To the Graduate Council:

I am submitting herewith a thesis written by William Gregory Upchurch entitled "Incidence of Antimicrobial Resistant Fecal *Escherichia Coli* in Tennessee Swine Units." I have examined the final 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

Alan Mathew, Major Professor

We have read this thesis
and recommend its acceptance:

Frank B Masincupp

Fred M Hopkins

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

# INCIDENCE OF ANTIMICROBIAL RESISTANT FECAL *ESCHERICHIA COLI* IN TENNESSEE SWINE UNITS

**A Thesis**

**Presented for the**

**Master of Science**

**Degree**

**The University of Tennessee, Knoxville**

**William Gregory Upchurch**

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## ABSTRACT

Sows and pigs from ten Tennessee swine units from various regions of the state were sampled to determine the incidence of resistant fecal *Escherichia coli* (*E. coli*) to selected antimicrobial agents. At each site 5 sows and 5 pigs per sow were randomly identified. At 7 days post partem, selected individuals were rectally swabbed to obtain samples for culture. Additional samples were collected from pigs at 35 and 63 days of age. Fecal samples were transferred to lactose MacConkey agar and cultured overnight. Ten lactose fermenting colonies, consistent with *E. coli* in color and morphology, were randomly chosen and subjected to apramycin, carbadox, gentamicin, neomycin, and oxytetracycline. Isolates were determined to be sensitive, intermediately resistant, or resistant according to the standardized diffusion test established by the National Committee for Clinical Laboratory Standards (NCCLS). Statistical comparisons were made using a Chi-square analysis to determine changes in resistance percentages of antibiotics over time, compare incidence between pigs and sows, and compare incidence between farms classified as low or high antimicrobial drug use. Changes in the percentage of resistant isolates over time were observed to be significant for all five antibiotics ($P < .0001$). Isolates expressed the greatest sensitivity to apramycin and carbadox, and expressed the greatest percentage of resistance to oxytetracycline. Resistant isolates were observed to be at their highest level in pigs at 35 days of age. Differences ($P < .0001$) in the percentage of isolates resistant to apramycin, carbadox, gentamicin, and neomycin as compared between

sows and pigs were observed. Differences ($P < .0001$) in the incidence of resistance were observed between farms of high and low antimicrobial drug use in pigs at 35 days of age for all antibiotics tested. These results indicate the need for limited and/or selective antibiotic use in order to preserve future effectiveness.

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# 1. INTRODUCTION

*Escherichia coli* (*E. coli*) was discovered in the late 1800's by Theodor Escherich (Bertschinger *et al.*, 1992). However, it was the 1930's before sulphonamides were introduced to combat bacterial organisms, primarily hemolytic streptococcus, and later before the discovery of the broad spectrum "wonder drug" penicillin (Anderson, 1968). With the use of penicillin, people felt the control of harmful bacteria was surely eminent. However, Mary Barber in 1947 determined that "the elimination of penicillin-sensitive staphylococci could be followed by their replacement by penicillin-resistant strains of independent origin" (Anderson, 1968). Later, transferable antibiotic resistance was discovered in 1957 by Japanese researchers, who witnessed the development of drug resistance in shigellae, which included resistance to tetracyclines (Anderson, 1968). Additionally, in 1959 and 1960 Japanese researchers discovered the potential transfer of multiple resistance patterns in humans (Anderson, 1968).

Controversy surrounding the administration of antibiotics to animals, the threat of development of resistance and the transfer of that resistance to humans has been a concern since penicillin was restricted for agricultural purposes in Britain in 1953 (Current Topics, 1994; Shlaes, Levy, and Archer, 1991; Solomons, 1978; Braude, 1978; Smith, 1966). Yet, despite the controversy, antimicrobial agents have been used extensively over the past four decades to control harmful bacteria and gain production advantages (Visek, 1978; Jukes, 1971; Jukes *et al.*, 1950). Many felt that with the development of antibiotic resistant bacteria in animals, benefits, such as growth

promotion would be lost. However, Smith, (1967); and Visek, (1978) disputed this theory by showing resistance to antibiotics had risen since their administration to animals, while the performance benefits of growth associated with antibiotics had remained constant.

Extensive research has been conducted over the past three decades concerning the effects of antibiotics on animals. As previously stated, early work evolved around the use of antibiotics as growth promotants, and their effects on the microflora of animals (Schaedler, 1973; Jukes, 1971; Taylor, 1957; Jukes *et al.*, 1950). Later research has been focused primarily on the emergence of drug resistant bacteria (Dawson *et al.*, 1984; Langlois *et al.*, 1984; Gaines *et al.*, 1980; Linton, 1978; Siegel *et al.*, 1974), transfer of resistance (Mancini and Behme, 1977; Burt and Woods, 1976; Howe and Linton, 1976), persistence of resistance (Langlois *et al.*, 1988; Langlois *et al.*, 1986; Hinton *et al.*, 1984; Langlois *et al.*, 1983; Langlois *et al.*, 1978), and the threat of increasing resistance among humans (Rockfeller University Workshop, 1994; Kunin, 1993). Yet, beyond these studies, research investigating resistance of *E. coli* to antimicrobial agents currently used by swine producers has not been conducted. The purpose of this study was to determine the incidence of resistant *E. coli* to antibiotics presently used by Tennessee swine producers.

## 2. LITERATURE REVIEW

### Intestinal flora of swine

*Preweaned pigs*: Pigs are born without the presence of *E. coli* in the gut (Katouli *et al.*, 1995; Kenworthy and Crabb, 1963). However, after the first day following birth, bacteria quickly begin to colonize the intestinal tract, with large numbers of *E. coli*, *Clostridium welchii*, streptococci, bacteroides and lactobacilli being observed (Smith and Jones, 1963; Kenworthy and Crabb, 1963; Smith, 1965). *Escherichia coli* are believed to quickly colonize the intestinal tract of the young pig and the young of other species of animals due to a favorable environment, most notably high gastric pH levels (Langlois, 1986; Hampson *et al.*, 1985; Smith, 1965; Smith and Jones, 1963; Smith, 1961). In all the above studies, high gastric pH levels were attributed to poor absorption of milk by the young animals. Once pH levels began to decline, so did levels of bacteria in the gut, excluding lactobacilli which remained high for the duration of the pig's life. Studies have proven that *E. coli* may flourish in the intestinal tract without producing clinical signs of scours (Hampson, Hinton, and Kidder, 1985). However, with the proliferation of certain harmful *E. coli* in the pig's gut, diarrhea may occur with devastating results (Fairbrother *et al.*, 1989).

Problems such as diarrhea in young pigs are associated with colonization of the small intestine by harmful enterotoxigenic *E. coli* (ETEC) as identified by Okerman, (1987); Moxley and Francis, (1986); Nataro *et al.*, (1985); and Moon *et al.*, (1983). These *E. coli* are identified by serogroups such as serogroup O8, which commonly carries the antigens K99 and K88, serogroup O9 which carries 987P and K99 antigens,

O157 and O149 serogroups which possess K88 antigens, and O141 which exhibits 987P antigens (Nagy *et al.*, 1990; Okerman, 1987; Wilson and Francis, 1986; Gaastra and de Graaf, 1982). These pathogenic *E. coli* adhere to the small intestinal mucosa by way of host specific proteinaceous appendages called fimbriae or pili (Fairbrother *et al.*, 1989; Sarmiento, Casey, and Moon, 1988; Gaastra and de Graaf, 1982; Moon *et al.*, 1980) and produce heat-labile (LT) and heat-stable (STa and STb) enterotoxins (Fairbrother *et al.*, 1989; Okerman, 1987; Nataro, 1985). In general, these ETEC adhere to the mucosa without causing much structural damage (Sarmiento, Casey, and Moon, 1988). Yet, toxins produced by the bacteria lead to the secretion of intestinal fluids and gases, the potential loss of villi, and malabsorption, all which may lead to diarrhea and possibly death (Fairbrother *et al.*, 1989; Sarmiento, Casey, and Moon, 1988; Gaastra and de Graaf, 1982). Other types of harmful bacteria which might be found in the small intestine are attaching effacing *E. coli* (AEEC) which have been recognized by Okerman, (1987) and Moon *et al.*, (1983). Attaching effacing strains of *E. coli,* which may possess adhesions such as O, K, or H antigens (Gaastra and de Graaf, 1982), have been observed to attach to absorptive epithelial cells in young pigs (Staley, 1969). In studies investigating the effects of AEEC (Moon *et al.*, 1983), extensive damage to absorptive cell microvilli was observed shortly after the attachment of AEEC. Damage sustained by epithelial cells from the attachment and effacing of *E. coli* is presumably the mechanism of digestive impairment and absorptive loss which leads to diarrhea (Moxley and Francis, 1986; Moon *et al.*, 1983).

*Postweaned pigs*: Changes in the intestinal flora of pigs have been found to occur as the pig gets older (Smith and Crabb, 1961). It has been determined that as animals get older decreases in the populations of bacteria are observed (Smith and Crabb, 1961). However, this disagrees with the findings of McAllister *et al.*, (1979), who observed *E. coli* and lactobacilli populations to increase throughout the small intestine after weaning. It was also reported that a high number of hemolytic *E. coli*, accompanied by light scours, developed immediately following weaning and persisted for 2 to 4 days. In postweaned pigs, the primary intestinal flora is comprised of lactobacilli (Smith and Crabb, 1961), though complexity of the intestinal flora may be greatly affected by the presence or absence of enterotoxigenic *E. coli* (Hinton *et al.*, 1985). Tomlinson, (1969) and Tomlinson and Lawrence, (1981) postulated that changes in pH following weaning were favorable for the proliferation of *E. coli* in the pig's gut. Hampson *et al.*, (1985) determined that there was a linear correlation between pH and coliform counts, but the linear correlation was not related to pig age or the presence of hemolytic coliforms. However, Hampson *et al.*, (1985) did report that pH values and coliform counts were lower in pigs that had been 'creep' fed from 10 days of age through weaning as compared to non-weaned pigs, and weaned non-creep fed pigs. However, it was determined that gastric pH levels were lower in weaned pigs resulting in a count of fewer viable coliforms.

Despite information detailing declines in the numbers of *E. coli* in the intestinal tract of weaned pigs, and studies showing no significant changes in the pigs intestinal flora following weaning at 3 weeks of age (Hinton *et al.*, 1985), diarrhea

associated ETEC has been well documented (Kenworthy and Crabb, 1963). It has been found that when ETEC strains such as 0149: K91, or K88 are present in the intestinal tract of pigs, they tend to dominate the intestinal flora (Hinton *et al., 1985*; Hampson, Hinton, and Kidder, 1985). As in preweaned pigs, enterotoxigenic *E. coli* establish themselves in the posterior portion of the small intestine by attaching host specific fimbriae and pilli to the mucosa, and colonizing toward the anterior portion of the small intestine (Fairbrother *et al.*, 1989; Sarmiento, Casey, and Moon, 1988).

