Title: A Comparison of Resistance Patterns on Swine Farms Using or Excluding Antimicrobial Products. **NPB#98-209**

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I. Abstract: Seven swine farms were selected to study the effects of use or exclusion of antibiotics on antibiotic resistance patterns in bacteria. Three farms were confirmed to exclude the use of all antibiotics in their production, while 4 farms used antibiotics in both subtherapeutic and therapeutic amounts, as deemed typical of US production. All antibiotic use on the latter farms was documented for a minimum of 12 months prior to the initiation of the study. On each farm, fecal samples were collected from 24 pigs from specific weight groups and sows. Ground pork samples, originating from pigs from each farm, were also obtained. Bacteria, including nonpathogenic *E. coli*, O157:H7 *E. coli*, and *Salmonella spp*. were isolated from samples and tested against a variety of antibiotics using a standardized minimum inhibitory concentration (MIC) analysis. Differences in resistance patterns were evident between farm types in nonpathogenic *E. coli*, with farms excluding antibiotics demonstrating lower MICs, fewer resistant organisms, and fewer organisms with multiple resistance patterns, compared to farms that used antibiotics. Differences also occurred, but were less pronounced, in salmonella, with isolates from farms that excluded antibiotics demonstrating lower MICs. O157:H7 *E. coli* were only isolated from 2 farms, both of which used antibiotics in production, thus a relevant analysis on that bacterium was not possible.

II. Introduction: Use of antibiotics remains commonplace in US livestock production 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. Numerous investigations have focused on the emergence of drug resistance, and a number of conferences have been assembled to address the risks of antibiotic use in the livestock industry. However, severe gaps in our knowledge of this area still remain, and little information is available from which to devise efficient strategies for control. While some groups have proposed limiting or even banning agricultural use of antibiotics, and such regulations have even been imposed in some European countries, there is yet little scientific evidence that such regulations will effectively reduce antibiotic resistance. It is especially important to determine if discontinuing the use of antibiotics in livestock production will affect resistance levels of foodborne pathogens, which have the greatest relevance to transfer of resistant elements to the general public. Thus research that addresses these areas is of great value.

III. Objectives: The objective of this study was to determine if differences in antibiotic resistance patterns occur between bacteria isolated from swine produced on farms that use antibiotics versus farms that exclude antibiotics.

IV. Procedures: Seven farms in the Midwest, Southeast, and Northeast US were selected for this study. Four farms (2 located in Indiana and 2 located in Tennessee) that used either subtherapeutic or therapeutic levels of antibiotics were identified from this group, and antibiotic use for the previous 12 months was documented. The three remaining farms, located in Iowa, Kentucky, and New Jersey were confirmed to exclude antibiotics and were thus categorized as antibiotic free. All farms were considered to be small by current commercial standards (herd size from 10 to 200 sows), with non-antibiotic farms representing both the smallest and the largest of the group. On each farm, 6 pigs from each of 4 weight groups (10, 50, 100, and 240 lb.) and 6 sows were randomly selected, and rectal swabs were used to collect fecal material for isolation of bacteria. To determine effects on subsequent pork products, ground pork was obtained from pigs originating from the test farms for isolation of bacteria. Nonpathogenic *E. coli*, O157:H7 *E. coli* (human hemorrhagic pathogen), and salmonella were cultured and identified using standard microbiological techniques. Isolated organisms were tested for sensitivity to commonly used antibiotics including ampicillin (a beta lactam drug), gentamicin (an aminoglycoside), oxytetracycline (a tetracycline), sulfamethazine (a sulfa drug), and ceftiofur sodium (a cephalosporin), using a minimum inhibitory concentration (MIC) dilution procedure according to standard procedures outlined by the National Committee for Clinical Laboratory Standards (NCCLS). Concentrations of antibiotics used in the analysis ranged from .25 to 256 micrograms/milliliter. If growth occurred at 256 µg/mL, the MIC was recorded as 512 µg/mL (next level of the dilution series). Antibiotic resistance, multiple resistance patterns, and the number of resistant isolates for each farm was determined using General Linear Models analysis for numerical data and Chi-square analysis for categorical data.

