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# A comparison of resistance patterns on swine farms using or excluding antimicrobial products

Melissa A. Beckmann

To the Graduate Council:

I am submitting herewith a thesis written by Melissa A. Beckmann entitled "A comparison of resistance patterns on swine farms using or excluding antimicrobial products." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Alan Mathew, Major Professor

We have read this thesis and recommend its acceptance:

Kelly Robbins, David Golden

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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# A COMPARISON OF RESISTANCE PATTERNS ON SWINE FARMS USING OR EXCLUDING ANTIMICROBIAL PRODUCTS

**A Thesis**

**Presented for the**

**Master of Science**

**Degree**

**The University of Tennessee, Knoxville**

**Melissa A. Beckmann**

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## ABSTRACT

The effects of farm use or exclusion of antibiotics on antibiotic resistance patterns of bacteria were compared using fecal samples from live swine. Four farms that used antibiotics and three farms that excluded antibiotics from production were selected and from each farm, 6 pigs from each of 4 weight groups (4.5, 23, 45, and 109 kg) and 5 sows were randomly selected for collection of fecal samples. *E. coli*, O157:H7 *E. coli*, and *Salmonella* spp. were isolated from fecal samples and tested for sensitivity to gentamicin, sulfamethazine, oxytetracycline, ceftiofur, and ampicillin using a standardized minimum inhibitory concentration (MIC) analysis. Resistance patterns were markedly different between farm types in *E. coli*, and moderately so in *Salmonella*. In both cases, isolates from farms that excluded antibiotics had lower ($P < 0.05$) MICs. The number of resistant isolates and those that demonstrated multiple resistance patterns was greater ($P < 0.05$) on farms that used antibiotics. *E. coli* from farms that excluded antibiotics had significantly lower ($P < 0.001$) MICs for gentamicin, sulfamethazine, oxytetracycline, and ampicillin and lower ($P < 0.10$) MICs for ceftiofur. Farm type differences were most evident for isolates from younger pigs for gentamicin, ceftiofur, and ampicillin but were also noted among all pig groups for sulfamethazine and oxytetracycline. In *Salmonella*, the MICs were higher from farms that used antibiotics particularly for oxytetracycline and ceftiofur ($P < 0.001$). O157:H7 *E. coli* were isolated from 2 farms, both of which used antibiotics in production, thus a relevant analysis on that bacterium was not possible. In total,

these data indicate that exclusion of antibiotics in swine production decreases antibiotic resistance in *E. coli*, and to a lesser extent resistance in salmonellae.

TABLE OF CONTENTS

## LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

# 1. INTRODUCTION

In the United States less than 2% of the population is directly involved in producing food for retail trade. Changes in the food animal industry are responsible in part for the gradual reduction in number of traditional farms. In the food animal industry there has been a move away from individual farms and toward more intensive or integrated animal production systems. These close confinement type operations reduce input of land, feed, and labor and maximize output of lean, healthy high quality animals (NRC 1999). The success of this transition in animal production may be partly due to the use of antibiotics. Antibiotics not only prevent and control diseases that might otherwise rapidly overwhelm densely populated groups of animals, but also increase the rate of growth and improve feed conversion efficiency.

The benefits provided by antibiotic use in animals brings with it new challenges in meeting the increasing demands (more high quality meat) and expectations (limited risk of contaminants such as antibiotic resistant bacteria) of the consuming public. A survey conducted by the Economic Research Service (ERS 1994) of the United States Department of Agriculture (USDA) revealed that out of all of the food safety issues, concern about pathogens in food was the issue most frequently cited during the period of 1937 to 1991 in news and media data bases. The issue of agricultural use of antibiotics and the evolution and transfer of antibiotic resistant bacteria has caused some segments of the medical community and consumer groups to suggest that antibiotics used in human medicine should not be used on animals, or that subtherapeutic use of antibiotics (low dose prophylaxis and growth

promoting amounts) should be banned. Decisive legislation concerning the use of antibiotics in food animal production and the threat to human medicine has been hindered by the lack of data with regard to cause and effect relationships between antibiotics and resistant bacteria. In the pork industry, for example, little is known about how either of the above mentioned recommendations will influence the amount of foodborne pathogens or more importantly resistant foodborne pathogens in market weight pigs and subsequent pork products. Some of the more recent drugs used in swine production have yet to be consistently monitored using standardized methods. New drugs provide an excellent opportunity for tracking the progression of antibiotic resistance through various pork production management schemes. This information could be used to devise efficient strategies that would prolong the efficacy of the antibiotics against pathogens while limiting the risk of inducing resistance. The purpose of this study was to determine if differences in antibiotic resistance patterns occur between bacteria isolated from swine produced on farms that have used old and new antibiotics versus farms that excluded antibiotics.

# 1. LITERATURE REVIEW

Domagk discovered the antibacterial action of sulfonamides in 1935 (Visek 1978). Experiments with these drugs caused depressed growth (Black et al. 1941; Kornberg et al. 1943) and vitamin deficiency signs in rats. These adverse effects were reversed with dietary supplementation of folic acid (Daft and Sebrell 1943) or biotin (Daft et al. 1942). It was then recognized that intestinal bacteria had the ability to synthesize vitamins (Elvehjem 1948) and be beneficial to the host unless they were pathogenic. Martin (1942) was the first to recognize that sulfonamides given to rats with infectious diseases increased survival rate and growth (Visek 1978). The potential for increasing the growth rate of farm animals with antibiotic agents was first suggested by Moore et al. (1946) and Morehouse and Mayfield (1946). The growth stimulation effect of antibiotics became fully appreciated during a search by Dugger (1948) for a microbial source of vitamin $B_{12}$ from *Streptomyces aureofaciens*. Researchers at Lederle Laboratories found that something other than Dugger's $B_{12}$ was causing what now is known as the antibiotic growth effect (CAST 1981).

The economic benefits (NRC 1999) brought about by antibiotic growth promotion have become the principal reason for the use of antibiotics in food animal production (Hays 1986). Antibiotics used at low (subtherapeutic) levels increase animal gains with less feed, and help prevent disease (Hays 1969; CAST 1981; Zimmerman 1986; NRC 1999). Subtherapeutic use is defined in the United States as

the use of an antibiotic as a feed additive at less than 200 g per ton of feed (NRC 1999). Eighty percent of the antibiotic drugs used in livestock and poultry are used at subtherapeutic concentrations (IOM 1989). In the U. S., nearly 100% of the chickens and turkeys, 90% of the swine and veal, 60% of the feedlot cattle, and 75% of dairy calves are fed antibiotics at some time during their lives (CAST 1981; Manchanda 1994).

Hog performance is improved with the use of subtherapeutic concentrations (NRC 1999). In hogs, antibiotics are used in about 90% of starter feeds, 75% of grower feeds and in more than 50% of the finisher feeds and in at least 20% of sow feeds (NRC 1999). It is estimated that the average increases in rates of gain for pigs from the use of antibacterials in their starter, grower and finisher feeds are about 25, 15, and 16% respectively. Corresponding estimates for feed utilization improvement are 9, 5, and 3% (Braude et al. 1953; Hays 1977). In breeding animals, feed-additive antibiotic drugs improve farrowing rate, litter size (Hays 1977), birth weight, and number of pigs weaned per litter (Hays 1986; NRC 1999). Stahly (1995) notes "In addition to enhancing pig performance, subtherapeutic concentrations of antimicrobials may also alter lean tissue growth." Others have made similar assertions (Hathaway 1990; Roth and Kirchgessner 1990).

Responses to antibiotics are much greater in young pigs (Hays 1969) and then decline as the animals mature (Hays 1985). Other factors that may affect an animal's response to antibiotics are the animal's genetic predisposition (Stahly 1996), the status of the pig's immune system (van den Broeck 1993), and the type and duration of

antibiotics used (Fagerberg et al.1979). For instance, agents that are widely distributed in the body and have a broad spectrum of antibacterial activity produce a greater growth response (Visek 1978). Responses may also vary with the type of carbohydrate in the diet (Stokstad et al. 1953; Harbers 1963) and changes in the diet (Schaedler and Dubos 1959).

The FDA has approved twenty-nine drugs (21 antibiotics and 8 chemotherapeutics) for use in hogs (Shepard et al.1992). To improve growth rate in hogs, 10 antibiotics (of microbial origin) and 2 chemotherapeutics drugs are used. The 10 antibiotics are arsenilic acid, bacitracin, bambermycins, chlortetracycline, efrotomycin, oleandomycin, penicillin, tiamulin, tylosin, virginiamycin, and the 2 chemotherapeutics are arsanilic acid and carbadox (NRC 1999). Avilamycin (Maxus$^{R}$) adds to the list of hog growth promotants (Roth and Kirchgessner 1993).

The following are prophylaxes: arsenilic acid, bacitracin, chlortetracycline, tiamulin, tylosin, and carbadox. Others that are approved for therapeutic use but not as growth promotants are antibiotics, amoxicillin, ampicillin, apramycin, erythromycin, gentamicin, lincomycin, neomycin, oxytetracycline, spectinomycin, streptomycin, tetracycline; and chemotherapeutics, aranilate sodium, roxarsone, sulfaethoxypryidazine, sulfachlorpydazene, sulfamethazine, and sulfathiazole (NRC 1999).