*Intestinal flora relationship between sows and pigs*: As stated previously, Smith and Crabb (1961) determined that as animals mature populations of bacteria within the intestine decrease, with lactobacilli dominating the intestinal flora, and moderately high numbers of *E. coli* being observed in sows. Yet, Smith and Crabb fail to state the period of time in which *E. coli* samples were taken from the sow. Arbuckle, (1968) and Callear and Smith, (1966) demonstrated that sows and gilts were free of the Abbotstown strain of *E. coli* during the late stages of pregnancy, but quickly acquired the pathogen upon entering the farrowing house. It was also determined that once sows obtained *E. coli* in their intestinal tract, they harbored a larger number of *E. coli* strains than did pigs (Katuouli *et al.*, 1995).

Arbuckle, (1968) postulated that *E. coli* strains associated with piglet diarrhea were wide spread throughout the farrowing house, with the pigs intestinal tract serving as the primary environment for *E. coli*. Craven and Barnum (1971) expressed concerns that *E. coli* might not be eliminated from production units following depopulation and clean-up. Thus, the sows and gilts main source of *E. coli* is through the ingestion of

piglet feces and pathogens residing within the farrowing unit. Research has proven that the transfer of bacteria from sows to their pigs is actually very limited (Arbuckle, 1968).

To further clarify the *E. coli* relationship between pigs and sows, Katoulli *et al.*, (1995) and Hinton and Linton, (1987) determined that pigs were able to maintain a "reasonably" individual *E. coli* population despite close contact with littermates and the sow. In addition, they observed few *E. coli* strains from sow feces that were common with those of their offspring, and determined that it was unlikely that the sow was the source of *E. coli* in baby pigs.

**Antimicrobial Drug Use**

*Antibiotics in animal feeds*: Antibiotics have been utilized in animal feeds for the past 40 years, and have significantly reduced production cost (Visek, 1978). However, the use of antibiotics has become increasingly controversial (Braude, 1978). Early researchers (Jukes *et al.*, 1950) showed that antibiotics could be used in swine diets to promote growth and performance. Information gathered from early studies helped researchers develop the theory that antibiotics used in feeds enable animals to grow faster due to effects on the microbial flora. Studies that substantiated this theory were conducted by Freeman *et al.*, (1975); Coates *et al.*, (1963). These studies indicated that animals being fed antibiotics did not grow faster when raised in a germ free environment. In addition, Hays (1969) demonstrated that growth performance

could be enhanced by a cleaner, more sterile environment, and the overall effects of supplemental antibiotics on performance were diminished.

Theories concerning the mechanisms of action of antimicrobial agents on bacteria are numerous. Visek, (1978) postulated that due to microbial presence in the gastrointestinal tract, animals must maintain more tissue with a higher cellular turnover rate. Thus, a higher maintenance level is required by the animal's body to replenish cells, and consequently utilizes nutrients that could be used for growth. He also presented other theories postulated by earlier researchers, concerning the effects of antimicrobials used as growth promotants. These theories included: (1) reduction of harmful bacteria within the swine's gut that have never produced clinical signs of disease; (2) toxins produced by enterotoxigenic bacteria are reduced; (3) enhanced absorption by the animal due to thinner cellular membrane; and (4) limited essential nutrients found in the bowel may be utilized by the host rather, than bacteria found within the host. However, as stated by Visek, "methods for testing the various hypotheses have not received general acceptance". Despite earlier research on the effects of antibiotics on the microflora in animals, little is known of the mechanisms by which antibiotics serve as growth promotants in animals (Smith, 1968). Furthermore, relationships between the host and bacteria exposed to antibiotics is not fully understood (Visek, 1978).

*Correlation of resistance transfer between animals and humans*: While many opposing theories have been postulated on the effects of antibiotics on microflora, probably the most controversial opinions involve speculation of resistance transfer

from animals to humans. Since the 1950's, governments, with the assistance of research scientists from universities, private industries, veterinarians, and medical professionals from around the world, have evaluated the risks of antibiotic use in animals. Information on the eminent dangers associated with antibiotics include: Agricultural Research Council, Britain, 1951; Medical Research Council, Britain, 1953; Agricultural Research Council, Britain, 1956 (Braude, 1978); Dr. Maxwell Finland, of Boston City Hospital, United States (Kiser, 1975). In 1960, British agricultural and medical research councils, collectively known as the Netherthorpe Committee, combined efforts to determine the effects of antibiotics on livestock, and their subsequent affects on humans (Braude, 1978). The council found that benefits associated with growth enhancement from the use of antibiotics had not diminished over eight years of studies. However, incidences of resistance development in livestock had been observed, and it was speculated that future problems associated with resistant bacterial populations could affect humans and animals. Based on their findings, the committee set forth liberal amendments to regulations already in place. However, in response to public outcry resulting from an outbreak of *Salmonella typhimurium*, a British scientific sub-committee, known as the Swan Committee, was organized to investigate antibiotic resistance, and the potential for transfer of resistance from animal to humans (Braude, 1978; Kiser, 1975). Initially, the Swan Committee found no eminent danger, and determined there was no need to revise the regulations set forth by the Netherthorpe Committee. However, after experiencing

media, public, and political pressure, the committee met a second time and proposed additional restrictive changes to antibiotic regulations in Britain.

In 1966, the United States Food and Drug Administration presented information concerning the potential health risks associated with veterinary and non-medical antibiotic use (Kiser, 1975). As reported earlier in the U.S. by Dr. Finland (Kiser, 1975), no eminent dangers to humans or animals from the use of antibiotics could be determined. In 1970, the U.S. Food and Drug Administration, appointed a task force to investigate antimicrobial drug use in livestock feeds (Kiser, 1975). Initially, information was gathered from a variety of sources in the United States. Included was information obtained from producers, special interest groups, and various university researchers. Members of the Swan Committee were also contacted for input. The report issued by the task force was considerably negative toward the use of antibiotics in livestock feeds and expressed many of the same opinions stated by the Swan Committee. After sharp criticism from scientists and livestock industry leaders, Dr. Shooter, the task force chairman, changed many of his earlier decisions pertaining to the threat of transfer of resistant disease from animals to humans (Kiser, 1975). As a result of numerous studies conducted throughout the mid 70's, and following recommendations provided by special investigative groups looking at the use of antibiotics in animal feeds, the Food and Drug Administration (FDA) in 1977 proposed the elimination of subtherapeutic use of chlortetracycline, oxytetracycline, and penicillin (Food and Drug Administration Center for Veterinary Medicine, 1993). These proposals were sharply criticized and prompted Congress to provide funds for

the National Academy of Sciences (NAS) to investigate antibiotic usage in animal feeds. The NAS determined that there was not sufficient evidence to prove or disprove previous proposals (FDA, 1993). Since 1978, the Natural Resources Defense Council, Inc. (NRDC) has petitioned to reinstate proposals suspending the use of penicillin and tetracyclines in animal feeds. However, the petition has been denied by Secretary of Health and Human Services on the ground that "eminent hazard" to humans could not be proven (FDA, 1993). Information provided by Solomons (1978) helps to verify this, as he reported that *E. coli* from animals were "poor colonizers of the human intestinal tract, even when exceptionally large doses of bacteria were ingested". As of 1989, research conducted by the National Academy of Sciences, Institute of Medicine, (NAS/IOM) determined that "direct evidence" linking human health risk to the subtherapeutic usage of antibiotics in animal feeds could not be proven (FDA, 1993).

By 1993, the FDA had restricted the use of several antibiotics in animal feeds, and was considering recommendations to eliminate the subtherapeutic use of fluoroquinolones, and to limit their therapeutic use as well. A few of the restricted antibiotics formally used to control disease in animals include penicillin-streptomycin, feed-grade neomycin, and sulfonamides (Current Topics, 1994). Nonetheless, there are still 29 antimicrobial compounds currently cleared for use in swine in the United States (Henry and Upson, 1994).

To date, the issue of resistance transfer from animals to humans and the threat of eminent danger has yet to be proven. However, it can be safely assumed that

further research pertaining to the transfer of resistance from animals to humans is needed. Furthermore, the monitoring of new antimicrobial resistance trends and research pertaining to new drug development needs to be promoted (Shales *et al.*, 1991).

*Selected antibiotics*: As already stated, producers often rely on antibiotics to enhance growth in pigs and to control harmful bacteria within their herds. Antibiotics may be classified as "over the counter drugs", "prescription drugs", or "extra-label drug use" (Henry and Upson, 1994). Over the counter drugs include antibiotics that may be purchased by individuals at farm supply stores without the consent of a veterinarian. Prescription drugs are antibiotics that may be purchased only with the written permission of a veterinarian. Extra label drugs are antibiotics that may be prescribed by veterinarians for purposes other than their normal intended use. All of the above drugs may be administered or categorized as therapeutic or subtherapeutic. Therapeutic antibiotics are principally used for the treatment of disease outbreak. Subtherapeutic antibiotics, often considered "feed-additive drugs", are continually administered for prophylaxis in animals.

The antibiotics selected for this study were all over the counter antibiotics (OTC). They were selected with the assistance of the Dr. Ralph Hall, with the University of Tennessee College of Veterinary Medicine, and Dr. Glen Conatser, State Swine Extension Specialist. These antibiotics were considered to be the most commoly used to control *E. coli* in Tennessee swine units. The antibiotics selected were apramycin, gentamicin, and neomycin, all representatives of aminoglycoside

compounds; oxytetracycline, representing tetracycline compounds; and carbadox, representing other antimicrobial compounds (Henry and Upson, 1994). Apramycin is approved for administration of up to 150g/ton of feed, and has a 28 day required withdrawal period. Neomycin is cleared for application of 70 - 140 g/ton of feed and has a 5 - 10 day withdrawal period. Gentamicin is cleared only for oral or injectable therapeutic use in neonatal pigs and can be administered as early as 3 days of age. Withdrawal periods for gentamicin are 10 days for oral dosages and 40 days for injectable dosages. Administration of oxytetracycline varies according to use. Oxytetracycline used for growth promotion is permitted in levels of 7.5 - 500 g/ton, while 7.5 - 50 g/ton is permitted for the control of bacteria. Oxytetracycline can legally be fed until slaughter. As with oxytetracycline, carbadox had two labeled uses. Carbadox utilized for growth stimulation is 10 -50 g/ton of feed, while 10 - 25 g/ton is permitted for the control of bacteria. Carbadox has the longest withdrawal period of 10 weeks (Henry and Upson, 1994).

