# PATTERNS OF ANTIMICROBIAL RESISTANCE AMONG ENTERIC BACTERIA FOUND IN MULTI-SITE GROUP-LEVEL COHORTS OF HUMANS AND SWINE

A Thesis

by

LINDA DIANE CAMPBELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2004

Major Subject: Epidemiology

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## ABSTRACT

Patterns of Antimicrobial Resistance among Enteric Bacteria Found in Multi-Site Group-Level Cohorts of Humans and Swine. (December 2004)

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The prevalence of antimicrobial resistance (AMR) phenotypes and genotypic characteristics (Class 1 integron and AMR gene cassettes) in commensal *Escherichia coli* (EC) and vancomycin resistant *Enterococcus faecium* (EF) isolated from humans and swine in a semi-closed, integrated farrow-to-fork population were evaluated in a cross-sectional study. Our objective was to establish baseline antimicrobial resistance patterns and to evaluate the stability of isolate recovery phenotype within multiple grab samples per collection day and over multiple biweekly samples collected during a period of several months. This data will serve as a baseline for continuing longitudinal studies within the population. These continuing studies should produce the first comprehensive epidemiological data to document the transmission dynamics of antimicrobial resistance in the farrow-to-fork continuum. Outcome variables assessed included: phenotypic resistance in EC, pan-susceptibility, multi-resistance and genotypic resistance. Potential predictor variables included: 1) host species, 2) unit, 3) unit type, 4) housing cohort by species, and 5) time of day. There were significant differences ($p<0.05$) between host species with swine at higher odds for both single and multiple resistance. There were also differences in resistance based on unit location, unit-type, and housing cohort within both humans and

swine. Our study found no significant differences ($p>0.05$) in resistance between swine workers and non-swine workers with the sole exception of resistance to cephalothin, with non-swine workers at 1.89 higher odds for resistance ($p=0.02$). A total of 17 VRE were isolated from human wastewater samples, and to the author's knowledge these represent the first environmentally isolated VRE in the U.S. Several unique multi-resistance phenotypes were observed and future evaluation of AMR phenotype in continuing longitudinal studies provides a unique opportunity to study phenotypic patterns and dissemination through the study population.

## DEDICATION

I would like to dedicate this thesis to my fiancé, Wesley. He has been a constant source of support, has inspired me to do my best, and has given me the encouragement I needed to accomplish my goals. I would also like to extend this dedication to the rest of my family and friends, especially my mother, Ellen Campbell. It is in no small part due to the support of these amazing people that I have been able to accomplish so many of my goals.

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First, I would like to thank my advisor, Dr. H. Morgan Scott, for his vision and determination, which brought this project into reality. He has challenged me throughout the duration of my studies, and as a result, I have a much broader understanding of the concepts of statistics and epidemiology and the form and structure that scientific literature should take. I would like to thank Dr. Roger Harvey for his help throughout the course of this project, for his insight and guidance and for his excellent editorial skills. I would also like to thank Dr. Kenneth Bischoff for his instruction in the lab and for his feedback and suggestions that were very helpful throughout my studies, even though he was far away.

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Finally, I would like to extend thanks to my fellow graduate students: Jonathan Hudson, for giving encouragement and advice; Summer Strickland, for her support and friendship; and both Ashley Loven and Summer Strickland, for doing everything first, so I would have an example to follow.

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# INTRODUCTION

The accidental discovery of penicillin in 1928 proved to be one of the greatest therapeutic advances of the century. Many believed that this discovery signaled the beginning of the end of infectious diseases caused by bacteria; instead, more than 70 years later antimicrobial resistance (AMR) in pathogenic bacteria is considered one of the greatest global threats to public health. Almost as rapidly as antimicrobials have been developed, marketed and prescribed, resistant bacteria have emerged. Widespread use of antimicrobials in the 1940's, while effective in treating many infectious diseases, was mirrored by the development and spread of AMR among bacteria. By the end of the 1960's transferable AMR had been detected and characterized (Witte, 2000). The use of antimicrobials provides selective pressure for, and in turn propagates resistant bacteria. In the presence of an antimicrobial, the resistant bacterium has a selective advantage and its numbers are amplified, while susceptible bacteria are inhibited or killed (Collier et al., 1998). Antimicrobial-resistant bacteria are now recognized to be both geographically widespread (worldwide) and of great public health importance. AMR leads to increasing difficulty in treating infections in human patients and propagates transmission of resistant bacteria within the hospital setting. Resistant bacteria in food-producing animals has lead to much debate and speculation about the role of transmission from animals to humans (van den Bogaard et al., 2001). Resistance to two