Antibiotic substances presently used in animal production fall into two broad groupings: broad and narrow spectrum antibiotics (Hays 1986) and (Merck Veterinary Manual 1986; Kucers et al., 1997). Broad–spectrum antibiotics such as Ǝ-lactams

prevent proper formation of bacteria cell wall making them generally effective in killing a wide range of bacteria. Narrow-spectrum drugs are usually highly selective by targeting biochemical pathways specific to a particular species of bacteria (NRC 1999).

Antibiotic action against bacteria varies among drug groups. Bactericidal drugs kill invading organisms (Merck Veterinary Manual 1986; Kucers et al. 1997). Bacteriostatic drugs prevent the growth of organisms, but do not kill them directly (NRC 1999).

Antibiotics are also classified as to how they are absorbed and their mode of action against microorganisms. Systemic antibiotics are absorbed in the intestines in large amounts. They include the tetracyclines (chlortetracycline, oxytetracycline, and tetracycline), erythromycin, lincomycin, and penicillin (procaine). Nonsystemic antibiotics are not absorbed from the intestines in significant amounts. This group includes bacitracin (zinc, manganese, or methylene disalicylate), neomycin sulfate, streptomycin chloride or sulfate, tylosin, oleandomycin, novobiocin, virginiamycin, and bambermycins (CAST 1981).

The exact mode of action of antibiotics in bringing about growth promotion is not thoroughly understood. Cromwell (1991) has summarized three possibilities. The first involves direct biochemical events that are affected by antibiotics: decreased nitrogen excretion (Roth and Kirchgessner 1993), efficiency of phosphorylation reactions in cells, and direct effects on protein synthesis (Vervaeke et al. 1979). The second involves direct effects on metabolism, including the effects of antibiotics on

the generation of essential vitamins and cofactors by intestinal microbes and the way that antibiotics affect the population of microbes that make these nutrients. In addition, the feeding of antibiotics is associated with decreased gut mass (Pepper 1953; Milner and Visek 1974), increased intestinal absorption of nutrients, and energy sparing. This reduces the nutrient cost for maintenance, so that a larger portion of consumed nutrients can be used for growth and production, thereby improving the efficiency of nutrient use for productive functions (Okumura et al.1978; Visek 1978). The third proposed mechanism of action is eliminating subclinical populations of pathogenic microorganisms (NRC 1999). This can be achieved with bactericidal drugs or subtherapeutic concentrations of antibiotics that increase specific immunological responses of the host to invading bacteria (Easmon and Desmond 1982; Veringa and Verhoef 1985; Hand et al. 1989).

Resistance of microorganisms to antibiotics develops through several mechanisms (reviewed in Davies and Webb 1998; Hickey and Nelson 1997; O'Grady et al. 1997). For instance, an alteration of the target for the antibiotic can protect the microbe from the drug by removing its substrate. Microbes can develop the enzymatic capability to degrade a drug, develop a mechanism to pump the drug out of the cell or develop an altered uptake system to prevent drug entry. Cells can also lose their ability to metabolize the drug into the actual inhibitory compound. Bacteria are known to retain defenses against antibiotics, such as tetracycline, that have been discontinued in a swine operation for 13 years (Langlois et al. 1986). In some of these cases compensatory mutations are linked to the drug resistant gene in such a way that

reversion to susceptibility would be cidal. The bacteria must retain the resistance to remain viable.

Some bacteria are "naturally resistant". For instance, penicillin does not work against salmonella (AHI 1998). Resistant bacteria have been isolated from apparently nonselective environments (Mach and Grimes 1982; Levy 1992). Normally, in a "wild type" bacterial population, approximately 2% are resistant to any given antibiotic (Novick 1981). Sometimes, by chance, bacterial genes mutate and as a result a viable resistant strain of bacteria will emerge. More commonly, resistance emerges through selective pressure applied through exposure to antibiotics. The most resistant bacteria are usually found in environments with the highest levels of antimicrobials such as hospitals (Datta 1969), fish farms (Sandaa et al. 1992), sewage, and wastewater (Fontaine and Hoadley 1976).

In animals and humans, conventional indigenous microflora prohibit the establishment of invading enteric pathogens by competitive exclusion (Dubos and Shaedler 1960, 1962; Hentges 1969). In the bowel of humans and animals there are more than $10^{10}$ bacteria per gram of feces. Most belong to a heterogeneous group of Gram-negative bacilli, called enteric bacilli the most abundant of which are the anaerobes *Clostridium* and *Bacteroides* (Anderson 1975). When subjected to prolonged or high doses of antibiotics, these commensal bacteria are killed or inhibited along with the pathogenic ones and resistant bacteria multiply to take their place (Gordon et al. 1959; Kobland et al. 1987).

Antibiotic resistance is the ability of certain bacteria, which are normally destroyed by a particular antibiotic, to survive exposure to that antibiotic. Resistance means that the bacteria no longer respond to the treatment. Susceptibility describes how sensitive bacteria are to antibiotics (AHI 1998).

Once resistance occurs through a mutational event, bacterial resistance can spread. Chromosomal resistance can be transferred to the progeny (Piddock 1996), or R (resistant) plasmids can be transferred from one bacterium to another (Hickey and Nelson 1997). Transferable resistance via R plasmids, the more common form, was first recognized by Japanese investigators (Watanabe 1963). R plasmids can spread among bacterial strains within species and between genera (Frieden et al. 1993), even under simulated natural conditions in the absence of antimicrobial agents (Kruse and SNrum 1994).

There are several mechanisms of plasmid transfer. Bacteria can engulf free DNA from dead cells through a process called transformation. A form of sexual transfer (conjugation) of genetic material by way of a narrow tube (pili) can also take place. Transduction is a phage (bacterial or viral) mediated transfer of genetic material. In addition, transposons, a class of DNA genes, can be shuttled between plasmids and chromosomal DNA (NRC 1999).

Resistance to one antibiotic may be genetically linked to resistance to one or more other antibiotics (CAST 1981; Hays 1986). Resistant organisms can accumulate resistance elements by which they become multi-drug-resistant. Resistance to

multiple agents is often encoded on a single plasmid, transposon, or integron and can be acquired en bloc (Murray 1994).

The time between introduction of an antibiotic and development of resistance is becoming shorter. This may be due to greater use of newer antibiotics (Mathew et al., 1998), and also to the fact that bacteria can now more easily modify existing drug resistance mechanisms rather than create mechanisms de novo, as was the case when antibiotics were first introduced (Tomasz 1994; Mathew et al., 1998).

It has been suggested but, with conflicting evidence, that plasmids which confer resistance affect the pathogenicity of organisms (Jarolmen 1971; Smith 1972). For instance, an R plasmid may also carry a gene for production of enterotoxin (Gyles et al. 1977; Hays 1986; Smith and Fatamico 1995).

A number of bacterial diseases of animals are transmissible to humans (zoonotic). It is of concern that either resistant bacteria may be capable of causing human disease that cannot be treated because the pathogens will be resistant to the drugs indicated for treatment (Levy 1992; Piddock 1996) or that non-pathogenic organisms could transmit their R plasmids to human flora or pathogens with the same results (Gyles et al. 1977; Levy 1992). The possible relationship between antibiotic resistance in the enteric flora of food producing animals and antimicrobial efficacy in the treatment of infections in humans has been studied over the past 50 years. Comprehensive discussion of antibiotic resistance and associated risks began with Great Britain's Neatherthorpe and Swann Committees and continued in the United

States by task forces of the FDA, various councils, and on two occasions by the National Academy of Sciences (Donnelly et al. 1996).

Case studies have shown that the passage of resistant organisms from animals to humans can occur and be perpetuated and amplified through food (Spika et al. 1987). Transfer of R plasmids among bacteria from animals to humans has been demonstrated (Smith, 1969, 1970, 1972; Jarolmen 1971; Levy et al. 1976; Levy 1978). However, the transfer does not take place as rapidly *in vivo* as it dies *in vitro* (Falkow 1975), possibly due to interference by normal gut flora (Anderson 1975).

The Institute of Medicine (IOM 1989) and the Office of Technology Assessment (OTA 1995) have reported on circumstantial evidence linking subtherapeutic use of antibiotic drugs in farm animals to potential human health hazards. The Institute of Medicine reported that the absolute number of antibiotic-resistant isolate bacteria appears to be greater when subtherapeutic doses are used in animal feed than when therapeutic doses are given (IOM 1989). Walton (1986) contends that antibiotic concentrations achieved in animals fed antibiotics at many of the subtherapeutic concentrations used in the field do not reach concentrations necessary for the selection of resistant strains. At least one experiment has shown that feeding therapeutic levels of chlortetracycline for 14 days increased antibiotic resistance and multiple antibiotic resistance more than the feeding of subtherapeutic chlortetracycline for 85 days (Langlois 1983). Experimenters that compared the antibiotic resistance of Gram-negative fecal bacteria from pigs in three herds with different histories of antibiotic exposure concluded that any form of antimicrobial

exposure would increase that prevalence of antimicrobial resistance and multiple resistance of fecal bacteria (Dupont and Steele 1987; Gellin et al. 1989).

Some scientists support the opinion that antibiotics used in food-animals are fundamentally benign to human health (Frappaolo 1986; van den Bogaard 1993). Others have pointed out that both fruits and vegetables (Levy 1984) have been associated with resistant bacteria, as well as animal protein. Statistics from the Department of Agriculture show that animal carcasses inspected just after slaughter have very low levels of contamination, which suggests that antibiotic use may be a factor in keeping bacteria counts low (AHI 1998). In a May 1999 interview, John Keeling, vice president of legislative and public affairs for the Animal Health Institute, offered the following quote " There is no documented case where antibiotic use in animals has caused treatment failure in people" (Ishmael 1999).