*Related studies*: Much research has been conducted comparing the effects of antibiotics in therapeutic and subtherapeutic levels on the microflora of animals and man (Sarmiento and Moon, 1988; Langlois *et al.*, 1984; Dawson *et al.*, 1984; Gains *et al.*, 1980; Ahart *et al.*, 1977; Hirsh *et al.*, 1974; Siegel *et al.*, 1974). Research has shown that both therapeutic levels (Langlois, 1978; Hirsh *et al.*, 1974) and subtherapeutic levels (Deneke *et al.*, 1985; Siegel *et al.*, 1974) of antibiotics may be utilized to enhance, or inhibit production. Both therapeutic and subtherapeutic levels have been proven to lead to increased percentages of resistant microflora in animals

and man. Early research involved considerable investigation into the effects of tetracyclines on bacteria. Therapeutic use of tetracyclines, including oxytetracycline, has proven to increase growth, in addition to increasing the percentage level of resistant bacteria (Dawson *et al.*, 1984). Some researchers feel that the use of therapeutic antibiotics increases resistance percentages of microbes to a greater extent than does subtherapeutic use of antibiotics (Langlois *et al.*, 1984; Dawson *et al.*, 1984; Ahart *et al.*, 1977). Langlois *et al.*, (1984) observed the incidence of multiple antibiotic resistance to be higher in pigs fed therapeutic levels of antibiotics for 14 days than in pigs fed subtherapeutic levels of antibiotics for the same period of time. It has been postulated that in cases where bacteria show tremendously high percentages of resistance to therapeutic antibiotics used in humans, the high resistant percentages were a result of very high levels of therapeutic dosages (Hirsh *et al.*, 1974). However, research has proven that even low levels of antibiotics administered to previously non-exposed animals will result in an increase of resistance (Dawson *et al.*, 1984). Deneke *et al.*, (1985) determined that attachment of K88 enterotoxogenic *E. coli* in the small intestines of pigs could be inhibited with very small dosages of oxytetracycline, while numbers of resistant K88 *E. coli* increased. These levels were determined to be well below the minimal inhibitory level (MIC) for *E. coli* to oxytetracycline.

Tetracycline products have proven to provide other benefits as indicated by Langlois *et al.*, (1978), who studied two herds of swine with different histories of antibiotic use to determine effects on reproductive performance. In a swine herd not exposed to antibiotics over a period of 12 years, the percentage of resistant isolates

were determined to decrease over time. However, reproductive performance, including pigs born, pigs weaned, conception rate, pig survival, and pig average weaning weights, all decreased significantly over time. In the other swine herd, which was continuously exposed to chlortetracycline, the percentage of resistant bacteria was significantly greater than the non-exposed herd. However, the antibiotic herd had significantly larger litters at birth and weaning. It should be mentioned that even after 42 months following antibiotic withdrawal, greater than 50% of the isolates tested were resistant to chlorotetracycline, while 90% were resistant in the herd exposed to antibiotics. The general pattern of increasing percentage of resistant bacteria accompanying the use of antibiotics, and the subsequent fall in percentage of resistant bacteria upon withdrawal of antibiotics can further be verified by Langlois *et al.*, (1984).

Studies investigating the effects of antibiotics, such as gentamicin and neomycin on the microflora of animals is very limited. Earlier research involving these two antibiotics showed only slight percentages of resistant bacteria, particularly in herds not exposed to antibiotics. Bacteria from herds exposed to antibiotics have shown marginal resistance to neomycin (Langlois *et al.*, 1984; Langlois *et al.*, 1978). No information for apramycin and carbadox was found.

*Antibiotic resistance after extended withdrawal periods*: Antibiotic resistance after extended withdrawal periods has been well documented (Langlois *et al.*, 1988; Langlois *et al.*, 1986; Hinton *et al.*, 1984; Langlois *et al.*, 1983; Langlois *et al.*, 1978). The extensive research conducted by Langlois at the University of Kentucky has

involved a swine herd that has not been exposed to antibiotics for over 13 years. Initial monitoring of resistance patterns, and persistence of resistant coliforms within the herd began 6 months following the removal of all therapeutic levels of antibiotics. Subtherapeutic levels of antibiotics had never been used within the herd. Bacterial samples were collected from the herd and the herd's environment. Bacteria determined to be positive for lactose use were exposed to selected antibiotics, including gentamicin, neomycin, and oxytetracycline, to determine resistance patterns. Resistance was determined using the Bauer Disk Diffusion Test (Bauer *et al.*, 1966). Bacterial resistance was observed to persist within the herd even after 13 years of withdrawal (Langlois *et al.*, 1986). Bacterial resistance to gentamicin and neomycin was observed to be less than 1% of the total coliform numbers, while 46% of isolates were resistant to tetracycline. In the same study, swine tested from an antibiotic exposed herd revealed no resistant bacteria to gentamicin, while 30% of isolates tested resistant to neomycin, and 92% of isolates were resistant to tetracycline.

Other studies showing the relationship between parents and offspring include Langlois *et al.*, (1986); Wierup, (1975); Sogard, (1973); and Linton *et al.*, (1972). These researchers presented data that indicated higher percentages of resistant and multiple resistant isolates in children, calves, and piglets as compared to their respective adults. As stated earlier, it is believed that resistant microflora may more easily colonize the intestinal tract of young animals compared to older animals (Langlois *et al.*, 1986; Wierup, 1975; Sogard, 1973; and Linton *et al.*, 1972).

**Antimicrobial Resistance**

*Origin of resistance*: Bacterial resistance has been determined to arise through two primary methods. First, bacteria may develop resistance to an antibiotic through spontaneous genetic mutations in genes located within the bacteria's chromosomes. In such cases, due to selective pressures of the antibiotic on sensitive bacteria, mutated resistant microflora are left to flourish and dominate in the host environment (Smith, 1971). Secondly, resistant bacteria may arise through the transfer of genetic information from one bacterial cell to another through conjugative transfer (Anderson, 1968; Smith, 1971). Fimbriae that are commonly referred to as "sex fimbriae" form a conjugative bridge between two cells by which the genetic information is transferred. Genetic information responsible for "transferable resistance", is located extrachromoasomally on plasmids, and is referred to as "resistance factors" or "R factors" (Anderson, 1968; Smith, 1971). R factors are comprised of two components, resistance transfer factors (RTF), and genetic information for resistance (Anderson, 1968). While RTF and genetic information can be transferred independently or in combination, transferable resistance cannot occur without a combination of both. "Contact between a strain carrying an RTF and another carrying a resistance determinant results in passage of the RTF into the resistant organism, in which it combines with the resistance determinant to form a transferable R factor" (Anderson, 1968). Mechanisms of bacterial resistance acquired through transfer of R factors will spread more rapidly through the host as compared to resistance acquired through mutations (Anderson, 1968).

Research conducted by Wierup (1975) investigating the potential for resistance transfer by *E. coli* in 5 and 30 day old calves revealed that 68% of all isolates tested were capable of transferring R factors of single, double, or multiple resistance. Antibiotics tested included tetracycline and neomycin. Furthermore, Wierup determined that the potential for R transfer was significantly higher in the 5 day old calves as compared to the 30 day old calves. Wierup continued to report findings of Larsen and Larsen (1972) who compared the percentages of *E. coli* strains possessing transferable R factors between cows and calves. Larsen and Larsen (1972) observed only 4 $R^+$ *E. coli* strains from 150 cow fecal samples compared to 154 $R^+$ *E. coli* strains from 217 calf fecal samples, thus indicating a greater potential for resistance transfer in young calves compared to older cows. In a related study conducted by Howe and Linton (1976) in which 568 resistant *E. coli* isolates were tested for transferable resistance, 22% of the isolates tested were determined to have transferable R factors for neomycin and 47% of isolates tested were determined to have transferable R factors for tetracycline. Additionally in that study, comparisons of O-type serogroups were made between humans and calves. In the comparison, the researchers determined there were 28 O serogroups common to humans and calves. Of these serogroups, none were determined to be capable of transferable resistance, while 14 were capable of transferable sensitivity.

*Mechanisms of resistance*: Anderson (1968) discussed in great detail studies outlining the mechanisms of transferable bacterial resistance, but recognized much more information was needed on resistance mechanisms. Three primary mechanisms

of resistance were discussed. First, bacteria through a series of structural changes may effectively limit the action of an antibiotic by inhibiting the antibiotic's entrance into the bacterial cell (Franklin, 1967; Unowsky and Rachmeler, 1966). Secondly, bacteria may produce enzymes that degrade or metabolize the antibiotic (Datta and Kontomichalou, 1965). Thirdly, bacteria may produce enzymes that inhibit an antibiotic by suppressing other enzymes required by the antibiotic to function properly (Shaw, 1967).

*Loss of resistance*: As stated by Anderson (1968), spontaneous loss of resistance by bacteria may occur. Generally, spontaneous resistance loss involves bacteria possessing multiple resistance patterns. Resistance is lost during transfer of R factors, as multiple resistance factors required in combination to continue transferable R factors are segregated and transferred independently to other bacteria. As greater segregation of the factors continue, percentages of transferable resistance and resistance patterns decrease. Furthermore, resistance may be lost or decreased by the loss of transferable R factors. Methods of such loss of transferability would include: (1) segregation of the resistance factors and resistance determinants in which each are transferred independently; (2) progeny from a segregated host cell receiving an independent determinant or R factors; (3) damage acquired by the resistance factors during transfer, in which the host progeny only receives the resistance determinants; and (4) "transduction to a fresh host, in which the determinant only is carried over by the transducing phage" (Anderson, 1968).

## 3. MATERIALS AND METHODS

*Animals*: Ten similar, high intensive, farrow to finish swine units were selected across Tennessee. Producers were interviewed to determine the history of antimicrobial drug utilization within the herd. Based on past and current drug use history, as stated by producers, herds were classified as high or low use. Producers were classified "low use" if they reported using therapeutic levels of antimicrobial agents during periods of disease outbreaks, and very limited subtherapeutic use of antibiotics in feeds. Producers classified as "low use" reported using subtherapeutic levels of oxytetracycline to help control respiratory disease. With the exception of oxytetracycline, "low use" producers reported no other application of subtherapeutic levels of antibiotics. Producers were classified "high use" if they reported continued use of subtherapeutic levels of antibiotics to decrease the potential of disease outbreaks, and utilized therapeutic antibiotics when outbreaks of disease did occur. At each site five sows, approximately seven days post farrowing, and five pigs nursing each of the sows were randomly identified. Due to randomization, sows and pigs selected were both purebred and multi-crossbred animals. Sows and pigs were rectally swabbed using the Culturette collection tubes (Culturettee Collection and Transport Systems, Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030). These tubes are sterile, disposable collection tubes containing a rayon-tipped swab and one ampule of 0.5 mL Amies bacterial transport medium (without charcoal). Following sampling, collection tubes were immediately placed on ice for transport to

the University of Tennessee agricultural campus located in Knoxville. Additional samples were taken from the same pigs at approximately 35 and 63 days of age.