This thesis follows the format and style of Foodborne Pathogens and Disease.

or more classes of antimicrobials in microbial pathogens like *Escherichia coli* is now commonplace in isolates from both veterinary and human medicine (Bass et al., 1999).

While no one argues the widespread geography and importance of AMR, the debate over whether animal agriculture is to blame for increased resistance in humans has raged for over 30 years (Snary et al., 2004). The first large-scale commissioned report to assess the role of food-producing animals in AMR transmission was published in 1969. The Swann report – based on the United Kingdom experience – was the first to link an outbreak of salmonellosis in humans to consumption of beef in which antimicrobials had been used (Swann, 1969).

With the advent of molecular techniques in the 1980's and 1990's a variety of 'fingerprinting' methods have been employed such as pulse field gel electrophoresis (PFGE), ribotyping, and various methods of polymerase chain reaction (PCR) to try to link resistant strains of bacteria isolated from human clinical specimens to animal agricultural sources. By identifying unique characteristics within the DNA, these methods attempt to relate the patterns observed both within and between species to establish potential relationships and/or similar characteristics. PFGE is particularly useful for bacterial sub-typing and is often considered the gold standard of molecular typing methods (Werner et al., 2003; van den Braak et al., 2000).

Many researchers (reviewed by Shea, 2003) and policy makers (Tollefson and Flynn, 2002) believe that the association between resistance traits found in animals and those found in humans provides sufficient evidence to implicate the use of antimicrobials in agriculture as a cause of resistance in humans and to justify policy

changes (e.g. discontinued use of growth promoters in animals) in antimicrobial regulation and use (Phillips et al., 2004). The United States Food and Drug Administration (U.S. FDA) is the government agency charged with the responsibility of evaluating all medicines allowed for human and veterinary use and its mission is to promote and protect public health. The U.S. FDA initially investigated the role of AMR in the United States as early as 1977, but was widely criticized for recommending actions to withdraw penicillin and tetracyclines from animal feed without adequate epidemiological evidence (Tollefson and Flynn, 2002). This struggle between the demand for more epidemiological evidence of transmission of AMR from animals to humans before taking action and the immediate need to protect human health in the absence of unequivocal 'proof' (i.e., the 'precautionary principle') has remained at the heart of the heated debate for over three decades.

Proponents of a scientific risk assessment framework argue that agencies such as the U.S. FDA and U.S. Centers for Disease Control and Prevention (CDC) have not adhered to the definition of hazard identification, which is the first step in quantitative risk assessment. At the U.S. FDA meeting on Draft Guidance held in 2002, Dr. Gregg Clayclamp, Director of U.S. FDA's Center for Veterinary Medicine (CVM) Scientific Support & Generic Animal Drug Staff, described hazard identification as "the process of determining whether exposure to an agent can cause an increase in the incidence of a health condition (U.S. FDA, 2002)." "Hazard identification is then a statement of possibility rather than probability" (Dean and Scott, 2004). Merely identifying AMR as a "hazard" is not sufficient for scientists who feel that the actual characterization of that

risk has not been amply demonstrated. Dr. Lyle Vogel of the American Veterinary Medical Association states, "[we] believe that the agency (U.S. FDA) has demonstrated that a hazard exists, however, the agency has not adequately characterized the risk to humans" (U.S. FDA, 1999).