V. Results: Nonpathogenic *E. coli* from farms that excluded antibiotics had significantly lower ($P < .001$) MICs for ampicillin, gentamicin, oxytetracycline, and sulfamethazine, compared to farms that used antibiotics. Resistance to ceftiofur followed a similar trend ($P < .10$) (Figure 1). Resistance to ceftiofur tended to be low, and thus even though differences occurred in MICs between the two farm types, all isolates were still within the range considered to be sensitive to that drug (using a clinical value of 4 µg/mL as the threshold for resistance). In general, the differences between farm types were most evident for isolates from younger pigs for ampicillin, gentamicin, and ceftiofur; whereas differences between farm types were noted among all pig groups for oxytetracycline and sulfamethazine (Figures 2 – 6). Farms that excluded antibiotics had fewer resistant isolates, using MIC thresholds of 32 µg/mL for ampicillin, gentamicin, and oxytetracycline, 4 µg/mL for ceftiofur, and 256 µg/mL for sulfamethazine (isolates demonstrating growth above those levels were considered resistant)

(Figure 7). Additionally, a greater number of *E. coli* from farms that used antibiotics demonstrated multiple resistance patterns ($P < .05$) compared to those from farms that excluded antibiotics from production (data not shown).

One hundred and forty-three salmonellae were isolated from 3 farms (2 farms that used and 1 that excluded antibiotics). The majority (92%) of these isolates were, *Salmonella* serovar Typhimurium (World Health Organization nomenclature), O-antigen Type B. Because only one non-antibiotic farm was represented by the salmonella data, an ANOVA analysis, as conducted for non-pathogenic *E. coli,* was not possible. Instead, a Chi-square analysis was conducted to determine differences between farm types. The minimum inhibitory concentration for ceftiofur was generally lower (Chi-square, $P < .001$) in salmonella from the farm that excluded antibiotics, compared to the other farms; although all isolates still demonstrated sensitivity (MICs below 4 µg/mL) to that drug (Figure 8). Resistance to gentamicin was also low on all farms, although some isolates from one farm that used antibiotics demonstrated higher MICs (Chi-square, $P = .13$). Similarly, the highest MIC's for oxytetracycline (Chi-square, $P < .001$) were found in salmonella from farms that used antibiotics. Approximately 2% of salmonella from farms that used antibiotics were determined to be clinically resistant to oxytetracycline (MIC above 32 µg/mL); whereas no resistant isolates were found from the farm that excluded antibiotics (data not shown). Resistance to sulfamethazine was not different (Chi-square, $P = .40$) between isolates from both farm types, with average MICs above 256 µg/mL for each farm type (MICs for salmonella are inherently greater for sulfamethazine compared to other test antibiotics).

Twenty-one O157:H7 *E. coli* were isolated in the study; however, all came from 2 farms that used antibiotics. Thus, comparison of resistance between farm types was not possible. A number of those isolates were resistant to ampicillin, and oxytetracycline (MIC of 256 µg/mL); while all were sensitive to ceftiofur, gentamicin, and sulfamethazine. We were unable to detect or isolate *E. coli* or salmonella of any kind from pork samples. An alternative strategy, using inoculation procedures will be employed to determine effects of meat origin on resistance patterns in bacteria of interest. In total, these data indicate that exclusion of antibiotics in swine production decreases antibiotic resistance in non-pathogenic *E. coli*, and to a lesser extent, resistance in salmonellae.