*Microbiological Procedures*: At the lab, contents from the collection tubes were streaked onto Lactose MacConkey agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030) and incubated at 37°C for 24 hours to identify *Escherichia coli* colonies. Selected colonies were consistent with *E. coli's* pink coloration and morphology on Lactose MacConkey agar. Randomly selected colonies were subjected to an A.P.I. test (Analytical Profile Index, bioMerieux Vitek, Inc., 595 Anglum Drive Hazelwood, MO 63042-2395). The A.P.I. "test" or "system" consists of a series of biochemical tests used to identify *Enterobacteriaceae* and other Gram-negative bacteria. Due to time restrictions and cost a limited number of *E. coli* were subjected to the A.P.I. test. However, 100% of the colonies tested were determined to be *E. coli.* In limited cases in which *E. coli* could not be clearly identified on the Lactose MacConkey plate, coliforms were transferred to a second Lactose MacConkey plate for identification. Colonies that could not be positively identified from either the first or second plating were discarded. Additionally, there were samples in which no coliforms could be found, and these samples were not included in the study. From each Lactose MacConkey plate, each representing an individual test animal, ten independent *E. coli* colonies were selected. Identified colonies were streaked onto Leuria Broth agar (10 g/L Bacto-tryptone, 5 g/L yeast extract, 10 g/L NaCl, adjusted to pH 7.5 w/ NaOH, and 17 g/L agar) and aerobically incubated at 37°C for 24 hours prior to storage at 5°C.

*Test Procedures*: A 4 mm wire loop was used to transfer bacteria from the LB media to a disposable pyrex tube containing *5 mL* of LB broth. Tubes were capped with a foam cork to allow aerobic growth of bacteria. Tubes were placed in racks at a 45° angle, in an Orbit Shaking Water Bath (model 3540) maintained at 37°C. Cells were permitted to grow to that of a .05 McFarland standard turbidity level as indicated by the National Committee for Clinical Laboratory Standards (NCCLS, 1976). Isolates were allowed a minimum of one hour incubation period to insure at least three generations growth. Isolates failing to reach the standard turbidity level were permitted to continue growing and were compared to the standard at 30 minute intervals.

Isolates exceeding the turbidity level were diluted to standard with additional sterile LB broth. Using a Ulster Scientific Inc. V3-series pipette (Ulster Scientific, Inc., P.O. Box 819, New Paltz, New York 12561-0819), 150 *uL* of cells were drawn from the pyrex tube and placed on a 4 inch disposable petri plate containing Mueler-Hinton agar as prescribed by NCCLS, (1976). Cells were spread over the entire plate using a sterile glass rod and spin table to acquire confluent growth. Plates were permitted 5-15 minutes to dry prior to application of the antibiotic discs. Discs were applied within 15 minutes to prevent over growth by the bacteria before the antibiotics could diffuse and take affect. Antibiotic discs were arranged alphabetically on the plate approximately 35 mm apart in a clockwise manner with the aid of sterile forceps. Placement of the discs allowed for zone sizes to reach their full potential while simplifying reading and recording of data. Antibiotics used in the study and their

respective disc potencies included apramycin, (ELI LILLY and Company, Indianapolis, IN 46285), 15 *ug*; carbadox (Sigma Chemical Company, P.O. Box 14508, St. Louis MO 63178), 20 *ug*; gentamicin (ICN Biomedicals Inc., 1263 South Chilicothe Road, Aurora, Ohio 44202), 10 *ug*; neomycin (ICN Biomedicals Inc., 1263 South Chilicothe Road, Aurora, Ohio 44202), 30 *ug*; and tetracycline, (ICN Biochemicals, Cleveland, Ohio 44128), 30 *ug*. Initially, carbadox, gentamicin, neomycin, and oxytetracycline susceptibility test discs (BBL, manufactured by Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030) were purchased. Apramycin sensi-disc were purchased from DIFCO Laboratories, (Detroit, Michigan). To reduce research cost, antibiotic discs were later produced in our lab. Antibiotics and paper discs were purchased through Baxter Diagnostics Inc. (1430 Waukegan Road, McGaw Park, IL 60085-6787). Using a Mettler AE160 scale, appropriate amounts of antibiotics were weighed to their specified disc potencies and added to a vial containing 10 mL of sterile water. Both water and vial were previously sterilized using a Barnstead Sterilizer at 17 psi and 120°C for 30 minutes. To insure proper mixing, carbadox was diluted in *20 mL* of water. Ten microliters of each antibiotic solution was applied to the respective paper discs, while twenty microliters of carbadox solution was applied, due to its higher dilution factor. Standard strains of *E. coli* (ATCC 25922) were exposed to sensi-discs manufactured by BBL and DIFCO, as well as discs produced in our lab. Plates were incubated for a period of 24 hours at 37°C and tested to compare zone sizes between purchased discs and discs produced within our lab. Standard zone sizes for each antibiotic were compared with those

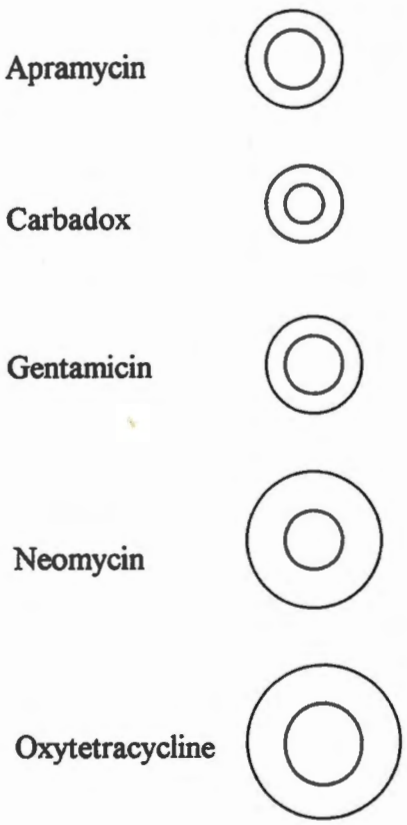
reported by NCCLS, (1990). Additionally, zone diameters were randomly tested throughout the study to ensure that diameters fell within the specified range. After application of discs, plates were incubated aerobically for 24 hours at 37°C. Sample zone sizes were then compared to standard control zone sizes as reported by the NCCLS, 1990 (Table1). With the bottom side of the plate facing upward, a transparent overlay marked with circular interpretive zone sizes corresponding to each antibiotic was placed over the antibiotic discs (Figure 1). Isolates were determined to be sensitive, intermediately sensitive, or resistant according to their zone diameter, as compared to the overlay. Zone sizes too close to determine were recorded as the higher sensitivity.

*Statistics*: The data generated by the above tests were analyzed using a Chi-square analysis. Statistical differences were compared for each antibiotic between pig and sow, low and high drug use, and between individual pigs at 7, 35, and 63 days of age.

**Table 1.** Zone diameter interpretive chart[1] for antibiotic susceptibility test discs for *E. coli* subjected to antimicrobial agents prevalently used by Tennessee swine producers.

| Antibiotic | ATCC 25922 | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|
| | | | | |
| Apramycin | 12-19 mm | 15 mm ≥ | 12-14 mm | ≤ 11 mm |
| Carbadox | NA | 12 mm ≥ | 10-11 mm | ≤ 12 mm |
| Gentamicin | 19-26 mm | 15 mm ≥ | 13-14 mm | ≤ 12 mm |
| Neomycin | 17-23 mm | 17 mm ≥ | 13-16 mm | ≤ 12 mm |
| Oxytetracycline | 24-30 mm | 19 mm ≥ | 15-18 mm | ≤ 14 mm |

[1]Measurements indicated for ATCC control strain reflect the acceptable range for kill zone diameters for the antibiotics tested. Antibiotic disc kill zone sizes for the ATCC strains indicate an acceptable level of antibiotic potency.




Kill zones exceeding the outer ring diameter represent isolates sensitive to the antibiotic. Kill zones extending past the inner ring and not exceeding the outer ring were determined to be intermediately resistant. Kill zones failing to exceed the inner ring were determined to be resistant. Objects are drawn to approximate size and reflect the method used for measuring kill zones.

**Figure 1.** Schematic of the transparent grid utilized to interpret antibiotic discs zone sizes.

## 4. RESULTS

*Pig and Sow Relationship*: Resistance was detected for all antibiotics at the day 7 sampling for both pigs and sows (tables 2-4). *Escherichia coli* were significantly more resistant ($P < .0001$) to apramycin, gentamicin, and neomycin in 7 day old pigs when compared to sows. *Escherichia coli* were more resistant to carbadox in the sow ($P < .0001$), and were observed to have a slightly greater resistance to oxytetracycline ($P < .05$) in the sow when compared to pigs.

*Resistance Summed Over Farms*: In pigs, differences in the percentage of sensitive, intermediately resistant, and resistant isolates were observed between day 7, 35 and 63 for all antibiotics tested (Tables 5-9). At day 7, isolates expressed some percentage of resistance to all five antibiotics tested. While resistance percentages remained low ($P < .0001$) in pigs at 7 days of age for apramycin (8.27%), carbadox (1.74%), gentamicin (8.84%), and neomycin (15.17%), isolates in pigs of this age were observed to be substantially more resistant ($p < .0001$) to oxytetracycline (80.51%).

At 35 days of age, (the initial weaning period), the percentages of resistant isolates to apramycin (44.32%), carbadox (29.15%), and gentamicin (34.78%) were observed to be significantly greater ($P < .0001$) than the percentage of resistant isolates in pigs at 7 days of age. Furthermore, the percentage of resistant isolates to neomycin (17.70%) and oxytetracycline (87.48%) in pigs at 35 days of age was observed to increase ($P < .0001$) when compared to isolates from pigs at 7 days of age.

**Table 2.** Percentage of sensitive *E. coli* isolates to selected antibiotics in pigs and sows summed over all farms.[1]

| Antibiotic | Pig | Sow |
|---|---|---|
| Apramycin [2] | 91.49 | 99.57 |
| | | |
| Carbadox [2] | 83.74 | 75.97 |
| | | |
| Gentamicin [2] | 23.74 | 21.65 |
| | | |
| Neomycin [2] | 47.82 | 44.59 |
| | | |
| Oxytetracycline [3] | 7.87 | 5.41 |
| | | |

[1]Samples were taken 7 days post parturition. Statistics reflect comparisons between pigs and sows for each antibiotic. No comparisons were made between antibiotics.