Even within government organizations such as the U.S. Department of Health and Human Services (DHHS), which includes the U.S. FDA and CDC, and the U.S. Department of Agriculture (USDA), there has been disagreement over the appropriate regulatory responses to AMR. According to the 1999 report entitled, *Food Safety: The Agricultural Use of Antibiotics and Its Implications for Human Health,* the USDA is not convinced enough knowledge is available to make substantive regulatory changes, and that further research is warranted, whereas the DHHS feels there is sufficient evidence to justify regulation (GAO, 1999).

In order to address both the disagreement between governmental agencies and the need for quantitative data regarding AMR, the U.S. FDA, CDC, and USDA established the National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS) in 1996. The role of NARMS is to prospectively monitor changes in antimicrobial susceptibilities of zoonotic enteric pathogens from human and animal clinical specimens, carcasses of food-producing animals at slaughter, and from retail food (Tollefson and Flynn, 2002). Typically, NARMS surveillance data are derived from human clinical enteric pathogen isolates whereas the animal isolates include both clinical and non-clinical sources, therefore, any comparisons and inferences remain difficult.

Although the NARMS collaboration has been a step forward in AMR research, there is still wide disagreement even among the NARMS collaborators. NARMS collaborators charged with protecting public health generally believe that adequate research exists to warrant regulatory changes, while those charged with protecting animal health generally argue that further research is warranted. Whereas the NARMS program is an important first step, there is still a need for expansion of research activities to include improved sampling along the entire farm-to-fork continuum (Isaacson and Torrence, 2002).

**Objectives**

To address this need, the research described hereafter was designed to provide initial phenotypic and genotypic characterization of the potential transmission dynamics of AMR in a uniquely integrated population of animals and humans with minimal outside influence occuring due to in- and out-migration and disparate food sourcing. The major objective of this study was to establish baseline antimicrobial resistance patterns of enteric bacteria from animals and humans within the study population; specifically, phenotypic and genotypic traits which are both unique and common to commensal *Escherichia coli* (EC) and *Enterococcus faecium* (EF) derived from the different sources. A secondary objective was to evaluate the stability of bacterial isolate phenotype within multiple grab samples per collection day and over multiple biweekly samples collected during a period of several months. We propose that continuing longitudinal studies in this population should produce the first comprehensive

epidemiological data that will document the transmission dynamics of antimicrobial resistance in the farrow-to-fork continuum.

# LITERATURE REVIEW

## Overview of Antimicrobial Resistance

An antimicrobial is defined as a drug, chemical or substance which kills or inhibits the growth of a microbe (CDC, 2004). There are several classes of antimicrobials which are used to treat infectious diseases caused by bacteria. These include the following: aminoglycosides, β-lactams, glycopeptides, fluoroquinolones, macrolides, streptogramins, sulphonamides, and tetracyclines. Amingolycosides target the 30S subunit of the bacterial ribosome and interferes with protein synthesis. β-lactams target enzymes required by the bacteria for synthesis of peptidoglycan and interfere with synthesis of the cell wall. Glycopeptides interfere with the synthesis of the bacterial cell wall by targeting the D-alanyl-D-alanine terminal dipeptide and preventing the reaction used to link peptidoglycan precursors. Fluoroquinolones inhibit synthesis of DNA gyrase, which is responsible for negative supercoiling of double-stranded DNA which balances the positive supercoiling of DNA replication. Without negative supercoiling irreparable breakages occur in the DNA strand. Macrolides target the 50S subunit of the bacterial ribosome and block the exit of the peptide chain. Streptogramins are related to macrolides and also target the 50S subunit and inhibit protein synthesis. Sulphonamides target the para-aminobenzoic acid molecule which is used to synthesize folic acid by the bacteria and blocks the synthesis of folic acid. Tetracyclines target the 30S subunit of the bacterial ribosome and inhibit protein synthesis by preventing transfer of amino acids to the ribosome. Some of the more commonly used antimicrobials within the aminoglycocide class include: kanamycin, streptomycin, gentamicin, spectinomycin,