It has been shown that factors other than exposure to subtherapeutic or therapeutic antimicrobials may be responsible for increases in pathogens and antimicrobial-resistant enteric bacteria in animals (Bolder and Mulder 1983; Dawson et al 1984; Langlois et al. 1986; Stern 1995). In swine, dirty quarters, stress of transport (Embry et al. 1962,1966, 1969), overcrowding in holding pens, rough handling before slaughter (Williams and Newell 1970; Corrier et al. 1990), decreases in temperature (Moro et al.1998), and feed and water removal (Bierer and Eleazer 1965; Smith 1977) have been reported to increase shedding of *Salmonella sp.* (Williams and Newell 1970; Rigby and Pettit 1980; Corrier et al. 1990), *Campylobacter spp.* (Stern and Line 1992; Jacobs-Reitsma et al. 1994) and *E. coli*

O157:H7 (Rasmussen et al., 1993), as well as the percentage of antimicrobial-resistant enteric bacteria shed into the environment (Molitoris et al. 1987), even in herds that have not been exposed to those drugs in over 13 years (Langlois et al. 1986; Dawson et al. 1984). These external factors upset the equilibrium of the intestinal function and flora lowering the resistance of otherwise healthy animals to pathogens (Mulder 1995). Pathogens that are orally consumed before and during crating and transportation may colonize the ceca where they may be retained throughout processing (Moran and Bilgili 1990).

Abuse of antibiotics is common in human medicine and also contributes to the pool of resistant bacteria (IOM 1989 and 1998; Amabile-Cuevas 1993; Hickey and Nelson 1997). At least half of the antibiotics prescribed in the United States are unnecessary or inappropriate according to Levy (1998). In many cases the antibiotics suggested are not specific to the infectious organisms or the recommended dosage and duration are wrong (CDC 1994; IOM 1998).

Conversely, data from the United States National Swine Survey collected by the National Animal Health Monitoring System were used to describe the use of feed additives in swine feeds. Of 3,328 feeds tested, only about of the 21% of the feeds contained additives in an off-label manner. One-half of these included greater concentrations of antibiotics than recommended or the wrong age of pigs were treated and the other half consisted of off-label combinations (Dewey et al. 1997).

Once a resistant human pathogen emerges, there are many factors that can contribute to its proliferation. Foremost is the density and mobility of the human

population. The increasing population, particularly of elderly and immunocompromised individuals, may provide greater opportunity for resistant pathogens to persist (Telzak et al. 1991). There have also been dramatic changes in consumer eating habits and the methods in which food is provided.

People are eating more frequently in restaurants and institutional cafeterias, increasing their contact with and exposure to one another. In addition food is produced, processed, handled and prepared in ways that concentrates activities to fewer and larger companies with extensive distribution capabilities (CDC 1994). All of these factors contribute to the spread of resistant organisms. The transfer of resistant organisms can take place whenever the conditions are right for bacterial growth (Hays 1986).

Research has shown that consumers are more sensitive to the subject of pathogens in food than any other food safety issue (ERS 1994). Technological advances in detection, surveillance and reporting have improved our ability to isolate and identify "new" pathogens or recognize foodborne diseases, formerly classified as cause "unknown" which may contribute to our perception of their increasing emergence (Smith and Fratamico 1995; NRC 1999). Not all reports accurately reflect the current status of the problem. For example, collection, analysis, and measurement of farm animal samples are not standardized in many studies and what information does exist cannot be pooled. By contrast, data on bacterial resistance in humans are of higher quality and are now on-line and widely available for many types of sophisticated analyses (AHI 1998). Others find the inherent problems of the tests of

antibiotic sensitivity and inconsistent interpretations of the results to be problematic (Wiedmann 1993). For example, the definition of resistance varies between countries as determined by minimum inhibitory concentration analysis. For ampicillin, resistance is determined in *E .coli* as ≥ 2, ≥ 4, ≥ 8, and ≥ 16 :g/mL for Sweden, Germany, the Netherlands, and the United States, respectively (Wiedmann 1993).

Some of the most important pathogens that have emerged lately that are frequently associated with food animals are, *Eschericia coli* O157:H7, a virulent strain not known to be resistant to antibiotics, vancomycin-resistant enterococci a pathogen resistant only to antibiotics used in human medicine, and *Salmonella* DT-104, a multi-resistant strain that has been linked to the use of antibiotics in food animals but still sensitive to some human therapeutics.

*Escherichia coli* O157:H7 which causes hemorrhagic colitis and hemolytic uremic syndrome: is an *E. coli* strain with increased virulence that has caused a number of fatalities in foodborne outbreaks (Smith and Fratamico 1975). This new *E. coli* serotype is believed to have evolved in Central America in the 1980s. It became pathogenic through the acquisition of virulence factors from a type of *Shigella* bacterium that causes severe dysentery in humans. *Shigella* is not found in animals (AHI 1998). The organism has the ability to adhere intimately to intestinal cells by an attaching and effacing mechanism and produces one or more types of phage-encoded Shiga-like toxins (Smith and Fratamico 1975). To date, this pathogen has not been implicated in antibiotic resistance problems and the most frequent outbreaks have been associated with vegetable and fruit juices rather than animal protein (NRC 1999).

There have been attempts to establish a possible link between the appearance of vancomycin-resistant enterococci (VRE) in humans and the food chain. Donnelly et al. (1996) point out that the *Van A* gene may have spread from humans to farm animals. Vancomycin belongs to the glycopeptide family of antibiotics along with avoparcin. Avoparcin has been used in the European Community as a growth promoter in animal feeds (Bates 1997). However, VRE has also appeared in the United States where avoparcin is only used for human medicine (Bingen et al. 1991; Frieden et al. 1993; Bates 1997). Vancomycin use has been on the rise for patients having major cardiovascular (Maki et al. 1992), orthopedic, and organ transplant surgeries, or low birth weight infants (Payne et al. 1994). The increased role of vancomycin as a defensive medicine may have attributed to intense selection pressure and rapid escalation in VRE (Hays 1996). In areas of the world where glycopeptides are used in animals, VRE is ten-fold less common, if it is present at all (Hayes 1996). The increase in VRE occurred in the USA in the absence of any use of glycopeptide antibiotics in animals (Hayes 1996). In North America, broad-spectrum tetracyclines, ionophores, and bacitracins continue to be the dominant feed additive antibiotics used on poultry and livestock (Donnelly et al. 1996).

The 5-drug-resistant (ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline) *Salmonella* (definitive type DT-104) is an isolate that is now reportedly resistant to ciprofloxin, the fluoroquinolone used in humans. Again, differences in terminology and measurement techniques may have confounded this issue. Whether or not this strain is resistant to ciprofloxin depends upon individual

definitions of resistance (NRC 1999). This strain appeared more than year before fluoroquinolones were actually used in animal production in the United Kingdom, and almost a decade before their use in poultry (1994) and cattle (1998) in the United States (AHI 1998). In the United States, a surveillance board named "National Surveillance for Antibiotic Resistance in Zoonotic Enteric Pathogens" has been established to track and oversee the effect of antibiotics in the development of resistant bacteria and to determine further use of these antibiotics in food animals. Surveillance data reviewed by FDA and CDC experts stated that, at the time of the report, there were no isolates of *Samonella* DT-104 that were resistant to ciprofloxacin in the United States (Glynn et al. 1998).

In the past, the common response to resistance has been to develop new classes of antimicrobials (Ishmael 1999). However, in a 1991 survey of pharmaceutical companies in the United States and Japan, 50 percent reported that they had substantially reduced or abandoned antibacterial research because the market for antibiotics was saturated. It is estimated that it takes several years to bring a new food animal drug to market (CAST 1981). Only 1 compound in 7,500 tested for initial activity reaches the market (AHI 1993). With the estimated investment of more than $300 million required to bring a new antibiotic to market, there is little incentive for such endeavors (Murray 1994).

Overall, the yearly cost exacted by drug-resistant infections in the United States is estimated to exceed $30 billion (Radetsky 1998). Public health community concerns are that antibiotics are approved for the use in animals before their efficacy

against human illnesses has been exhausted, thus speeding up the development of resistance. Many believe that antibiotics that are used in humans should not be used in animals, and this was one of the recommendations of the Swann Committee in 1969 (NRC 1999; Swann 1969). However, follow-up tests have shown that different drugs from the same antibiotic class used in animals and humans leads to cross-resistance because bacteria are unable to distinguish between the two (Piddock 1996). In addition, a single drug can select resistance to several chemically unrelated agents (Piddock 1996).

Another recommendation of the Swann Committee was to restrict the use of antibiotics to a prescription only basis. This failed to influence the amount of antibiotics used in England, because some larger producers employ their own veterinarians (CAST 1981). World Health Organization (WHO 1997) is in favor of phasing out the use of antibiotics; particularly penicillin, tetracyclines, and others used to treat human diseases. However, from experiments on the withdrawal of tetracycline and penicillin, it does not appear that total restriction has an impressive influence on reducing the level of resistant bacteria either. The level of tetracycline resistant fecal coliforms in a 13 year, antibiotic-free herd declined from above 90% but the resistance level is still between 20 and 55% without antibiotics and 5 generation turnovers of the herd (Hays 1986).