[2]Chi-square analysis ($P < .0001$)

[3]Chi-square analysis ($P < .05$)

**Table 3**. Percentage of intermediately resistant *E. coli* isolates to selected antibiotics in pigs and sows summed over all farms.[1,2]

| Antibiotic | Pig | Sow |
|---|---|---|
| Apramycin [3] | 0.24 | 0.00 |
| | | |
| Carbadox [3] | 14.53 | 21.21 |
| | | |
| Gentamicin [3] | 67.42 | 77.27 |
| | | |
| Neomycin [3] | 37.01 | 46.75 |
| | | |
| Oxytetracycline [4] | 11.62 | 9.31 |
| | | |

[1]Due to a possible error in determining intermediate zone sizes for gentamicin, we suspect a greater percentage of isolates determined as intermediately resistant to gentamicin are actually sensitive.

[2]Samples were taken 7 days post parturition. Statistics reflect comparisons between pigs and sows for each antibiotic. No comparisons were made between antibiotics.

[3]Chi-square analysis ($P < .0001$)

[4]Chi-square analysis ($P < .05$)

**Table 4**. Percentage of resistant *E. coli* isolates to selected antibiotics in pigs and sows summed over all farms.[1]

| Antibiotic | Pig | Sow |
|---|---|---|
| Apramycin [2] | 8.27 | 0.43 |
| | | |
| Carbadox [2] | 1.74 | 2.81 |
| | | |
| Gentamicin [2] | 8.84 | 1.08 |
| | | |
| Neomycin [2] | 15.17 | 8.66 |
| | | |
| Oxytetracycline [3] | 80.51 | 85.28 |
| | | |

[1]Samples were taken 7 days post parturition. Statistics reflect comparisons made between pigs and sows for each antibiotic. No comparison were made between antibiotics.

[2]Chi-square analysis ($P < .0001$)

[3]Chi-square analysis ($P < .05$)

**Table 5**. Percentage of sensitive, intermediately resistant, and resistant fecal *E. coli* to apramycin in pigs.[1,2]

| PIG AGE[2] | SENSITIVE | INTERMEDIATE | RESISTANT | ISOLATES |
|---|---|---|---|---|
| Day 7 | 91.49 | 0.24 | 8.27 | 2478 |
| Day 35 | 55.35 | 0.33 | 44.32 | 2412 |
| Day 63 | 77.31 | 0.21 | 22.48 | 1406 |
| TOTAL | | | | 6296 |

[1]Percentages reflect data summed over all farms for the three sample ages.
[2]Time effect: Chi-square analysis ($P < .0001$)

**Table 6**. Percentage of sensitive, intermediately resistant, and resistant fecal *E. coli* to carbadox in pigs.[1]

| PIG AGE[2] | SENSITIVE | INTERMEDIATE | RESISTANT | ISOLATES |
|---|---|---|---|---|
| Day 7 | 83.74 | 14.53 | 1.74 | 2478 |
| Day 35 | 55.02 | 15.84 | 29.15 | 2412 |
| Day 63 | 60.31 | 13.44 | 26.24 | 1406 |
| TOTAL | | | | 6296 |

[1]Percentages reflect data summed over all farms for the three sample ages.
[2]Time effect: Chi-square analysis ($P < .0001$)

**Table 7.** Percentage of sensitive, intermediately resistant, and resistant fecal *E. coli* to gentamicin in pigs.[1,2]

| PIG AGE[3] | SENSITIVE | INTERMEDIATE | RESISTANT | ISOLATES |
|---|---|---|---|---|
| Day 7 | 23.74 | 67.42 | 8.84 | 2477 |
| Day 35 | 12.40 | 52.82 | 34.78 | 2412 |
| Day 63 | 49.13 | 33.17 | 17.70 | 2412 |
| TOTAL | | | | 6295 |

[1]Percentages reflect data summed over all farms for the three sample ages.

[2]Due to a possible error in determining intermediate zone sizes for gentamicin, we suspect a greater percentage of isolates determined as intermediately resistant to gentamicin are actually sensitive.

[3]Time effect: Chi-square analysis ($P < .0001$)

**Table 8**. Percentage of sensitive, intermediately resistant, and resistant fecal *E. coli* to neomycin in pigs.[1]

| PIG AGE[2] | SENSITIVE | INTERMEDIATE | RESISTANT | ISOLATES |
|---|---|---|---|---|
| Day 7 | 47.82 | 37.01 | 15.17 | 2478 |
| Day 35 | 49.13 | 33.17 | 17.70 | 2412 |
| Day 63 | 44.17 | 34.00 | 21.83 | 1406 |
| TOTAL | | | | 6296 |

[1]Percentages reflect data summed over all farms for the three sample ages.
[2]Time effect: Chi-square analysis ($P < .0001$)

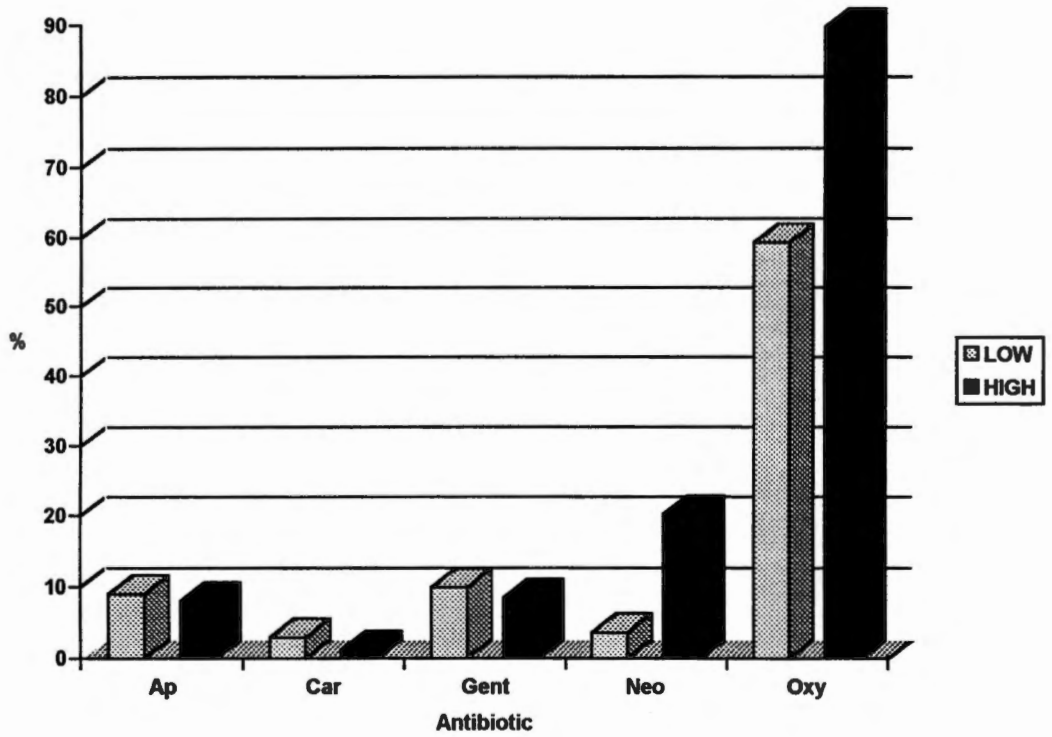
**Table 9**. Percentage of sensitive, intermediately resistant, and resistant fecal *E. coli* to oxytetracycline in pigs.[1]

| PIG AGE[2] | SENSITIVE | INTERMEDIATE | RESISTANT | ISOLATES |
|---|---|---|---|---|
| Day 7 | 7.87 | 11.62 | 80.51 | 2478 |
| Day 35 | 7.01 | 5.51 | 87.48 | 2412 |
| Day 63 | 1.00 | 1.99 | 97.01 | 1406 |
| TOTAL | | | | 6296 |

[1]Percentages reflect data summed over all farms for the three sample ages.
[2]Time effect: Chi-square analysis ($P < .0001$)

Upon completion of the grower phase, (63 days of age), the percentages of resistant isolates to apramycin (22.48%), carbadox (26.24%), and gentamicin (17.70%) were observed to decrease ($P < .0001$) when compared to isolates tested from pigs at 35 days of age. However, the percentage of resistant isolates to neomycin (21.83%) and oxytetracycline (97.01%) increased ($P < .0001$) when compared to isolates tested from pigs at 35 days of age.

*High vs. Low Antimicrobial Drug Use*: A total of 3,735 isolates from farms recognized as low drug use, and 8,655 isolates from farms classified as high drug use were collected from pigs at 7 days of age and subjected to antimicrobial agents (Figure 2). No differences ($P > .1$) in the percentage of resistant isolates to apramycin were observed between farms of low (8.97%) and high (7.97%) antimicrobial drug in pigs at 7 days of age. Differences ($P < .01$) were observed in the percentage of resistant isolates to carbadox between farms of low (2.95%) and high (1.21%) drug use. Differences ($P < .0001$) in the percentage of resistant isolates to gentamicin were observed between farms of low (9.91%) and high (8.38%) drug use. The largest differences in the percentage of resistant isolates to antibiotics between farms of low and high antimicrobial drug use in 7 day old pigs were observed with neomycin and oxytetracycline. In farms considered low drug use, 3.61% of isolates were determined to be resistant to neomycin , while in farms recognized as high drug use, 20.16% of isolates tested were resistant to neomycin ($P < .0001$). For the low use farms, 59.30% of the isolates tested were determined to be resistant to oxytetracycline, while 89.66%