neomycin, and amikacin (Davies and Wright, 1997). Macrolides include tylosin, erythromycin, clarithromycin, and azithromycin (Martel et al., 2002). Lincosamides include lincomycin and clindamycin (Martel et al., 2002). β-lactams encompass the penicillins and cephalosporins such as ceftiofur and cephtriaxone. The sulphonamide class contains sulphamethoxazole and potentiated sulphonamides such as trimethoprim (of the diaminopyrimidine drug class) and sulphamethoxazole. Streptogramins include quinupristin/dalfopristin (Synercid®) and virginiamycin (Klare et al., 2003). Drugs within the tetracycline class include chlortetracycline, oxytetracycline, tetracycline, limecycline, and doxycycline (Chopra and Roberts, 2001). Fluoroquinolones include ciprofloxacin, ofloxacin, norfloxacin, lomefloxacin, levofloxacin, enoxacin, and sparfloxacin (Bakken, 2004).

Antimicrobials are intensively used in human medicine, veterinary medicine and animal agriculture (Bailar III and Travers, 2002; Catry et al., 2003). Antimicrobial usage is considered the most important factor promoting the emergence, selection, and dissemination of resistant organisms in both veterinary and human medicine (van den Bogaard et al., 2001). As a result of widespread use of antimicrobials and subsequent dissemination of resistant bacteria, AMR has been documented worldwide in both human and veterinary settings.

In order to further investigate the complex subject matter of AMR and risks for transmission, relevant subject matter will be presented concerning: 1) the role and use of antimicrobials in human medicine and animal agriculture, 2) the acquisition of resistance within bacteria, 3) the role of resistant commensal EC and EF, 4) phenotypic expression

of resistance, 5) genotypic traits in resistance, and 6) integron-mediated resistance. The body of evidence concerning the risk of transmission of resistant bacteria from animals to humans will be critically reviewed.

**Use of Antimicrobials in Human Medicine**

Antimicrobial usage in human healthcare has been widespread since the discovery of antimicrobial agents in the 1940's. Approximately 22.5 kilograms of antimicrobials are produced in the United States each year with human use accounting for half of the yearly consumption by mass (Levy, 1998). An estimated 80-90% of antimicrobials used in human healthcare are given in the outpatient setting (Cizman, 2003). While only 10% of all antimicrobials are used in the hospital setting, approximately 2 million individuals admitted to acute care hospitals in the United States are subsequently diagnosed with nosocomial (hospital-acquired) infections that are increasingly resistant to antimicrobial agents (Diekema et al., 2004).

Increasing resistance to antimicrobials is of growing concern, especially with recent multi-resistant strains which can be difficult to treat (Shea, 2003). Multi-resistant *Staphylococcus auereus* and vancomycin-resistant EF are increasingly a problem in the hospital setting, often leading to therapy failure and increased morbidity and mortality (Catry et al., 2003). The economic impact of treating resistant infections was estimated by the Institute of Medicine (1998) to be $4 to $5 billion annually.

Historically, physicians that practice in clinical settings have focused on the health of the individual rather than the population at large. This focus has perhaps led to over-use and misuse of antimicrobials. An estimated 20 to 50% of all antimicrobial

usage may be questionable (Cizman, 2003). Due to concerns regarding the judicious use of antimicrobials in the healthcare setting, the CDC unveiled a campaign targeted at the reduction of AMR (CDC, 2002).

Many of the antimicrobials used in human medicine belong to the same class as those commonly used in animal agriculture. Worldwide, the β-lactams are among the most widespread antimicrobials used, but fluoroquinolones, sulphonamides, macrolides, tetracyclines, aminoglycosides, and glycopeptides are also widely administered (Phillips et al., 2004). The use of similar antimicrobials in human medicine and animal agriculture has led to widespread concern over the potential transmission of resistant bacteria from animals to humans.

**Use of Antimicrobials in Animal Agriculture**

Antimicrobials are used in animal agriculture to prevent and treat illness as well as to promote growth. In many food production systems it is often more efficient to treat the entire group of animals via feed or water rather than each animal individually. As a result, large quantities of antimicrobials may be administered; however, direct data on the exact amount of antimicrobials used in specific animal production settings are often not available to the public. This has resulted in difficulties in determining both the types and amounts of antimicrobials used in food animal production. According to the World Health Organization (WHO), the total amount of antimicrobials used in food animals is not precisely known, although it is estimated that about half the total mass of the antimicrobials produced globally are used in farming, particularly in pig and poultry production (Stohr, 2000). Estimates of antimicrobial usage in the United States in food

animals for both therapeutic and non-therapeutic uses range from 8.2 million to 13.2 million kilograms per year (Isaacson and Torrence, 2002). Approximately 90% of the aforementioned amount is used in animal agriculture for growth promotion and prophylaxis rather than to treat infection (Khachatourians, 1998).