Some critics (WHO 1997; Witte 1998) of current commercial production methods suggest that antibiotics are necessary only because of the stressful rearing conditions, and that the return to smaller individual farms would obviate the need for

antibiotics (CAST 1981; Hays 1986; Roura et al. 1992). However, returning to that type of animal rearing could result in environmental extremes, more exposure to parasites and the associated susceptibility to diseases, and ultimately, an increase in therapeutic use (Braude 1978; Hays 1986). The use of antibiotics has resulted in a healthier animal population with fewer parasites and diseases. In addition, animal welfare is improved and the environment benefits as well (Roth and Kirchgessner 1993; Donnelly et al. 1996). Food can be produced on fewer acres. Feed additives reduce nitrogen excretion due to a more efficient utilization of the feed in pigs (Roth and Kirchgessner 1993). Few people agree that the return to individual farms would be a justifiable solution at this point.

Since the beginning of the use of antibiotics in feed, the average enhancement in rate of growth appears to have remained relatively constant (Peo 1962; Teague et al. 1966; Visek 1978). Thus, either the mechanisms of growth promotion are unrelated to antimicrobial factors or factors causing antibiotic resistance (Visek 1978; CAST 1981; NRC 1999), or this observation is an illusion masked by the use of newer antibiotics (Mathew 2000 personal communication). Whatever the case may be, more people seem to be in agreement that the solution lies with development of new drugs or the use of alternative growth promotants.

Chopra et al. (1996) have reviewed the search for antimicrobials effective against multiple resistant bacteria. The development of new prophylactic and therapeutic procedures discussed include the design of analogs of existing antibiotics that resist enzymatic inactivation by bacteria, analogs that disable or elude recognition

by bacterial efflux pumps, and analogs that inhibit protein synthesis on ribosomes that express resistance to older tetracyclines. Novel antibiotics that specifically target products associated with infection *in vivo* appear to hold the most promise. These drugs target infection processes and will be highly specific for each pathogen and less disruptive to the commensal flora. In order for these to be used effectively, more rapid and accurate microbial diagnostics than are now available will have to be invented.

Other research has focused on alternatives to antibiotics. Copper was found to be useful as both a growth promotant and an antibacterial (at higher levels) (Sollman 1957). However, multiple-antibiotic resistance (MAR) was linked with metal tolerance in a study by Calomiris et al. (1984). They reported that simultaneous selection for metal and antibiotic resistance might occur in some microorganisms. Positive correlations between copper, lead and zinc tolerance and MAR were found (Kunkle et al 1981; Kelley et al. 1996). Some analysts have predicted that substitutes like these will also fall under regulatory scrutiny and possibly be banned (Edwards 1972).

Given the time and difficulties involved in research and bringing new antimicrobials or growth promotants to market, the food animal industry has initiated its own quality assurance programs to address consumer concerns and to improve accountability of antibiotic use by producers. For instance, in the swine industry, The National Pork Producers Council developed the Pork Quality Assurance Program (PQA) (NPPC 1997). It is a 3 level, 10 step voluntary program to help producers understand appropriate uses of medications. The program applies principles from the

Hazard Analysis and Critical Control Points (HACCP) to the production of pork to aid producers in monitoring and controlling farm drug use problems by identifying 10 critical control points. The PQA program was originally intended to reduce the incidence of drug residues in meat, which is rarely a concern anymore but it is equally effective in the control of the diseases and reducing the incidence of resistant organisms.

The PQA program is practical for all types of pork production facilities, even those that raise hogs organically. For a growing number of farmers, organic production is becoming an increasingly viable alternative to traditional and industrial farming. Producers are motivated to "go organic" for a number of reasons. Some enjoy the challenge and opportunities of competing in new market. Market analysts have reported that the organic industry has grown by 20% per year in the last five years (Carter 1999). For others, the motivation is provided through financial incentives. Organic products fetch premium prices in specialty markets and demand often exceeds supply. In the United Kingdom, the Ministry of Agriculture has set up the Organic Aid Scheme to provide financial assistance to farmers converting to organic methods.

## 3. MATERIALS AND METHODS

*Animals*: Seven swine farms from the United States were selected to study the effects of use or exclusion of antibiotics on antibiotic resistance patterns in bacteria. Producers were interviewed to determine the history of antimicrobial drug utilization within their herds. Three farms (Iowa, Kentucky, New Jersey) excluded antibiotics in their production. Four farms (2 in Tennessee and 2 in Indiana) used antibiotics in both subtherapeutic and therapeutic amounts. Antibiotic use on the latter farms was documented for a minimum of 12 months prior to the initiation of the study (Table 1). All farms were at least 62 km apart except for the two farms in Indiana.

*Sample Collection*: At each farm, 6 pigs from each of 4 weight groups (4.5, 23, 45, and 109 kg) and 5 sows were randomly selected for collection of fecal samples. Sterile dacron-tipped swabs were used to collect rectal fecal samples from pigs by their owners. The swabs were placed in disposable screw cap vials (16 x 125 mm, Fisherbrand® Fisher Scientific Company, Pittsburgh, PA) filled with Cary-Blair transport medium (BAM 1995) and shipped immediately on ice in coolers to the Dept. of Animal Science at the University of Tennessee, Knoxville. Ground pork from one market weight pig from each farm was also sent by way of an iced cooler. All samples were received at the laboratory within 48 hours of collection.

*Microbiological Procedures*: The tips of the swabs were cut off into individual sterile plastic tubes containing 5 mL of cryoprotectant, a freezer storage solution

containing 10% sterile, inactivated horse serum, 20% sterile glycerol, and 70% sterile trypticase soy broth. The contents were mixed by vortexing and divided into 5, 1 mL aliquots then stored at –80° C until used. One dacron-tipped swab was used to swab the surface of the ground pork samples and was also inserted about an inch into the ground pork. The swabs were in contact with the meat for about 30 seconds then placed in cryoprotectant solution as above.

*Isolation of microorganisms*: All cultivation media were prepared without the use of antibiotics to prevent possible selection for resistant organisms. One exception was made with Modified Trypticase Soy Broth (mTSB) an enrichment broth containing novobiocin, recommended for the recovery of *E. coli* O157 (BAM 1998).

*Isolation of E. coli*: For the isolation of *E. coli*, a 1 mL aliquot of each sample was thawed at room temperature and incubated at 37° C for 24 hours in 5 mL of Mueller Hinton Broth (MH, BBL, Becton Dickinson and Company, Cockeysville, MD). One loopful of the culture was streaked for isolation onto MacConkey II MUG agar (MAC II, BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated at 44° C for 24 hours. A control *E. coli* plate was also streaked (ATCC 25922, American Type Culture Collection, Rockville, MD).

MAC II agar contains bile salts to inhibit Gram positive organisms, a neutral red pH indicator and MUG (4-methylumbelliferyl-ß-D-glucoronide) for presumptive identification of *E. coli* and differentiation between *E. coli* and *E. coli*

O157: H7. *E. coli* metabolize lactose in this agar which reduces the pH causing the indicator to turn pink. In addition, *E. coli* are characterized by production of ß-D-glucoronidase an enzyme which hydrolizes MUG to yield 4-methylumbelliferone, a compound which fluoresces blue-green under long-wave (366) UV light. *E. coli* O157:H7 do not produce this enzyme, so they are pink but do not fluoresce. MAC II agar had a two-fold purpose, not only did it have 2 indicator ingredients for *E. coli* which prevented extra labor in further confirmation testing, but it helped reveal possible *E.coli* O157:H7.

*Isolation of E. coli O157:H7*: For the isolation of *E. coli* O157:H7, the samples were pooled by pig weight within farms. A 1mL aliquot of sample from each pig of the same weight and farm was thawed at room temperature and combined. One mL of the pooled sample was placed in 5 mL of mTSB and incubated at 37° C for 24 hours for enrichment. One mL of enrichment was used in immunomagnetic separation with Dynabeads® (Dynal, 5 Delaware Drive, Lake Success, NY). Dynabeads® anti-*E. coli* O157 beads are coated with specific antibodies designed to bind to the target bacteria. The bead-bacteria complexes were magnetically separated from the suspension, removed with a pipette, and streaked for isolation on differential agar plates of Sorbitol MacConkey agar (SMAC, BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and MAC II and incubated at 37° C for 24 hours. One control plate of each medium was streaked with *E. coli* O157:H7 ( Jack in the Box strain kindly provided by Dr. Laslo Csonka, Purdue University). Suspect colonies were colorless on SMAC, as *E. coli* O157:H7 do not

ferment sorbitol and were pink but glucoronidase negative on MAC II (BAM 1995). Homogenous, typical colonies were tested using Analytical Profile Index 20E (API, bioMerieux Vitek, Inc., Hazelwood, MO). API 20E tests consist of a series of biochemical tests used to identify Enterobacteriaceae and other Gram-negative bacteria. Presumptive non-sorbitol fermenting *E. coli* colonies were identified and assumed to be O157:H7.