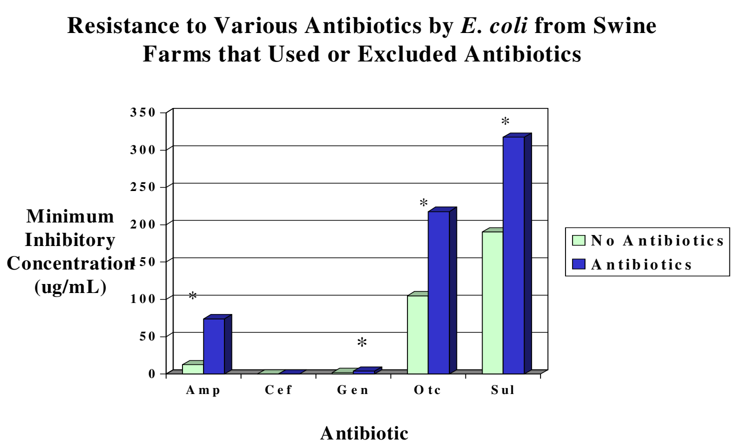

Figure 1. Minimum inhibitory concentrations (MIC) for various antibiotics tested against non-pathogenic *E. coli* isolated from farms that used and farms that excluded antibiotics. **Amp** = ampicillin, **Cef** = ceftiofur, **Gen** = gentamicin, **Otc** = oxytetracycline, **Sul** = sulfamethazine. Asterisks above bars indicate differences between farm types (Antibiotic use versus antibiotic exclusion, P < .05).

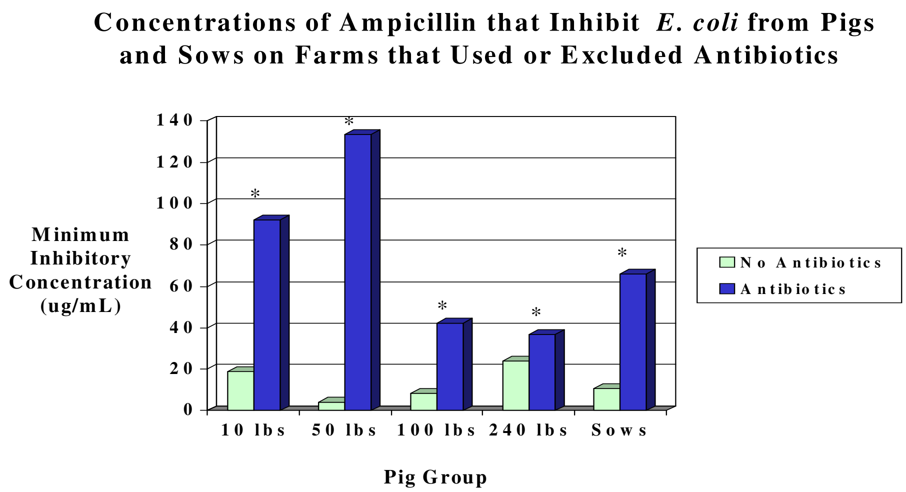

Figure 2. Minimum inhibitory concentrations (MIC) of ampicillin for non-pathogenic *E. coli* isolated from pigs of various sizes and sows on farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

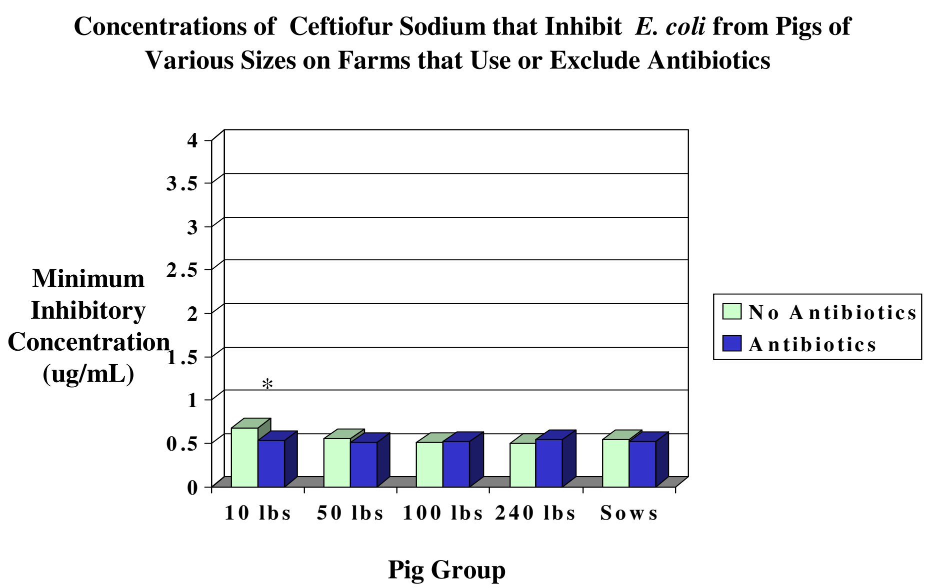

Figure 3. Minimum inhibitory concentrations (MIC) of ceftiofur sodium for non-pathogenic *E. coli* isolated from pigs of various sizes and sows on farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