Several classes of antimicrobials used in animal agriculture are closely related to those used in human medicine: β–lactams (penicillins and cephalosporins), sulphonamides both with/without trimethoprim, tetracyclines, macrolides, lincosamides, streptogramins, and quinolones (Phillips et al., 2004). Most antimicrobials in swine production are used in feed at relatively low concentrations for growth promotion or disease prophylaxis (McEwen and Fedorka-Cray, 2002). Most swine are treated after weaning when they are most vulnerable to infectious disease (McEwen and Fedorka-Cray, 2002). A minimum of 17 classes of antimicrobial agents are approved for growth promotion and feed efficiency in the United States (Anderson et al., 2003). Of these, the most commonly used in swine production are tetracyclines, macrolides (tylosin), and sulfa compounds such as sulphamethazine (McEwen and Fedorka-Cray, 2002). The previously mentioned classes of antimicrobials present a concern for potential transmission risk of resistant bacteria from food animals to humans.

**Acquisition of AMR in Bacteria**

There are two forms of AMR in bacteria: intrinsic and acquired. Intrinsic or natural resistance is widespread in bacteria and results from evolutionary adaptations of bacteria to the environment. The inability of the antimicrobial to penetrate a bacterial cell and the lack of a target for the antimicrobial agent to act against are all natural

bacterial adaptations which result in AMR. Acquired resistance may be the result of either a single or multiple step mutation, or may result from the lateral transfer of resistance traits between bacteria of the same species, of a different species, or acquired from the environment by a process known as transformation (McManus, 1997). Acquired resistance in bacteria is usually the result of acquisition of mobile extrachromosomal DNA elements such as plasmids, transposons and integrons rather than mutation (Heinemann, 1999).

A plasmid is a circular body of double stranded DNA which is separate from the chromosome and carries genes that encode various traits such as virulence and AMR (Kaye et al., 2000). There are two types of plasmids: conjugative and non-conjugative. Conjugative plasmids transfer resistance via their sex pilli whereas non-conjugative plasmids must have direct contact for transfer to occur. In non-conjugative transfer, both the donor bacteria and the recipient bacteria have a copy of the transferred plasmid. Conjugative transfer is an important mechanism in AMR because transfer can occur in a wide range of bacterial species and can be spread to unrelated organisms. Moreover, a single plasmid can contain multiple genes conferring resistance to multiple classes of antimicrobials (Akkina and Johnson, 1999).

Bacteria can also acquire DNA via transduction and transformation. Transduction occurs when DNA is transferred via bacteriophages, which are viruses that attack bacteria. Bacteriophages are very tightly packaged and do not have much room to carry extra DNA. They also have a very narrow host range, and as a result transduction is a less important mechanism for resistance gene transfer (Akkina and Johnson, 1999).

Transformation occurs when bacteria pick up free DNA from the environment. While the presence of DNA is common after cell lysis, the compatibility between the free DNA and the intact recipient is narrow (McManus, 1997). Additionally, free DNA in the environment would be highly susceptible to digestion by nuclease. As a result, transduction and transformation are not thought to contribute significantly to the dissemination of AMR (McManus, 1997).

A transposon is a genetic element that contains an insertion sequence at each end. The insertion sequences allow the gene to jump to different locations on the chromosomal DNA, from plasmid to plasmid or from chromosome to plasmid (McManus, 1997). Movement of a transposon is known as transposition and represents an important facet of AMR transfer because resistance genes can be moved from a non-conjugative plasmid or chromosome to a conjugative plasmid, and hence easily transferred to other bacteria (Akkina and Johnson, 1999).