*Isolation of Salmonella Typhimurium*: For the isolation of *Salmonella*, the samples were pooled by pig weight within farms. A 1mL aliquot of sample from each pig of the same weight and farm was thawed at room temperature and combined. One mL from each pooled mixture was incubated in 5 mL of Brain Heart Infusion (BHI, Difco Laboratories, Detroit, MI) and incubated at 37° C for 24 hours to revive cells. In addition, a *Salmonella Typhimurium* control (ATCC 4232) was incubated in the same way. After incubation, one mL of the BHI culture was placed in 9 mL of sterile Lactose Broth (LB) for pre-enrichment. LB contains lactose which non-*Salmonella* organisms ferment causing the pH to decrease. After 60 min at room temperature, the pH of the medium was adjusted to 6.8 with sterile 1 N NaOH or 1 N HCL, returning the solution to that which favored a higher ratio of *Salmonella* to non-*Salmonella* organisms (BAM 1995). The LB cultures were incubated for 24 hours at 35° C. For enrichment, one mL of the pre-enrichment was transferred into 10 mL of Tetrathionate Broth (TT, Difco) and incubated at 42° C for 24 hours. TT broth is a selective media with bile salts to inhibit Gram-positive organisms. Tetrathionate is formed in the medium by the

addition of the iodine-iodide solution and inhibits the normal intestinal flora of fecal specimens (Draughon 1999). One loopful of the enrichment was streaked for isolation onto Xylose-Lysine-Tergitol 4, Brilliant Green, and Bismuth Sulfite agars each (XLT4, BG, and BS, Difco) and incubated for 48 hours at 35° C. These agars all vary in selectivity and were chosen to increase the chances of recovering as many *Salmonella* as possible.

XLT4 is a highly selective media for non-typhi salmonellae. Tergitol 4 supplement inhibits non-*Salmonella* organisms. This agar contains 3 fermentable sugars, xylose, sucrose, and lactose. Phenol red serves as a pH indicator to detect acid (yellow) production from fermentation. When xylose is exhausted, *Salmonella* decarboxylate lysine which causes alkaline pH production (red) agar (Ebner 1999). Sodium thiosulfate (tergitol) and ferric ammonium citrate are differential hydrogen sulfide indicators that make the *Salmonella Typhimurium* colonies black. Black colonies on red medium were selected for further testing,

BG agar contains brilliant green dye that inhibits Gram-positive bacteria and a majority of Gram-negative bacilli. Phenol red indicates acid production from the fermentation of lactose or sucrose. *Salmonella* were characterized by pink-white colonies on red medium.

BS agar also contains brilliant green dye and bismuth to inhibit Gram-positive bacteria and many Gram-negative enteric organisms, except most *Salmonella* and some *Shigella* species. Ferrous sulfate is an indicator of hydrogen sulfide

production. *Salmonella* were characterized by brown, black, or dark green colonies that sometimes had a metallic sheen on green medium.

Presumptive colonies were subjected to serological identification using Bacto-Salmonella O, H and Vi antisera (Bacto Salmonella O Antiserum Poly A-I and Vi, Difco Laboratories, Detroit, MI). Duplicate samples of these colonies were sent to another laboratory at the University of Tennessee, Knoxville for type identification using a random primer PCR DNA fingerprint analysis.

*Microdilution Tray Preparation*: Eight typical *E. coli* colonies were randomly selected and as many *S. Typhimurium* and presumptive *E. coli* O157 colonies as could be found were transferred into 5 mL of Cation Adjusted Mueller Hinton II Broth (MH II, BBL). The tubes were incubated in a water bath (Orbit shaker bath, Lab-line Instruments, Inc., Melrose Park, IL) at 37° C for 30 min. to 2 hours, or until turbidity of the contents was visibly equal to 0.5 McFarland standard. (National Committee for Clinical Laboratory Standards 1990). The cultures were used for minimum inhibitory concentration determination using approved standards set by the NCCLS. Antibiotics used in the susceptibility tests were ampicillin (Sigma Chemical Co., St. Louis, MO), ceftiofur sodium (Naxcel® Pharmacia & Upjohn Company, Kalamazoo, MI), gentamicin (ICN Biochemicals, Inc., Aurora, OH), oxytetracycline (Oxytetracycline dihydrate, USP, ICN Biochemicals, Cleveland, OH), and sulfamethazine (Sigma). Veterinarians, pork producers, and extension agents interviewed reported that these antibiotics were most commonly used in swine operations. Aqueous stock solutions of these

antibiotics were made in a ten-fold concentration and stored at –80° C for filling the microdilution trays as needed (NCCLS 1998). The assay potencies of the antibiotics were 1000 μg/mg for ampicillin, ceftiofur and sulfamethazine, 998 μg/mg for oxytetracycline, and 595 μg/mg for gentamicin. Sterile water was used to dissolve the antibiotics except oxytetracycline, which was first dissolved in absolute ethyl alcohol over heat. Prior to use, stock antibiotic solutions were thawed and diluted to four-fold concentrations. The wells of sterile microdilution trays (Costar® styrene, u bottom, 96 well microdilution trays, Corning Inc., Corning NY) were first filled with 50 μL of MH II before adding 50 μL of antibiotic solutions by way of two-fold serial dilutions. This resulted in 7 rows of wells, each decreasing in concentration by one-half of the previous row. The eighth row was left void of antibiotics to serve as a measure of bacteria viability. Completed trays were sealed with package tape and stored at –80° C.

The trays were thoroughly thawed at room temperature before the addition of innoculum. Once the bacterial suspension reached the 0.5 McFarland turbidity standard, 20 μL were diluted in a 1:10 mixture of sterile, purified water and MH II. Within 15 minutes, 50 μL were pipetted into each well. The last column of wells was reserved for the control strain and served as a test for antibiotic dilution accuracy. The final concentration of bacteria per well was approximately $5 \times 10^4$ CFU/mL (NCCLS 1990). Minimum inhibitory concentrations were visually determined using a reflective stand (Microtiter© reading stand, Cooke Engineering Company, Alexandria, VA). Final dilution ranges of antibiotics from the top row

of the microdilution trays to the last row are listed on Table 2 along with the NCCLS established endpoints at which resistance was recorded.

*Statistics*: Antibiotic resistance, measured by MIC and the number of resistant isolates for each farm type was determined using General Linear Models analysis for numerical data. Least squares means by LSD mean separation were used to compare composite MICs from both farm types, the percentage of resistant *E. coli* between farm types, and the percentage of resistant *E. coli* isolated from pigs of various sizes between farm types. Chi-square analysis frequency procedure was conducted to determine differences between farm types using MICs of *Salmonella* isolates.

# 4. RESULTS

The use or exclusion of antibiotics on farms affected sensitivity and resistance of swine *E. coli*. Farms that excluded antibiotics had lower ($P < 0.001$) MICs for ampicillin, gentamicin, oxytetracycline and sulfamethazine. There were no significant differences between the 2 farm types in sensitivity to ceftiofur although, the composite MIC for *E. coli* from antibiotic exclusion farms (A-) was 0.03 μg/mL higher than that of the antibiotic use farms (A+) (Table 3).

A- farms had lower percentages ($P < 0.001$) of resistant *E. coli*. For each antibiotic tested, composite MICs from A+ farms were higher ($P < 0.05$), except for ceftiofur to which there were no resistant isolates from either farm type (Figure 1). Otherwise, the percentage of gentamicin resistant *E. coli* was lowest and the percentage of oxytetracycline resistant *E. coli* was greatest among both farm types. A- farms had 0.31 percent gentamicin resistant *E. coli* and A+ farms had 1.4 percent. The percentage of oxytetracycline resistant *E. coli* from these farms was 41 and 86 percent respectively.

Mean MICs only varied among pig sizes on A- farms for ceftiofur, oxytetracycline and sulfamethazine (Table 4). Variation in MICs between some pigs of different sizes on A+ farms occurred with all antibiotics except ceftiofur. For both farm types, when differences did exist, MICs were numerically higher for isolates from pigs that weighed 23 kg or less. In contrast, the greatest MICs among isolates from A+ farms for oxytetracycline were found in larger pigs. Isolates from 109 kg

pigs and sows had numerically higher MICs. There were no differences between MICs of isolates from pigs of different sizes for ceftiofur from A+ farms.

The percentages of ampicillin resistant *E. coli* were only different ($P < 0.001$) among pigs of different sizes on A+ farms. The greatest percentages of resistant *E. coli* were among pigs less than 45 kg. Market weight pigs (109 kg), 45 kg pigs, and sows had the fewest ampicillin resistant isolates (Figure 2). The percentage of ampicillin resistant isolates from market weight pigs of both farm types was not different ($P > 0.05$).

The percentages of gentamicin resistant *E. coli* were only different ($P < 0.001$) between pigs of different weights on A+ farms. Isolates from pigs less than 23 kg were higher ($P < 0.05$) than pigs of greater weights.

Only one weight group of pigs (45 kg) from A- farms had gentamicin resistant *E. coli*; however, there were no significant differences between weight groups on these farms.

The percentage of gentamicin resistant isolates from pigs of 45 and 109 kg pigs and sows were statistically the same ($P > 0.05$) for both farm types.

The percent of oxytetracycline resistant *E. coli* differed among pigs of different weights of both farm types ($P < 0.05$).

Among A+ farms, *E. coli* from pigs 45 kg and less had a lower ($P < 0.05$) percentage of oxyteracycline resistance than market weight pigs and sows (Figure 4). In contrast, the percentage of oxytetracycline resistant *E. coli* declined as pig age

increased ($P < 0.05$) in A- farms and in all age groups the percentage was lower compared to A+ farms.

The percentage of sulfamethazine resistant *E. coli* among pigs of various weights was different ($P < 0.001$) between both farm types (Figure 5) for all weight groups except 45 and 109 kg.