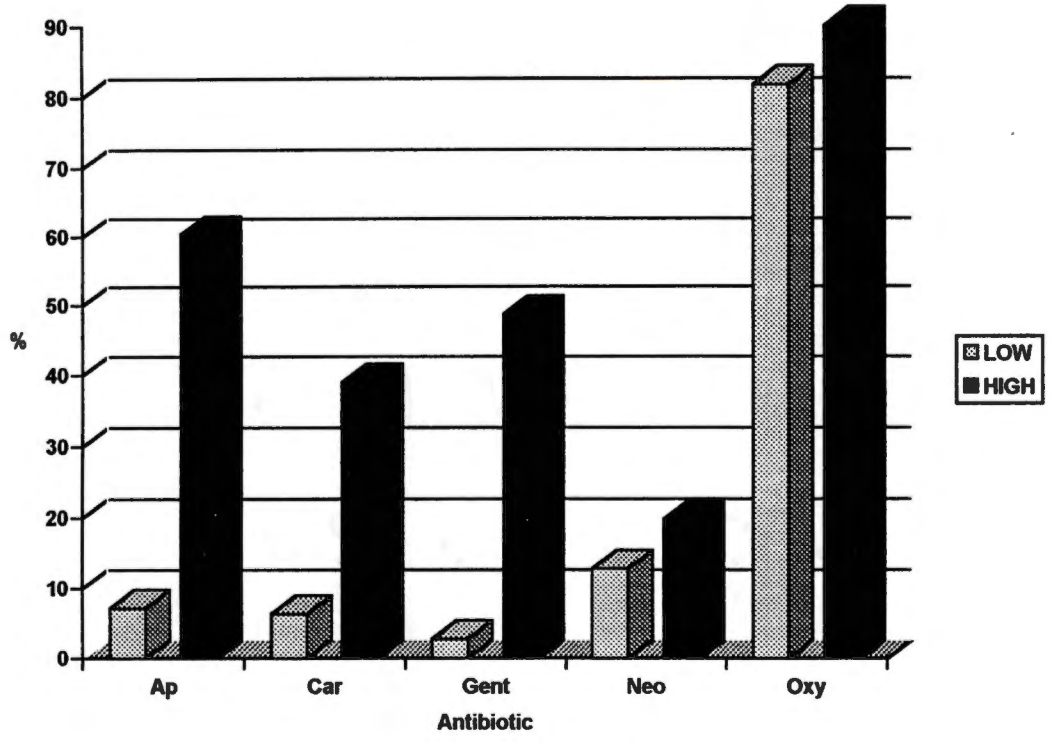

[1]Percentages are of resistant fecal *E. coli* isolates to selected antibiotics from pigs at 7 days of age. A total of 747 isolates were tested from farms identified as low antimicrobial drug use, and 1,731 isolates were tested from farms identified as high antimicrobial drug use. Statistics are based on Chi-square analysis.

Ap (apramycin): low versus high use; ($P > .1$
Car (carbadox): low versus high use; ($P < .01$)
Gent (gentamicin): low versus high use; ($P < .0001$)
Neo (neomycin): low versus high use; ($P < .0001$)
Oxy (oxytetracycline): low versus high use; ($P < .0001$)

**Figure 2.** Comparison of the percentages of resistant *E. coli* isolates in Tennessee swine units categorized as low or high antimicrobial use.[1]

of the isolates were resistant to oxytetracycline in farms recognized as high drug use ($P < .0001$).

Samples taken from pigs at 35 days of age resulted in collection of 3,645 isolates tested from farms considered low drug use, and 8,415 isolates collected from farms recognized as high drug use. The largest differences in the percentage of resistant isolates between farms of low and high drug use were observed in pigs at 35 days of age when compared with the other age groups. Significant differences ($P < .0001$) in the percentage of resistant isolates from pigs at 35 days of age were observed between farms of low and high antimicrobial drug use for all antibiotics tested (Figure 3). In units recognized as low use, 7.13% of isolates collected from pigs at 35 days of age were determined to be resistant to apramycin, while 60.43% of isolates tested from farms of high use were determined to be resistant to apramycin. In low use units, 6.31% of isolates were resistant to carbadox, compared to 39.04% of isolates for high use units. In low use farms, 2.74% of isolates were resistant to gentamicin, and 48.66% of isolates to gentamicin in farms recognized as high drug use. From pigs at 35 days of age housed in the low drug use units, 12.76% of isolates were resistant to neomycin, while 19.85% of the isolates were resistant to neomycin in high drug use farms. The highest percentages of resistance were observed with oxytetracycline. Pigs at 35 days of age, housed on farms reporting low antimicrobial drug use, 81.76% of isolates were resistant to oxytetracycline. In pigs 35 days old, housed on farms categorized as high drug use, 89.96% of isolates were observed resistant to oxytetracycline.

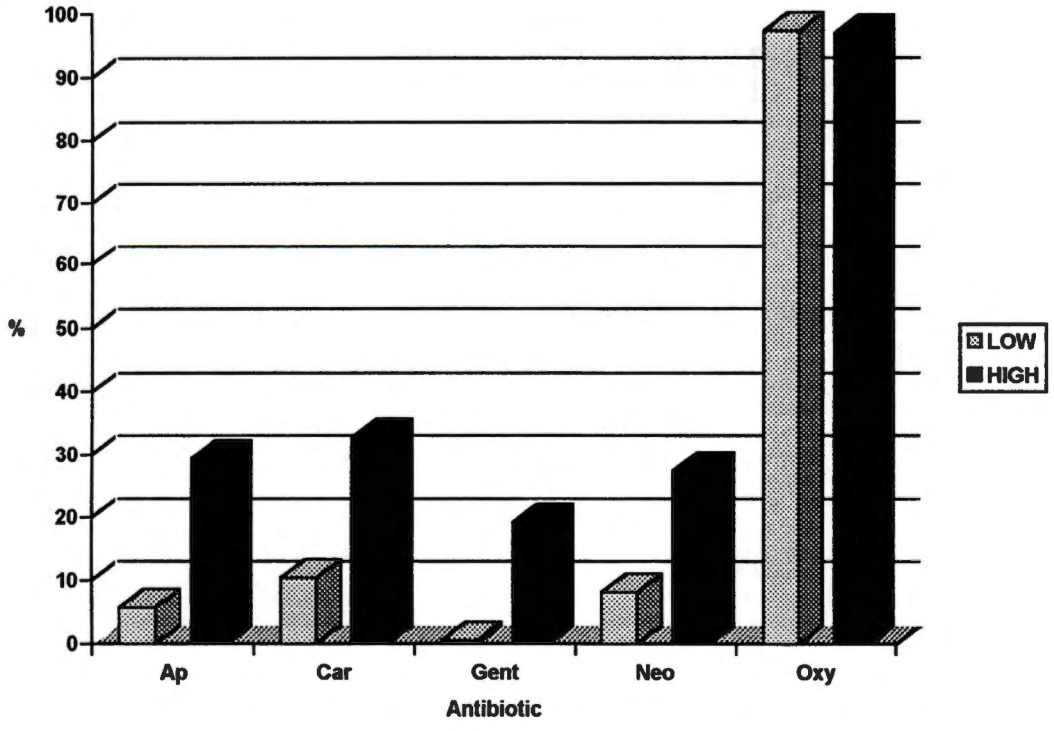



[1]Percentages are of resistant fecal *E. coli* isolates to selected antibiotics from pigs at 35 days of age. A total of 729 isolates were tested from farms identified as low antimicrobial drug use, and 1,683 isolates were tested from farms identified as high antimicrobial drug use. Statistics are based on Chi-square analysis.

Ap (apramycin): low versus high use; ($P < .0001$)
Car (carbadox): low versus high use; ($P < .0001$)
Gent (gentamicin): low versus high use; ($P < .0001$)
Neo (neomycin): low versus high use; ($P < .0001$)
Oxy (oxytetracycline): low versus high use; ($P < .0001$)

**Figure 3.** Comparison of the percentages of resistant *E. coli* isolates in Tennessee swine units categorized as low or high antimicrobial drug use.[1]

A total of 2,035 isolates were collected from pigs at 63 days of age for the farms of low drug usage, and 4,995 isloates were collected from farms of high drug usage. Significant differences ($P < .0001$) in the percentages of resistant isolates were observed between farms of low and high drug use for apramycin, carbadox, gentamicin, and neomycin (Figure 4). No differences ($P > .1$) in the percentages of resistant isolates to oxytetracycline between low and high drug use were observed. From pigs at 63 days of age, housed in farms of low drug use, 5.65% of the isolates were determined resistant to apramycin, compared with 29.33% from farms of high drug usage. From low usage farms, 10.32% of isolates were resistant to carbadox, while 32.73% of isolates from high usage farms were resistant to carbadox. It was observed that 0.49% of isolates from pigs at 63 days of age were resistant to gentamicin in the farms of low usage, while 19.22% of the isolates from 63 day old pigs housed in units of high drug usage were resistant. From farms reporting low drug usage, 8.11% of the isolates subjected to neomycin were resistant, while 27.43% of the isolates from high use farms were resistant. The highest resistance percentages were observed for isolates from pigs at 63 days of age and subjected to oxytetracycline. No differences ($P > .1$) in the percentage of resistant isolates to oxytetracycline were observed between farms of low and high drug usage. In low use farms, 97.30% of the isolates exposed to oxytetracycline were resistant. In swine units categorized as high antimicrobial drug usage, 96.90% of isolates were resistant to oxytetracycline.




[1]Percentages are of resistant fecal *E. coli* isolates to selected antibiotics from pigs at 63 days of age. A total of 407 isolates were tested from farms identified as low antimicrobial drug use, and 999 isolates were tested from farms identified as high antimicrobial drug use. Statistics are based on Chi-square analysis.

Ap (apramycin): low versus high use; ($P < .0001$)
Car (carbadox): low versus high use; ($P < .0001$)
Gent (gentamicin): low versus high use; ($P < .0001$)
Neo (neomycin): low versus high use; ($P < .0001$)
Oxy (oxytetracycline): low versus high use; ($P > .1$)

**Figure 4.** Comparison of the percentages of resistant *E. coli* isolates in Tennessee swine units categorized as low or high antimicrobial drug use.[1]

## 5. DISCUSSION

A total of 6,296 isolates from 750 pig fecal samples, and 462 isolates from 50 sows fecal samples were subjected to apramycin, carbadox, gentamicin, neomycin, and oxytetracycline to determine the incidence of resistant *E. coli* in Tennessee swine units. Significant differences in the percentages of resistant isolates were observed between sows and their pigs for each of the antibiotics tested. We initially believed that pigs would acquire resistant microflora from the sows feces. However, Callear and Smith (1966) and Arbuckle (1968) determined that sows and gilts entering the farrowing unit were actually free of *E. coli*, and that sows obtained *E. coli* from pig feces and the environment of the farrowing house. This was confirmed by Hinton and Linton (1987) and Katouli *et al.*, (1995) who determined that young pigs were able to maintain their own unique population of microflora, even while in close contact with the sow and littermates. Isolates from pigs were observed to have a higher percentage of bacterial resistance to apramycin, gentamicin, and neomycin compared with isolates from sows. High percentages of resistant bacteria to antibiotics in young animals are not uncommon. Linton *et al.*, (1972) Sogard (1973), Wierup (1975), and Langlois *et al.*, (1986) have conducted similar studies in which bacteria obtained from young animals were determined to more resistant to an antibiotic than were bacteria collected from adults. Langlois (1986) postulated that bacteria from young animals may show a higher percentage of resistance, as *E. coli* can more easily and rapidly colonize their intestinal tract. In addition, Larsen and Larsen (1972) and Wierup (1975) determined that resistance may be greater in young animals as bacteria in their intestinal tract have

the potential for increased resistance transfer. Such rapid colonization would allow for the development and transfer of resistant bacteria. *Escherichia coli* isolated from sows were found to be significantly more resistant to carbadox and oxytetracycline. The high resistance percentages for sows and pigs (85.28% and 80.51% respectively) may be explained by the continuous use of tetracycline products in swine feeds over the past three decades (Langlois *et al.*, 1978; Langlois *et al.*, 1983; Hinton *et al.*, 1984; Langlois *et al.*, 1986; Langlois *et al.*, 1988). Producers often utilize tetracycline products in sow diets to enhance reproductive performance. In addition, the use of subtherapeutic levels of tetracycline to promote growth and performance in pigs has long been established (Jukes *et al.*, 1950; Dawson *et al.*, 1984).