Concentrations of Gentamicin that Inhibit *E. coli* from Pigs and Sows on Farms that Used or Excluded Antibiotics

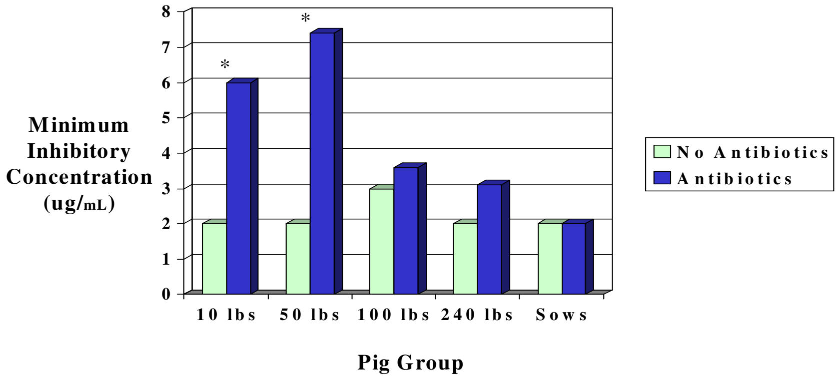

Figure 4. Minimum inhibitory concentrations (MIC) of gentamicin for non-pathogenic *E. coli* isolated from pigs of various sizes and sows on farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

Concentrations of Oxytetracycline that Inhibit *E. coli* from Pigs and Sows on Farms that Used or Excluded Antibiotics

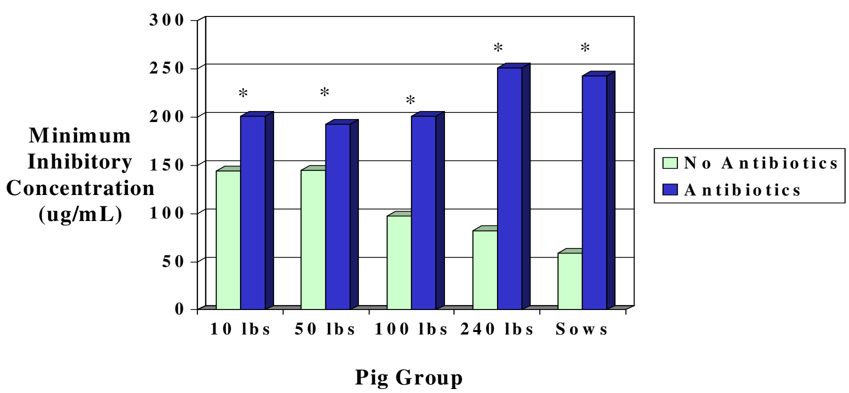

Figure 5. Minimum inhibitory concentrations (MIC) of oxytetracycline for non-pathogenic *E. coli* isolated from pigs of various sizes and sows on farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

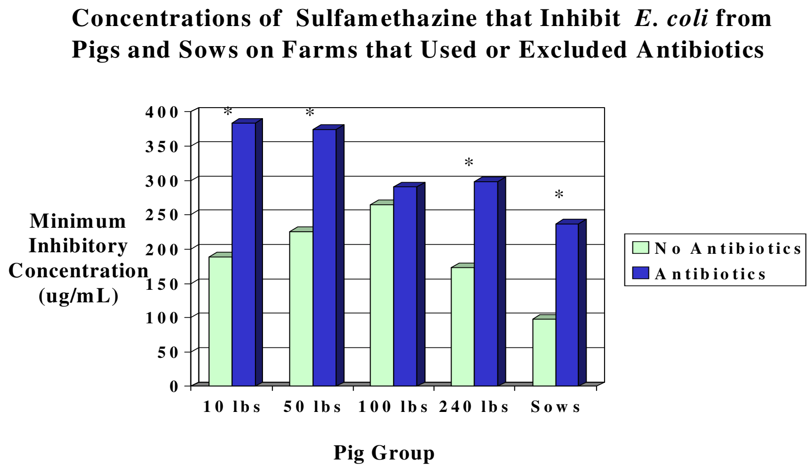

Figure 6. Minimum inhibitory concentrations (MIC) of sulfamethazine for non-pathogenic *E. coli* isolated from pigs of various sizes and sows on farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