The most distinct difference ($P < 0.05$) among pigs from both farm types were between pigs that were 23 kg or less and pigs 45 kg or greater. Sows from A- farms had the lowest percentage (38%) of sulfamethazine resistant *E. coli*, whereas sows from A+ farms had percentages of resistant *E. coli* that did not differ ($P > 0.05$) from those of the 23 and 45 kg pigs of that farm type.

One hundred and thirty-two *Salmonella Typhimurium* (World Health Organization nomenclature), O-antigen Type B were isolated from 3 farms. Isolates originated from all except market weight pigs from two A+ farms, and only from 23 kg pigs of one A- farm.

Chi-square analysis frequency procedure was conducted to determine differences between farm types using MICs of isolates from 23 kg pigs (Figure 6). Data are represented by 35 isolates from the A+ farms and 11 isolates from the A- farm.

No differences in sensitivity to ampicillin (Chi-square, $P = 0.63$), gentamicin (Chi-square, $P = 0.13$), or sulfamethazine (Chi-square, $P = 0.40$) existed between farm types. Differences did occur in sensitivity to ceftiofur ($P < 0.001$) and

oxytetracycline ($P < 0.001$). As with *E. coli*, *Salmonella* from farms that excluded antibiotics were more susceptible to these 2 antibiotics.

All *Salmonella* isolates from 25 kg pigs on all farms were susceptible to ampicillin, ceftiofur, and gentamicin using NCCLS minimum inhibitory concentrations. One salmonellae from an antibiotic use farm was resistant to oxytetracycline. All *Salmonella* isolates were resistant to sulfamethazine (data not shown).

Twenty-three presumptive *E. coli* O157:H7 were isolated, but all originated from farms that used antibiotics. Thus, comparison of resistance between farm types was not possible. Eleven of the isolates were from 23 kg pigs on one farm, 10 were from sows of another and 2 were from 45 kg pigs of yet another farm. The frequency of antibiotic resistance for each group of isolates were tabulated (Table 5). Of interest, are the similarities in resistance of isolates from the 2 Indiana farms that were only 62 km apart.

Even though this was a particularly small data set, the lack of variability in sensitivity or resistance may be unusual. Isolates were either 100 percent susceptible or 100 percent resistant to each antibiotic tested.

No *E. coli* or *Salmonella* of any kind were detected from pork sample.

# APPENDIX

TABLE 1. LABEL USE OF ANTIBIOTICS ADMINISTERED ON FARMS THAT USED ANTIBIOTICS 12 MONTHS PRIOR TO TESTING

| ANTIBIOTICS USED | INDIANA FARM # 1 | INDIANA FARM # 2 | EAST TN FARM | WEST TN FARM |
|---|---|---|---|---|
| Apramycin<br>Trade Name: Apralan | | | Subtherapeutic use in creep feed | Subtherapeutic use in weanling to 7kg pig feed |
| Bacitracin<br>Trade Name: BMD | | | | Growth promotion use in 77kg to 109 kg pig feed |
| Carbadox<br>Trade Name: Mecadox | Growth promotion use in weanling to 14kg pig feed | Subtherapeutic use in < 34kg pig feed | Growth promotion use in nursery feed | Growth promotion and subtherapeutic use in 11kg to 18kg pig feed |
| Chlortetracycline<br>Trade Name: AUREOMYCIN® | Therapeutic use in 40% of finishing pigs | | Growth promotion use and subtherapeutic use in all pigs and sows (depending of gestation status) | Subtherapeutic use in 18kg to 77kg pig feed |
| Lincomycin<br>Trade Name: Lincomix® | Therapeutic use in breeding stock as needed | Therapeutic use in breeding stock as needed and in pigs 2x per year | | Growth promotion use in 7kg to 11kg pig feed and therapeutic use in 1-3 day old pigs |
| Oxytetracycline<br>Many Trade Names:<br>Terramycin® | | Therapeutic use in sows as needed | | Therapeutic use in sows as needed |
| Penicillin<br>Trade Name: Procaine Penicillin G | Therapeutic use for growing, finishing, and breeding stock as needed | Therapeutic use in sows as needed | | |
| Tylosin<br>Trade Name:TYLAN® | | | Growth promotion use in growing and finishing feeds | |
| Virginiamycin<br>Trade Name: Stafac® | | Growth promotion use and subtherapeutic use in pig feed | | |
| Isoflupredone acetate<br>Trade Name PREDEF®<br>Isoflupredone | | Therapeutic use in sows as needed | | |

TABLE 2. MINIMUM INHIBITORY CONCENTRATIONS (MIC) AT WHICH ENTEROBACTERIACEA RESISTANCE IS INDICATED (NCCLS) AND ANTIBIOTIC CONCENTRATIONS RANGES USED

| Antibiotics used in MIC analyses | NCCLS resistance concentrations (μg/mL) | Range of antibiotic concentrations used (μg/mL) |
|---|---|---|
| Ampicillin | ≥ 32 | 0-128 |
| Ceftiofur | ≥ 8 | 0-32 |
| Gentamicin | ≥ 16 | 0-128 |
| Oxytetracycline | ≥ 16 | 0-128 |
| Sulfamethazine | ≥ 256 | 0-256 |

TABLE 3. COMPARISON OF MINIMUM INHIBITORY CONCENTRATIONS OF *E. COLI* FROM SWINE FARMS THAT USED OR EXCLUDED ANTIBIOTICS USING MEAN COMPOSITE MICS

| Antibiotic | Type of farm | Mean MIC (μg/mL) |
|---|---|---|
| Ampicillin | A+ | 74.20 ± 3.25[a] |
| | A- | 13.20 ± 3.33[b] |
| Ceftiofur | A+ | 0.52 ± 0.01 |
| | A- | 0.55 ± 0.01 |
| Gentamicin | A+ | 4.41 ± 0.33[a] |
| | A- | 2.12 ± 0.34[b] |
| Oxytetracycline | A+ | 217.73 ± 3.43[a] |
| | A- | 105.14 ± 3.53[b] |
| Sulfamethazine | A+ | 316.85 ± 6.43[a] |
| | A- | 190.12 ± 6.57[b] |

[1] LS mean ± SE

[a, b] superscripts indicate differences between farm types for a given antibiotic ($P < 0.05$).

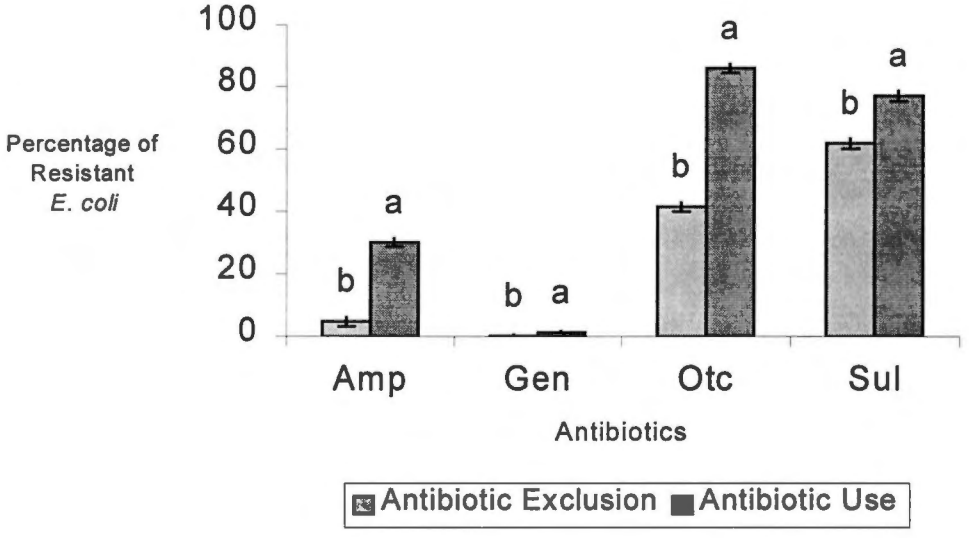


Figure. 1. Percentage of resistant *E. coli* from farms that used or excluded antibiotics. Data are Least squares means and represent 1,258 isolates. Bars within antibiotic that do not share like superscripts differ ($P < 0.05$). Amp = ampicillin, Gen = gentamicin, Otc = oxytetracyclin, Sul = sulfamethazine.