Significant changes in the percentage of resistant *E. coli* over time for each antibiotic were also observed. *Escherichia coli* identified from pigs at 7 days of age were determined to be slightly to marginally resistant to apramycin (8.27%), carbadox (1.74%), gentamicin (8.84%), and neomycin (15.17%). *Escherichia coli* isolated from 7 day old pigs were observed to be much more resistant to oxytetracycline (80.51%). We were suprised by the resistance percentages of *E. coli* in the 7 day old pig, particularly considering the fact that pigs are born with no *E. coli* (Kenworthy and Crabb, 1963). Obviously, rapid colonization of the pig's gut occurs soon after birth, as indicated by Smith and Jones (1963), Kenworthy and Crabb (1963), and Smith (1965).

Samples taken from pigs at 35 days of age showed the greatest increases in resistance percentages. Though it was often very difficult to determine the antibiotics

and level utilized by producers during this period, we suspect that pigs had been exposed too, or were currently receiving their highest levels of therapeutic and subtherapeutic antibiotics at this time. It has been proven that the continuous subtherapeutic or therapeutic use of antibiotics in increasingly higher levels will result in the development of resistant bacteria (Siegel *et al.*, 1974; Hirsh *et al.*, 1974; Ahart *et al.*, 1977; Gains *et al.*, 1980; Dawson *et al.*, 1984; Langlois *et al.*, 1984; Sarmiento and Moon, 1988).

Pigs that were 63 days old were either approaching or had entered the grower phase as indicated by producers. We suspect levels of antibiotics being administered to pigs at this age had been reduced, especially with regard to therapeutic application. The percentage of isolates resistant to apramycin (22.48%), carbadox (26.24%), and gentamicin (17.70%) were observed to decrease compared to isolates from the pigs at 35 days of age. In this case, declines in resistance percentages may be attributed to two possible factors. First, decreases in the use of antibiotics such as gentamicin, used primarily in pigs 1 to 10 days of age to control baby pig scours; and second the decline of *E. coli* in pigs with increased age (Smith and Crabb, 1961). Isolates resistant to neomycin (21.83%) and oxytetracycline (97.01%) were again observed to rise when compared to samples taken at 35 days of age. Increases in the percentage of resistance to neomycin is not fully understood as it is a member of the aminoglycoside, as is apramycin and gentamicin, to which isolates appeared more sensitive during the day 63 sampling period. One theory as to why there was an the increase of resistant isolates to neomycin may be that neomycin has been extensively utilized by producers

for two decades, and that a large base population of resistant bacteria now exists. This is analogous to what was observed with oxytetracycline. Furthermore, the use of tetracyclines through the finishing phase as a growth promotant, and as a feed additive in sow diets, allows for the proliferation of *E. coli* resistant to oxytetracycline.

Additional comparisons were made for antibiotics between farms categorized as high or low antimicrobial drug use. Isolates collected from pigs at 7 days of age showed no differences between low and high use in the percentage of resistance to apramycin. This might have been expected as apramycin is used in nursery and grower/finisher diets. A greater percentage of bacterial resistance to carbadox, gentamicin was observed in farms of low use as compared to farms recognized as high use. Higher resistance percentages in the low use farms may be the result of increased resistance as a result of higher levels of therapeutic drug use. Ahart *et al.*, (1977), Langlois *et al.*, (1984), and Dawson *et al.*, (1984), determined that swine receiving high therapeutic levels of antibiotics were more likely to develop the same or higher levels of resistance than swine fed subtherapeutic levels of antibiotics. This may be particularly true in the case of higher resistance percentages to gentamicin, where gentamicin is used as a therapeutic antibiotic in neonatal pigs to control scours. Though differences were found between low and high use for the percentage of resistant isolates to carbadox, percentages remained low. Again, carbadox is used in nursery and grower/finisher diets. A greater percentage of resistance to neomycin and oxytetracycline was observed in the farms categorized as high use compared with low use farms. The higher percentages of bacterial resistance to these two antibiotics in

pigs at 7 days of age from farms of both low and high drug use, reflects a long history of their use within the units. Even in cases of long periods of antibiotic withdrawal, bacterial resistance to oxytetracycline and neomycin have been proven to persist (Langlois *et al.*, 1978; Langlois *et al.*, 1983; Hinton *et al.*, 1984; Langlois *et al.*, 1986; Langlois *et al.*, 1988).

The greatest differences in resistance percentages between farms of low and high use were observed in isolates collected from 35 day old pigs. Resistance percentages to selected antibiotics were all significantly lower in farms recognized as low use. Limited therapeutic and subtherapeutic use during the nursery phase would account for the differences. However, the resistance percentages for low and high use farms were observed to increase dramatically when compared to pigs sampled at 7 days of age. This again may confirm earlier research indicating higher levels of antibiotic application lead to the development of higher levels of antibiotic resistance.

Isolates collected from pigs at 63 days of age were observed to decrease in the percentage of resistance to apramycin, carbadox, and gentamicin for both the low and high use farms when compared to isolates taken from pigs 35 days of age. However, significant differences were determined between low and high use for the previously mentioned antibiotics, with the high use farms showing a greater percentage of resistance. The decline in resistance to these antibiotics may again be the result of increasing age or the decline in antibiotic use. Significant differences were observed between low and high use for neomycin, in which high use farms were observed to have a higher percentage of resistant isolates. However, once again resistance to

neomycin was observed to increase in isolates taken from pigs at 35 days of age as was observed with oxytetracycline. Both neomycin and oxytetracycline have a long history of use by swine producers, and as indicated here, bacterial populations have been able to develop a considerable amount of resistance to them as a result.

# 6. IMPLICATIONS

Antibiotics may be effectively used for the control of harmful bacteria and as growth promotants in swine. However, with a continued increase of therapeutic and subtherapeutic antibiotics applied to swine, significant levels of antibiotic resistance by bacteria will develop. In such cases of increased resistance by *E. coli* to antibiotics used by producers, the ability of such antibiotics to control disease outbreaks may be diminished. Producers in this study were observed to limit the percentage of resistant *E. coli* within their pigs by decreasing the usage of antibiotics. Such trends by producers may not only enhance the effectiveness of antibiotics upon future use, but may also evolve into a required management practice by all livestock producers as consumer safety issues arise. As scientist continue to debate eminent danger of antibiotic resistance transfer from livestock to humans, public pressure concerning product safety may dictate future legislation. Such legislation could significantly restrict the agricultural usage of many needed antibiotics.

# LITERATURE CITED

Ahart, J. G., G. C. Burton, and D. C. Blenden. 1977. The influence of antimicrobial agents on the percentage of tetracycline-resistant bacteria in faeces of humans and animals. J. Appl. Bact. 44:183.

Anderson, E. S. 1968. The ecology of transferable drug resistance in the enterobacteria. Ann. Rev. Microbiol. 22:131.

Anonymous. 1994. FDA panel urges restricted use of fluoroquinolones in animals. Am. Soc. Microbiol. 60:350.

Arbuckle, J. B. R. 1968. The distribution of certain *Escherichia coli* strains in pigs and their environment. Br. Vet. J. 124:152.

Bauer, A. W., W. M. M. Kirby, J. L. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45:493.

Bertschinger, H. U., J. M. Fairbrother, N. O. Nielsen, and J. F. Plhlenz. Diseases of Swine 7th ed. Ames, IA: Iowa State Univ. Press; 1992. *Escherichia coli infections* pp. 487-97.

Braude, R. 1978. Antibiotics in animal feeds in great Britain. J. Anim. Sci. 46:1425.

Burt, S. J. and D. R. Woods. 1976. R factor transfer to obligate anaerobes from *Escherichia coli*. J. Gen. Microbiol. 93:405.

Callear, J. F. F. and I. M. Smith. 1966. The effect of furazolidone on *Escherichia coli* infection of newborn piglets. Br. Vet. J. 122:169.

Coates, M. E., R. Fuller, G. F. Harrison, M. Levine and S. F. Suffolk. 1963. A comparison of the growth of chicks in the Fustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Brit. J. Nutr.17:141.

Craven, J. A. and D. A. Barnum. 1971. Distribution of porcine fecal coliflora throughout a barn. Can. J. Comp. Med. 35:324.

Dawson, K. A., B. E. Langlois, T. S. Stahly, and G. L. Cromwell. 1984. Antibiotic resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentrations of chlortetracycline. J. Anim. Sci. 58:123.

Datta, N. and P. Kontomichalou. 1965. Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. Nature 208:239.

Deneke, C. F., G. M. Thorne, A. D. Larson, and S. L. Gorbach. 1985. Effect of tetracycline on the attachment of $K88^+$ enterotoxigenic *Escherichia coli* to porcine small-intestinal cells. J. Infect. Dis. 152:1032.

Fairbrother, J. M., A. Broes, M. Jacques, and S. Lariviere. 1989. Pathogenicity of *Escherichia coli* O115:K"V165" strains isolated from pigs with diarrhea. Am. J. Vet. Res. 50:1029.

Food and Drug Administration Center for Veterinary Medicine. 1993. Information for consumers. Antibiotics in animal feeds. Center for Veterinary Medicine, Rockville, Maryland.

Franklin, T. J. 1967. Resistance of *E. coli* to tetracyclines: Changes in permeability to tetracyclines in *E. coli* bearing transferable resistance factors. Biochem. J. 105:371.

Freeman, B. M., A. C. C. Manning, G. F. Harrison and M. E. Coates. 1975. Dietary aureomycin and the response of the fowl to stressors. Brit. J. Poul. Sci. 16:395.

Gaastra, W. I. M. and F. K. de Graaf. 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. 46:129.

