.1	179.4	4.50
35	52.9	68.3	81.5	69.6	3.46
51	--------	61.5	74.7	100.4	4.76

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Enterococcus faecalis* isolated from pigs prior to and following weaning, through 51 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

Table 7. Sensitivity to apramycin by *Enterococcus faecalis* isolated from pigs exposed to high or low room sanitation

Days of age	SW-PW-HS	SW-P0-HS	SW-PW-LS	SW-P0-LS	S0-PW-HS	S0-P0-HS	S0-PW-LS	S0-P0-LS	SEM
81	215.3	512.0	512.0	472.1	675.6	430.5	455.1	699.4	10.35
135	292.0	407.3	280.1	724.1	296.1	352.1	362.0	442.6	8.29
136	146.0	181.0	304.4	256.0	186.1	215.3	219.8	198.1	5.37

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Enterococcus faecalis* isolated from growing pigs.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics, HS= High sanitation, LS= Low sanitation. SEM = maximum standard error for Lsmeans within row.

Table 8. Sensitivity to oxytetracycline by *Enterococcus faecalis* isolated from pigs exposed to high or low sanitation*

Days of age	SW-PW-HS	SW-P0-HS	SW-PW-LS	SW-P0-LS	S0-PW-HS	S0-P0-HS	S0-PW-LS	S0-P0-LS	SEM
81	32.0	144.0	128.0	64.0	130.7	76.6	65.8	49.9	5.59
135	70.0	80.4	76.1	49.5	108.4	67.6	135.3	35.3	4.45
136	146.0	99.0	107.6	128.0	128.0	140.1	118.6	50.2	4.63

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Enterococcus faecalis* isolated from growing pigs.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics, HS= High sanitation, LS= Low sanitation. SEM = maximum standard error for Lsmeans within row.

Table 9. Sensitivity to apramycin by *Salmonella* Typhimurium isolated pigs derived from sows with or without previous exposure to antibiotics

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
23	2.6	3.7	3.0	2.7	0.34
28	4.8	6.0	3.8	3.9	0.39
35	--------	2.0	2.0	2.4	0.25

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Salmonella* Typhimurium isolated from postweaned pigs through 35 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

Table 10. Sensitivity to oxytetracycline by *Salmonella* Typhimurium isolated from pigs derived from sows with or without previous exposure to antibiotics

Days of age	SW-PW	SW-P0	S0-PW	S0-P0	SEM
23	3.4	4.1	3.8	3.8	0.13
28	4.0	4.1	3.8	3.8	0.13
35	--------	2.0	2.0	2.1	0.10

*Data are Least squares means of minimum inhibitory concentrations (MIC) measured in micrograms per milliliter for *Salmonella* Typhimurium isolated from postweaned pigs through 35 days of age.
SW= sows with previous exposure to antibiotics, S0= sows without antibiotic exposure, PW= pigs treated with antibiotics, P0= pigs not treated with antibiotics. SEM = maximum standard error for Lsmeans within row.

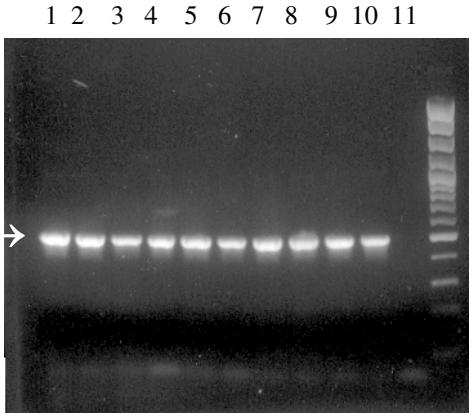

Figure 1. Detection of aac(3)-IV gene via PCR. Electrophoresis gel showing amplified resistance gene products from apramycin-resistant wild type *E. coli* derived from test pigs. Lanes 1 – 10 are from resistant *E. coli* and indicate an amplified DNA band corresponding to the known aac(3)-IV gene (arrow). Lane 11 is derived from a sensitive test *E. coli* strain and in which the gene was not observed.

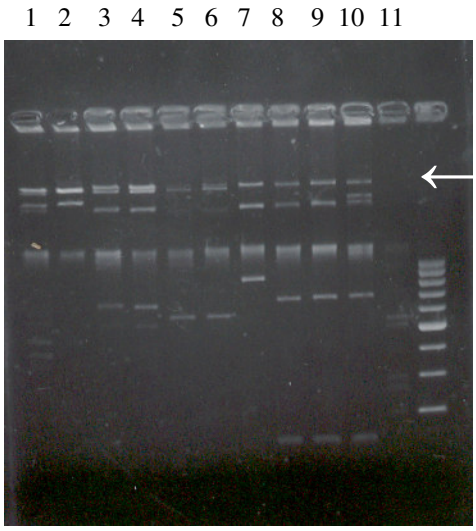

Figure 2. Plasmid patterns from *E. coli* derived from test pigs. Electrophoresis gel showing plasmid patterns from apramycin-resistant wild type *E. coli* derived from test pigs (lanes 1 – 10), and a sensitive control strain (lane 11). Note the consistent band in resistant isolates (arrow), which later proved to contain the aaa(3)-IV apramycin resistance gene.

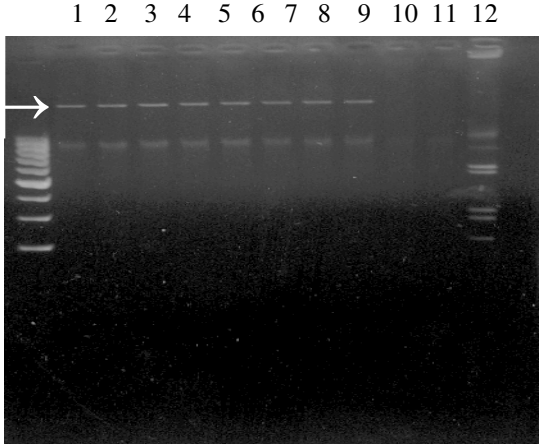

Figure 3. Electroporation of resistance plasmid into sensitive *E. coli* strain. Electrophoresis gel indicating the transformation of *E. coli* control strain (JM109) with plasmids originally from resistant wild type *E.* coli derived from test pigs (arrow). Plasmids were found to carry the aaa(3)-IV apramycin resistance gene and consequently bestowed resistance to JM109 (lanes 1-9). Lane 10 shows the original JM109 (sensitive to apramycin and without electroporated plasmid) and lane 11 shows sensitive *E. coli* control strain V517 also not carrying the resistance plasmid.