TABLE 4. COMPARISON OF MINIMUM INHIBITORY CONCENTRATIONS OF *E. COLI* FROM SWINE OF DIFFERENT WEIGHTS FROM A+ AND A- FARMS, USING MEAN MICS ($\mu$g/mL)

| | | Pig Weight Groups (kg) | | | | |
|---|---|---|---|---|---|---|
| Antibiotic | Farm type | 4.5 | 23 | 45 | 109 | Sows |
| Ampicillin | A+ | $92.21^{b}$ | $133.37^{a}$ | $42.17^{d}$ | $37.04^{de}$ | $66.11^{c}$ |
| | A– | $19.02^{ef}$ | $3.91^{f}$ | $8.33^{f}$ | $24.00^{def}$ | $10.74^{f}$ |
| Ceftiofur | A+ | $0.52^{b}$ | $0.51^{b}$ | $0.52^{b}$ | $0.53^{b}$ | $0.52^{b}$ |
| | A– | $0.67^{a}$ | $0.54^{b}$ | $0.51^{b}$ | $0.50^{b}$ | $0.53^{b}$ |
| Gentamicin | A+ | $5.92^{a}$ | $7.41^{a}$ | $3.60^{b}$ | $3.12^{b}$ | $2.00^{b}$ |
| | A– | $2.00^{b}$ | $2.01^{b}$ | $2.58^{b}$ | $2.00^{b}$ | $2.00^{b}$ |
| Oxytetracycline | A+ | $201.04^{b}$ | $192.51^{b}$ | $201.33^{b}$ | $250.82^{a}$ | $242.93^{a}$ |
| | A– | $143.83^{c}$ | $144.85^{c}$ | $96.62^{d}$ | $81.75^{d}$ | $58.66^{e}$ |
| Sulfamethazine | A+ | $384.21^{a}$ | $374.47^{a}$ | $290.64^{b}$ | $298.31^{b}$ | $236.64^{c}$ |
| | A– | $189.24^{de}$ | $226.07^{cd}$ | $264.88^{bc}$ | $172.75^{e}$ | $97.68^{f}$ |

A+ = farms that used antibiotics; A– = farms that excluded antibiotics

LS means within antibiotics and not sharing like superscripts differ ($P < 0.05$)

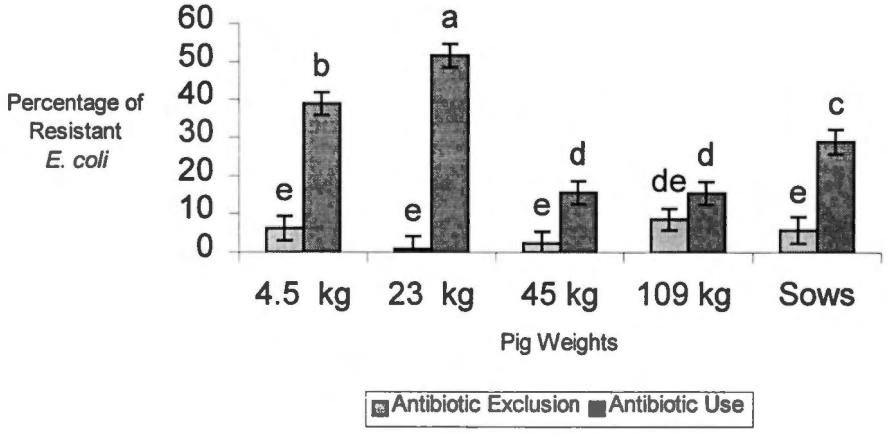


Figure. 2. Percentage of ampicillin resistant *E. coli* between pigs of various weight groups from farms that used or excluded antibiotics. Data are Least squares means and represent 1,258 isolates. Bars not sharing like superscripts differ ($P < 0.05$).

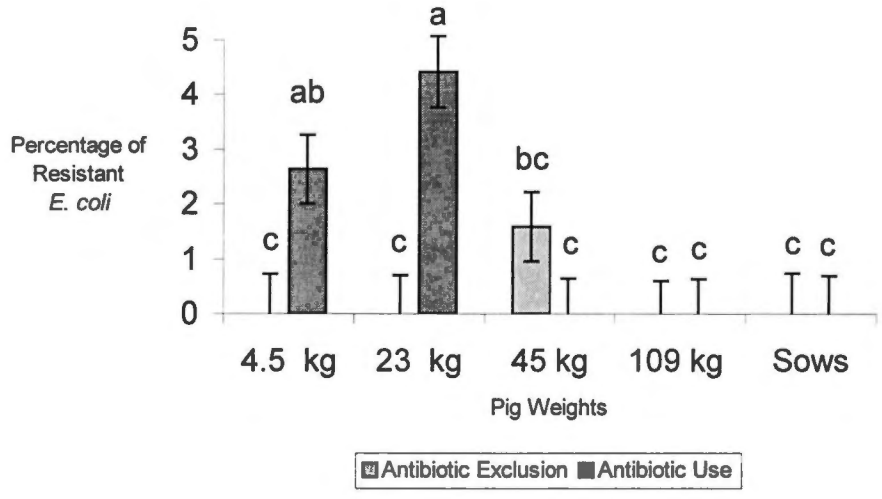


Figure. 3. Percentage of gentamicin resistant *E. coli* between pigs of various weight groups from farms that use or exclude antibiotics. Data are Least squares means and represent 1,258 isolates. Bars not sharing like superscripts differ ($P < 0.05$).

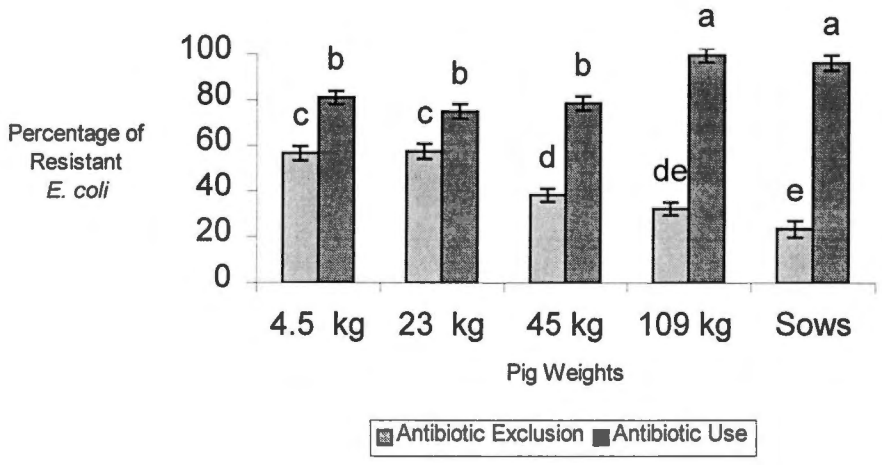


Figure. 4. Percentage of oxytetracycline resistant *E. coli* between pigs of various weight groups from farms that used or excluded antibiotics. Data are Least squares means and represent 1,258 isolates. Bars not sharing like superscripts differ ($P < 0.05$).

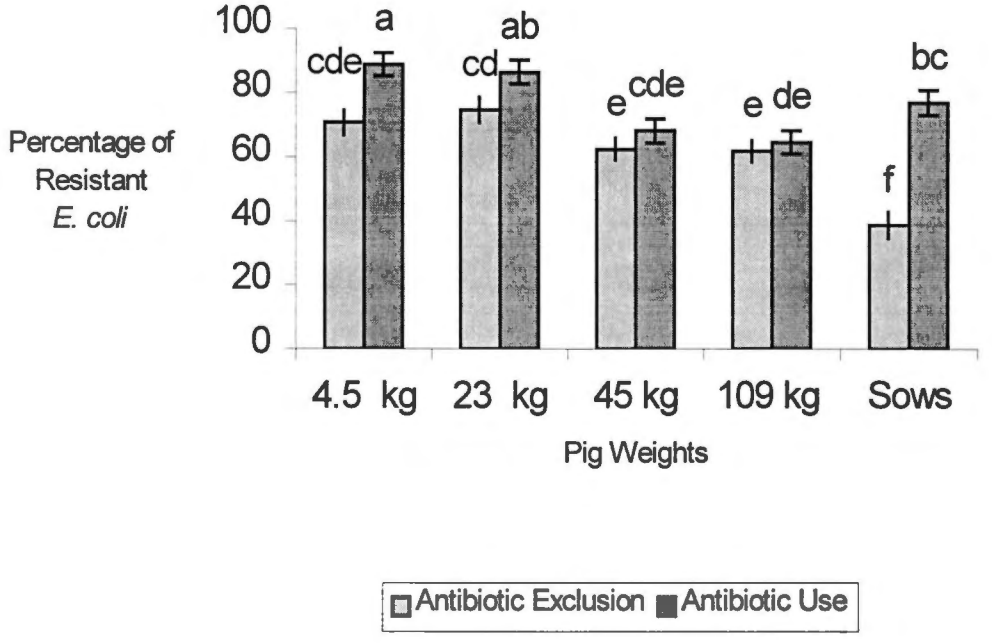


Figure. 5. Percentage of sulfamethazine resistant *E. coli* between pigs of various weight groups from farms that used or excluded antibiotics. Data are Least squares means and represent 1,258 isolates. Bars not sharing like superscripts differ ($P < 0.05$).

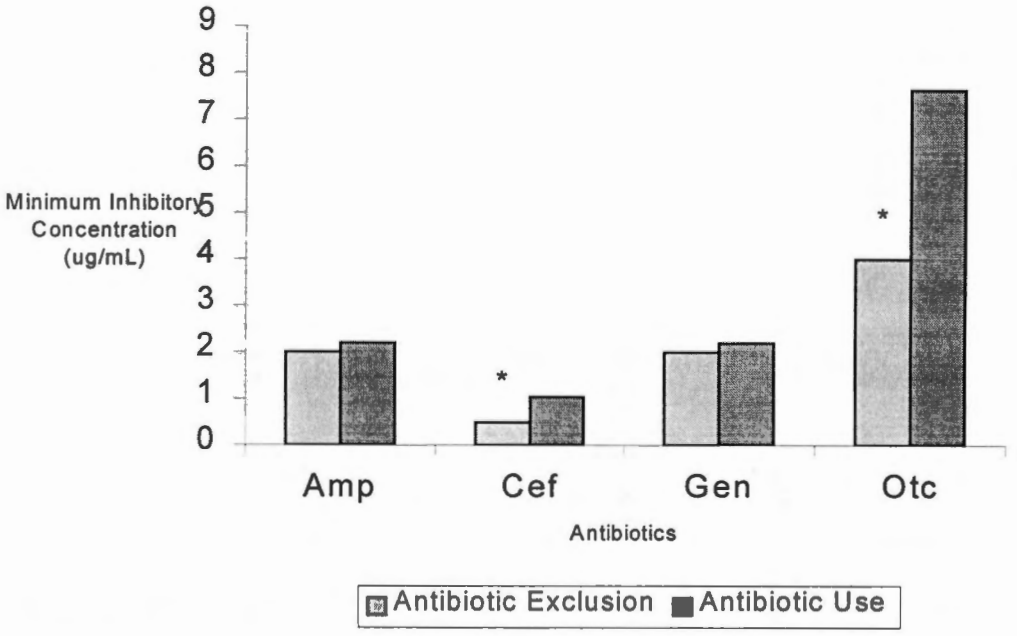


Figure. 6. Sensitivity to various antibiotics by 46 *Salmonella Typhimurium* isolates from 23 kg pigs (pooled by weight) from farms that used or excluded antibiotics. Asterisks above bars indicate differences between farm types ($P < 0.0001$). Sensitivity to sulfamethazine was not different between farms types ($P > 0.0001$), with MIC's ranging near 500 $\mu$g/mL.