Gaines, S. A., L. D. Rollins, R. D. Williams, and M. Selwyn. 1980. Effect of penicillin and virginiamycin on drug resistance in lactose-fermenting enteric flora. Antimicrob. Agents Chemo. 17:428.

Gross, R. J. and B. Rowe. 1985. *Escherichia coli* diarrhoea. J. Hyg. Camb. 95:531.

Hampson, D. J., M. Hinton, and D. E. Kidder. 1985. Coliform numbers in the stomach and small intestine of healthy pigs following weaning at three weeks of age. J. Comp. Path.

Hampson, D. J., M. Hinton, and D. E. Kidder. 1985. Coliform Numbers in the stomach and small intestine of healthy pigs following weaning at three weeks of age. J. Comp. Path. 95:353.

Hays, V. W. 1969. Biological basis for the use of antibiotics in livestock production. National Academy of Science, Pub. 1697.

Henry, S. C. and D. W. Upson. 1994. Diseases of Swine 7th ed. Ames, IA: Iowa State Univ. Press; 1992. *Therapeutics* pp. 837-846.

Hinton, M., D. J. Hampson, E. Hampson, and A. H. Linton. 1985. A comparison of the ecology of *Escherichia coli* in the intestine of healthy unweaned pigs and pigs after weaning. J. Appl. Bact. 58:471

Hinton, M., P. D. Rixon, V. Allen, and A. H. Linton. 1984. The persistence of drug resistant *Escherichia coli* strains in the majority faecal flora of calves. J. Hyg. Camb. 93:547.

Hinton M. and A. H. Linton. 1987. The ecology of *Escherichia coli* in healthy newborn piglets. Br. Vet J. 143:541.

Hirsh, D. C., G. C. Burton, D. C. Blenden, and R. Tsutakawa. 1974. The effect of tetracycline upon establishment of *Escherichia coli* origin in the enteric tract of man. J. Appl. Bact. 37:327.

Howe, K. and A. H. Linton. 1976. The distribution of O-antigen types of *Escherichia coli* in normal calves, compared with man, and their R plasmid carriage. J. Appl. Bact. 40:317.

Jukes, T. H. 1971. The present status and background of antibiotics in the feeding of domestic animals. Ann. N.Y. Acad. Sci. 182:362.

Jukes, T. H., E. L. R. Stokstad, R. R. Taylor, T. J. Cunha, H. M. Edwards, and G. B. Meadows. 1950. Growth promoting effect of aureomycin on pigs. Arch. Biochem. 26:324.

Katouli, M., A. Lund, P. Wallgren, I. Kuhn, O. Soderlind, and R. Mollby . 1995. Phenotypic characterization of intestinal *Escherichia coli* of pigs during suckling, postweaning, and fattening periods. Appl. Environ. Microbiol. 61:778.

Kenworthy, R. and W. E. Crabb. 1963. The intestinal flora of young pigs, with reference to early weaning, *Escherichia coli* and scours. J. Comp. Path. 73:215.

Kiser, J. S. 1976. A perspective on the use of antibiotics in animal feeds. J. Anim. Sci. 42:1058.

Kunin, C. M. 1993. Resistance to antimicrobial drugs-a worldwide calamity. Ann. Intern. Med. 118:557.

Langlois, B. E., K. A. Dawson, I. Leak, and D. K. Aaron. 1988. Antimicrobial resistance of fecal coliforms from pigs in a herd not exposed to antimicrobial agents for 126 months. Vet. Microbiol. 18:147.

Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly. 1986. Antibiotic resistance in pigs following a 13 year ban. J. Anim. Sci. 62:18.

Langlois, B. E., K. A. Dawson, T. S. Stahly, and G. L. Cromwell. 1984. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J. Anim. Sci. 58:666.

Langlois, B. E., G. L. Cromwell, T. S. Stahly, K. A. Dawson and V. W. Hays. 1983. Antibiotic resistance of fecal coliforms after long-term withdrawal of therapeutic and subtherapeutic antibiotic use in a swine herd. Appl. Environ. Microbiol. 46:1433.

Langlois, B. E., G. L. Cromwell and V. W. Hays. 1978[a]. Influence of chlortetracycline in swine feed on reproductive performance and on incidence and persistence of antibiotic resistant bacteria. J. Anim. Sci. 46:1369.

Langlois, B. E., G. L. Cromwell and V. W. Hays. 1978[b]. Influence of type of antibiotic and length of antibiotic feeding period on performance and persistence of antibiotic resistant enteric bacteria in growing-finishing swine. J. Anim. Sci. 46:1383.

Larsen, H. E. and J. L. Larsen. 1972. Occurrence and distribution of drug-resistant *Escherichia coli* in feces from cattle and swine. Nord. Vet. Med. 24:651.

Linton, A. H., B. Handley, and A. D. Osborne. 1978. Fluctuations in *Escherichia coli* O- serotypes in pigs throughout life in the presence and absence of antibiotic treatment. J. Appl. Bact. 44:285.

Linton, K. B., P. A. Lee, M. H. Richmond, and W. A. Gillespie. 1972. Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community. J. Hyg. Camb. 70:99.

Mancini, C. and R. J. Behme. 1977. Transfer of multiple antibiotic resistance from bacteroides fragilis to *Escherichia coli*. J. Infect. Dis. 136:597.

McAllister, J. S., H. J. Kurtz, and E. C. Short. 1979. Changes in the intestinal flora of young piglets with post-weaning diarrhoea or edema disease. J. Anim. Sci. 49:868.

Moon, H. W., E. M. Kohler, R. A. Schneider. 1980. Prevalence of pilus antigens, enterotoxin type and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. Infect. Immun. 27:222.

Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Giannella. 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infect. Immun. 41:1340.

Moxley, R. A. and D. H. Francis. 1986. Natural and experimental infection with an attaching and effacing strain of *Escherichia coli* in calves. Infect. Immun. 53:339.

Nagy, B., T. A. Casey, and H. W. Moon. 1990. Phenotypes and genotypes of *Escherichia coli* isolated from pigs with postweaning diarrhea in Hungary. J. Clin. Microbiol. 28:651.

Nataro, J. P., I. C. A. Scaletsky, J. B. Kaper, M. M. Levine, and L. R. Trabulsi. 1985. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect. Immun. 48:378.

National Committee for Clinical Laboratory Standards. 1976. Performance standards for antimicrobic disk susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.

Okerman, L. 1987. Enteric infections caused by non-enterotoxigenic *Escherichia coli* in animals: Occurrence and pathogenicity mechanisms. a review. Vet. Microbiol. 14:33.

Rockfeller University Workshop. 1994. Special report. Multiple-antibiotic resistant pathogenic bacteria. N. Engl. J. Med. 330:1247.

Sarmiento, J. I., T. A. Casey, and H. W. Moon. 1988. Postweaning diarrhea in swine: Experimental model of enterotoxigenic *Escherichia coli* infection. Am. J. Vet. Res. 49:1154.

Sarmiento, J. I. and H. W. Moon. 1988. Postweaning diarrhea in swine: Effects of oxytetracycline on enterotoxigenic *Escherichia coli* infection. Am. J. Vet. Res. 49:1160.

Schaedler, R. W. 1973. The relationship between the host and its intestinal microflora. Proc. Nutr. Soc. 32:41.

Shales, D., S. Levy, and G. Archer. 1991. Antimicrobial resistance: New directions. Am. Soc. Microbiol. 57:455.

Shaw, W. V. 1967. The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant *Escherichia coli*. J. Biol. Chem. 242:687.

Siegel, D., W. G. Huber, and F. Enloe. 1974. Continuous non-therapeutic use of antibacterial drugs in feed and drug resistance of the gram-negative enteric florae of food-producing animals. Antimicrob. Agents Chemo. 6:697.

Solomons, I. A. 1978. Antibiotics in animal feeds-human and animal safety issues. J. Anim. Sci. 46:1360.

Smith, H. W. 1961. The development of the bacterial flora of the faeces of animals and man: The changes that occur during ageing. J. Appl. Bact. 24:235.

Smith, H. W. 1965. The development of the flora of the alimentary tract in young animals. J. Path. Bact. 90:495.

Smith, H. W. 1966. The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals. J. Hyg., Camb. 64:465.

Smith, H. W. 1967. The effect of the use of antibacterial drugs, particularly as food additives on the emergence of drug-resistant strains of bacteria in animals. New Zealand Vet. J. 15:153.

Smith H. W. 1968. Anti-microbial drugs in animal feeds. Nature 218:728.

Smith, H. W. and W. E. Crabb. 1961. The faecal bacterial flora of the faeces of animals and man: Its development in the young. J. Path. Bact. 82:53.

Smith, H. 1971. The effect of the use of antibacterial drugs on the emergence of drug-resistant bacteria in animals. Adv. Vet. Sci. Comp. Med. 15:67.

Smith, H. W. and J. E. T. Jones. 1963. Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. J. Path. Bact. 86:387.

Sogard, H. 1973. Incidence of drug resistance and transmissible R factors in strains of *E. coli* from faeces of healthy pigs. Acta Vet. Scand. 14:381.

Staley, T. E, E. W. Jones, and L. D. Corley. 1969. Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. Am. J. Pathol. 56:371.

Taylor, J. H. 1957. The mode of action of antibiotics in promoting animal growth. Vet. Rec. 69:287.

Tomlinson, J. R. 1969. Post-weaning enteritis and dysentery. Vet. Rec. 85:298.

Tomlinson, J. R. and T. L. J. Lawrence. 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs. Some field observations. Vet. Rec. 109:120.

Unowsky, J. and M. Rachmeler. 1966. Mechanisms of antibiotic resistance determined by resistance-transfer factors. J. Bacteriol. 92:358.

Visek, W. J. 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447.

Wierup, M. 1975. Antibiotic resistance and transferable antibiotic resistance of *Escherichia coli* isolated from Swedish calves 5 and 30 days old. Nord. Vet. Med. 27:77.

Wilson, R. A. and D. H. Francis. 1986. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res. 47:213.

# VITA

William Gregory Upchurch was born December 8, 1963, is the son of Kay and Lacy Upchurch. He received his elementary and secondary education in Crossville, Tennessee, graduating from Cumberland County High School in May of 1983. He then entered the University of Tennessee, Knoxville majoring in Animal Science. Greg graduated from the University of Tennessee with a B.S. in Animal Science December 1987. Upon graduation he married Cindy Turner and resided in Crossville. After managing Roanoke Farms in Fentress county Tennessee and serving with the University of Tennessee Extension Service in Putnam county, he returned to the University of Tennessee, Knoxville in August 1993 to begin graduate work. He received his Master of Science with a major in Animal Science December 1995.

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