Integrons are genetic units that include the determinants of the components of a site-specific recombination system capable of capturing and mobilizing genes that are contained in mobile elements called gene cassettes (Hall and Collis, 1995). The essential components of an integron are an *Int* gene which encodes a site-specific recombinase belonging to the integrase family, an adjacent site *attl*, which is recognized by the integrase and is the receptor site for gene cassettes, and a promoter suitably oriented for the expression of the cassette-encoded genes (Hall and Collis, 1995). The integron is composed of a 5' conserved sequence (CS) (the integrase gene) and a 3' CS, which can vary for the four different classes of integrons; classes 1 through 4 (Roe et al.,

2003a). The majority of integrons described to date are Class 1 integrons and the majority of those are associated with *su1I,* a gene commonly found within the 3' conserved sequence (Figure 1) (Fluit and Schmitz, 1999; Naas et al., 2001). AMR gene cassettes are not always present in integrons, and the integrase gene (*Int*) can excise gene cassettes as covalently closed supercoiled circular molecules. Integron gene cassettes can be deleted, rearranged and duplicated within the integron (Fluit and Schmitz, 1999).

Integron mediated AMR is a major mechanism for transfer of resistance traits within both Gram-negative and Gram-positive bacteria (Leverstein-van Hall et al., 2002; O'Brien, 2002; Roe et al., 2003b; Mathai et al., 2004). Integrons have been found to harbor the majority of resistance genes within the mobile resistance elements (transposons and plasmids), which allow for transfer of resistance and multi-resistance between bacteria (Levesque et al., 1995; Hall and Collis, 1998; Leverstein-van Hall, 2002; Roe et al., 2003a). Within the integron, more than 60 gene cassettes have been found that confer antimicrobial resistance to a variety of agents (White et al., 2001). Of these, the most prevalent genes are those coding for aminoglycoside and trimethoprim resistance (Fluit and Schmitz, 1999; White et al., 2001). Integrons also represent an important mechanism for transfer of resistance characteristics from commensal to pathogenic organisms and have been found to harbor multiple resistance genes at one time (Goldstein et al., 2001; Maguire et al., 2001; Zhao et al., 2001).

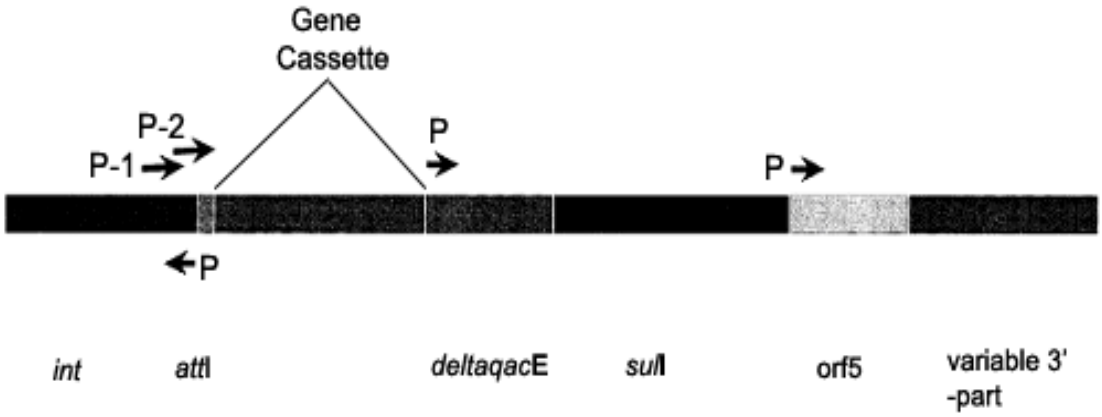


**Figure 1. Schematic of a Class 1 integron*.**

*Figure adapted from Fluit and Schmitz, 1999. 5' conserved sequence includes the *int* and *attI* regions of the Class I integron
*int* stands for the integrase gene, which excises gene cassettes as covalently closed supercoiled molecules and is believed to control gene integration as well
*attI* = adjacent recombination site
P1 and P2 are promoters which are responsible for expression of the integron
Gene cassette indicates the position of placement for the strongest level of resistance within the integron. Gene cassettes are not always part of an integron.
3' conserved sequence includes the *deltaqacE, sul*I and orf5
*deltaqacE is a gene cassette which carries a promoter region*
*sulI* is described in the text