TABLE 5. PERCENTAGE OF RESISTANT ISOLATES OF 23 POSSIBLE *E. COLI* O157:H7

| Antibiotic | Pooled weights and locations of pigs | Percentage of resistant isolates |
|---|---|---|
| Ampicillin | 23 kg pigs (IN #2) | 100% |
| | 45 kg pigs (east TN) | 0 |
| | Sows (IN #1) | 100% |
| Ceftiofur | 23 kg pigs (IN #2) | 0 |
| | 45 kg pigs (east TN) | 0 |
| | Sows (IN #1) | 0 |
| Gentamicin | 23 kg pigs (IN #2) | 0 |
| | 45 kg pigs (east TN) | 0 |
| | Sows (IN #1) | 0 |
| Oxytetracycline | 23 kg pigs (IN #2) | 100% |
| | 45 kg pigs (east TN) | 100% |
| | Sows (IN #1) | 100% |
| Sulfamethazine | 23 kg pigs (IN #2) | 100% |
| | 45 kg pigs (east TN) | 100% |
| | Sows (IN #1) | 100% |

## 5. DISCUSSION

Mean MICs for ampicilin, gentamicin, oxytetracyline, and sulfamethazine were more than twice as high for *E. coli* from A+ farms compared to A_ farms. Lack of ceftiofur resistance on either farm type may be attributed to the more recent incorporation of ceftiofur into animal use, and its infrequent, therapeutic use in swine.

The A_ farms were intended to represent practical non-selective hog production environments and serve as a type of control for comparison with A+ farms. Occasionally *E.coli* isolates from A_ farms exceeded 2% resistance, which was Novick's (1981) description of a normal "wild type" population.

Oxytetracycline was the only antibiotic common to the A+ farms. It was used also used therapeutically to treat sows on two of the A+ farms. Another tetracycline derivative, chlortetracycline, was used almost continuously on three out of four A+ farms.

The selective pressure of one or more related or unrelated antibiotics used on farms may have selected for resistance mechanisms that caused resistance to the antibiotics analyzed in this study. If so, it is likely that a certain percentage of these isolates contained multiple resistant mechanisms

The vast differences between farm types might also be explained by distinctive husbandry practices, other than the use or exclusion of antibiotics. For example, more intensive farms, (on which antibiotics may be more likely used), more commonly have confinement buildings. The close contact among animals

and limited exposure to external influences might select for resistant bacteria. Producers that raise hogs without the use of antibiotics are more likely to have free range or open air type farms that might allow for a more natural balance of resistant to susceptible bacteria. External factors known to increase antimicrobial-resistant enteric bacteria, such as described by Embry et al. (1962,1966,1969) Williams and Newell (1970), Corrier et al. (1990), or Moro et al. (1998), may have affected the bacterial resistance of each farm type.

An non-statistical analysis of data from the three A_ farms using the mean MICs was conducted (data not shown). It was found that the percentage of ampicillin, oxytetracycline and sulfamethazine resistant *E.coli* differed significantly between farms. It is likely that more variables other than the presence or absence of antibiotics should be considered before banning or restricting their use in food animals. Factors such as poor farm hygiene, questionable feed sources, lax biosecurity and minimal lot drainage, may be responsible for variances in occurrence, prevalence and persistence of resistant bacteria.

One important trend regarding the prevalence of resistance among all of the farms tested was its gradual reduction across increasing pig weights. This phenomenon has been reported by others (Hays 1969, Langlois 1986, Mathew 1998), but may bear repeating due to its relevance to market weight pigs, which are ready for human consumption. The one exception to this trend was with oxytetracycline. Ninety-nine percent of the *E. coli* from market weight hogs and 96% from sows were resistant to oxytetracycline, whereas only 80% of *E. coli* from

the smaller pigs were resistant. High levels of oxytetracycline resistant bacteria have been encountered in similar research. Upchurch (1995) summarized his results, and those of others, by pointing out that tetracyclines have been used in swine feeds for more than three decades in all weight classes of pigs and for both reproductive and growth enhancement. Sows are usually free of *E. coli* entering the farrowing unit but become infected as a result of contact with young offspring in the farrowing house (Hinton and Linton 1987). Baby pigs' GI tracts may be rapidly colonized with resistant bacteria because they lack exclusive commensal flora. Additionally, sows from two of the A+ farms were routinely given therapeutic doses of oxytetracycline post-farrowing which may have affected our results. In addition, sows are kept longer than pigs, approximately 3 years in intensive systems and possibly longer in others. Thus, they might be expected to contain an accumulation of flora representing the entire farm environment

Differences in percentages of gentamicin and ampicillin resistant *E. coli* occurred in our study. However, for A_ farms there were no differences between the different weight groups. This may be a reflection on the lack of confinement practices of this type of farm. For instance, these pigs might not have been segregated as to size or age. In intensive production systems, all-in-all-out is a common management scheme. Each new pig population is isolated from others and relocation to larger pens is preceded by stringent sanitation of the new quarters. Apparently this is an important control point in reducing the amount or spread of

resistant bacteria. Segregation may be partly responsible for the reduction in resistance frequency in market weight pigs on A+ farms.

As with oxytetracycline, the high percentages of sulfamethazine resistant *E. coli* from both farm types might be explained by the longevity of sulfanamides in the animal health industry. The antibacterial activity of the sulfa drugs started the antibacterial revolution and has been exploited for over 60 years (Visek 1978).

Of note from these data is the uncommonly low percentage (39%) of sulfamethazine resistant *E. coli* isolated from sows of A_ farms. Sample collection may have occurred following a recent turnover in sow population in one or more of the A_ farms. Many specialty breeding stock suppliers, such as those that would supply stock to A_ farms, follow stringent "organic" guidelines which would reduce the occurrence of resistant organisms even further. For example, certain rearing facilities have attained organic status through decades of antibiotic exclusion and the use of only organically raised feeds.

Zoonotic pathogens, *Salmonella Typhimurium* and possibly *E. coli* O157:H7 were isolated from some pigs of every weight group except market weight pigs. The importance of this observation cannot be fully realized because there were too few data and a dearth of comparative research.

Most research concerning market weight animals seems to have been at processing plants prior to or directly after slaughter. As such, data from these efforts might not represent near market weight populations on farms, as were sampled in this study. Research has shown that the stresses involved in transport to

market increase pathogen load and antimicrobial-resistant enteric bacteria in animals (Embry et al. 1962,1966,1969).

The *S. Typhiumurium* isolates apparent sensitivity to all of the antibiotics tested except sulfamethazine might be attributed to efficient prevention and control measures on farms. Many of the food additives listed from the A+ farms were specified for the control and prevention of salmonellosis.

The multidrug resistant (ampicillin, oxytetracycline, sulfamethazine) presumptive *E. coli* O157:H7 isolates may have been a localized phenonomen. Twenty-one of 23 originated from neighboring farms. *E.coli* O157:H7 are not frequently associated with swine or pork products. The frequency of ampicillin resistant *E. coli* was less than 37% for both of these farms (data not shown). Thus, it would seem that these pathogens might have "acquired" resistance to ampicillin elsewhere before contaminating the two farms in this study.

# 6. IMPLICATIONS

Antibiotic resistance transfer from animal to human pathogenic bacteria is perceived as an eminent threat to human health. Livestock producers must be able to identify and eradicate practices that contribute to the emergence and spread of resistant organisms if they expect legislative officials to support their use of antibiotics on farms. With continued research it is becoming apparent that abrupt discontinuance of antibiotics does not eliminate resistant bacteria. Exclusion of antibiotics from the onset of production is simply not a practical alternative for more than a handful of "niche" market competitors due to the evolution of the food animal production industry.

Producers that excluded antibiotics in this study were observed to have less than one half of the percentage of resistant *E. coli* in their pigs compared to producers that used antibiotics. However, the presence of high numbers of resistant *E. coli* for the two oldest antibiotics (oxytetracycline and sulfamethazine) among these farms may indicate that once resistant mechanisms become established they are not likely to disappear with the removal of selective pressure. Significant differences between antibiotic exclusion farms in resistance to ampicillin may imply that other management practices outside of the exclusion of antibiotics influence resistant bacterial populations. Further research will be necessary to characterize these confounding factors.

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