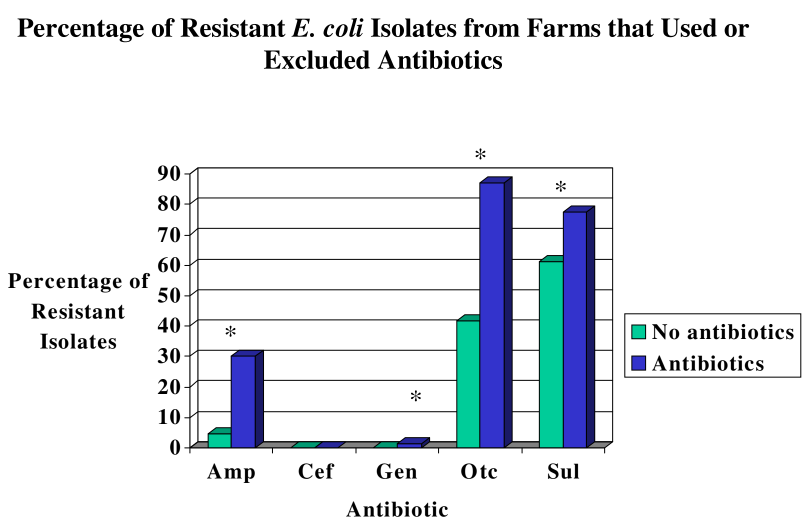

Figure 7. Percentage of *E. coli* isolates that were resistant, from farms that used or excluded antibiotics. **Amp** = ampicillin, **Cef** = ceftiofur sodium, **Gen** = gentamicin, **Otc** = oxytetracycline, **Sul** = sulfamethazine. Resistance was determined based on growth above 32 µg/mL for ampicillin, gentamicin, and oxytetracycline, 4 µg /mL for ceftiofur, and 256 µg /mL for sulfamethazine. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, P < .05).

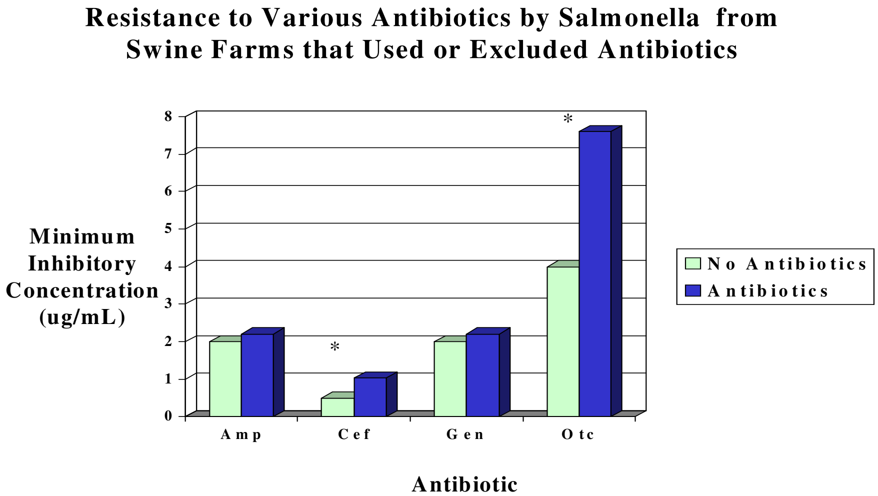

Figure 8. Minimum inhibitory concentrations (MIC) for various antibiotics tested against salmonella isolated from farms that used and farms that excluded antibiotics. **Amp** = ampicillin, **Cef** = ceftiofur, **Gen** = gentamicin, **Otc** = oxytetracycline. Asterisks above bars indicate differences between farm types (antibiotic use versus antibiotic exclusion, $P < .0001$). Sulfamethazine sensitivity was not different between farm types, with MIC's ranging near 500 ug/mL (beyond range of this graph) for both antibiotic and non-antibiotic farms.