## The Role of Commensal *Escherichia coli* in AMR

Commensal bacteria, such as *Escherichia coli* (EC) and *Enterococcus faecium* (EF), which are naturally occurring host enteric flora, constitute an enormous potential reservoir of resistance genes for pathogenic bacteria (Bartoli et al., 2004; Turnidge, 2004). The prevalence of resistance in the commensal bacteria of humans and animals may reflect the selective pressure of antimicrobial usage and is a potential indicator of resistance in future infections (Anderson et al., 2003). EC has served as an indicator of AMR for Gram-negative enteric bacteria and the emergence of strains showing multi-

resistance is a public health concern (Guerra et al., 2003). EC and EF are used internationally as indicator bacteria for AMR because of their high prevalence in animal feces, and because they harbor several resistance determinants (Witte, 2000; Catry et al., 2003).

EC is a member of the family Enterobacteriaceae. Members of this family of bacteria are part of the normal intestinal flora of animals and humans. EC is a Gram-negative, oxidase-negative, non-spore forming, non-acid fast straight rod (0.3-1.0 x 1.0-6.0μm). EC is able to grow on simple nitrogen and carbon compounds and grows well in aerobic and anaerobic environments. It has been shown to be prolific in the environment as a result of fecal contamination and can survive for weeks in optimal conditions in the environment (Brenner, 1984). EC is typically found in the lower intestinal tract (ileum and large intestine) and is an important commensal of both animals and humans. There has historically been disagreement among scientists over the ability of animal strains of EC to colonize humans (Phillips et al., 2004). Recent research has indicated that transfer may be limited due to reduced ability of animal strains to colonize the human alimentary tract (reviewed by van den Bogaard et al., 2001). Studies employing molecular methodologies have found strain differences in human and animal isolates even when the two species are in close contact (Kariuki et al., 1997; Kariuki et al., 1999; van den Bogaard et al., 2001). The abundance of EC implicates them as likely candidates for the spread of resistance genes and vectors between the bacterial populations of animals and humans; however, their abundance also makes such spread difficult to trace (O'Brien, 2002). Within the Gram-negative bacteria, a substantial

portion of the resistance genes present on plasmids and transposons are incorporated into Class 1 integrons (Winokur et al., 2001; Leverstein-van Hall et al., 2002).

**The Role of *Enterococcus faecium* in Resistance Transmission**

EF is a species of the genera *Enterococcus*, which are Gram-positive cocci (~1μm diameter). EF are facultatively anaerobic, occur singly, in pairs, or short chains, and show no hemolysis on blood agar after 24 hours (may see alpha hemolysis after 48 h). EF is a normal inhabitant of the intestinal tract of animals and humans as well as the genital tract of humans. EF has been show to be extremely hardy and can survive on environmental surfaces for several weeks at a time (Brenner, 1984).

EF, specifically vancomycin resistant EF (VRE) are a major concern for public health and are a source of heated debate regarding transmission dynamics between animals and humans. VRE first appeared in humans in the United States in 1998 (Martinez et al., 2003). There is no known reservoir of VRE in the United States other than colonized patients in the hospital setting and transmission represents horizontal spread from patient to patient usually in the hospital setting (Martinez et al., 2003). In the United States, VRE are the third most prevalent source of nosocomial bacteremia, and nearly 25% of *Enterococci* isolated from nosocomial infections (hospital setting) are resistant to vancomycin (Houghton, 2002). The *vanB* gene is the most prevalent resistance trait genotype found among *Enterococci* in the United States and induces varying levels of resistance to vancomycin (Low et al., 2001).

In Europe, food-producing animals are the likely reservoir of one type of VRE; namely, EF strains with the *vanA* AMR gene (Salisbury et al., 2002). It is believed that

is a result of the use of the growth promoter avoparcin in food animals in Europe (Salisbury et al., 2002). *VanA* resistance is typically plasmid borne with genes carried within a conjugative transposon and confers high level resistance to vancomycin (Low et al., 2001). The lack of a reservoir of VRE in the United States as compared to Europe has led to much speculation about the dynamics of VRE transmission between animals and humans.

**Phenotypic Expression of AMR**

More than $10^{21}$ diverse types of bacteria live and compete with each other on and in people, animals, and within the environment (O'Brien, 2002). These organisms interact with their environment and respond to stress by modifying their physiological status. When faced with a stressor, some organisms readily adapt while others die off. In the case of exposure to antimicrobials, those organisms which are able to survive may become resistant to antimicrobials and this resistance can then be propagated within the general bacterial population. Further, resistance can be inherited and can persist for generations after the initial exposure (Heinemann, 1999). Phenotypic susceptibility patterns of indicator bacteria such as EC and EF from healthy animals are suggested to be good predictors of resistance in the bacterial population at large (Catry et al., 2003). Minimum inhibitory concentration (MIC), or breakpoint susceptibility testing, is an *in vitro* diagnostic procedure for antimicrobial susceptibility testing of Gram-positive and Gram-negative bacteria, and has been a widely used method since its inception in the early 1980's (Larkin et al., 2004). By use of a 96-well plate dosed with selected

antimicrobials in a microbroth dilution series, investigators are able to assess the level at which a bacterium is able to resist treatment with a particular antimicrobial agent.

**Genetic Linkage of AMR**

Most AMR genetic traits occur in linked arrays rather than as a result of chance or mutation (Summers, 2002). When exposed to an antimicrobial agent, a bacterium with genetically linked resistance mechanisms may possibly become resistant to multiple antimicrobials, even to ones unrelated to the agent given (Barza, 2002; Summers, 2002). As an example, antimicrobial co-selection appears to play a role in vancomycin resistance. In Denmark, VRE resistant to macrolides and tetracyclines have been documented (Aarestrup, 2000). After the ban of avoparcin, VRE was expected to decline dramatically, however, the decline was not realized until the use of tylosin was halted. Glycopeptide resistance (GRE) remained around 20% in swine (Aarestrup, 2000). This finding suggests that co-selection may be the culprit as the resistance genes for both agents are located on the same conjugative plasmid (Aarestrup, 2000; Hasman and Aarestrup, 2002).

Exposure to other chemicals, such as heavy metals, can also select for AMR bacteria. Antimicrobial co-resistance has been recorded in the microbial population of ecosystems contaminated by metals in the absence of antimicrobial selection (Levin et al., 1997). Furthermore, the mechanisms of genetic linkage such as plasmids, transposons, and integrons are likely ancient in terms of their evolution and appear to significantly pre-date the use of antimicrobials by humans; this, in turn, may aid the transmission of resistance characteristics within the population (Summers, 2002). This

also may indicate the important role of co-selection and co-resistance in the ecology of AMR and transmission potential from animals to humans and vice-versa.

**Transmission of Resistant Bacteria from Animals to Humans**

In the era of modern medicine it is widely argued that use of antimicrobials in animal agriculture has led to increased antimicrobial resistance in humans. While this argument has potential merit, there is a distinct lack of empirical evidence with which to characterize the basis for potential transmission between animals and humans. Finding the "smoking gun" of antimicrobial resistance is important, but virtually impossible due to the widespread distribution of resistance genes, and because most drugs are used in multiple animal species as well as humans (Isaacson and Torrence, 2002).

Many researchers appear to have adopted a model of transmission from animals to humans which neglects the fundamental ecology of humans interacting with animals in complex production systems (Barber et al., 2003). Moreover, uni-directional models ignore other potential reservoir species as well as environmental interactions between potential hosts and the pathogen or commensal bacteria of interest (Halling-Sorenson et al., 1998; Meyer et al., 2000; Summers, 2002). Few studies have addressed the risk of antimicrobial use and the potential maintenance of resistance in swine populations (Dunlop et al., 1998a).

Further, evidence that antimicrobial resistant bacteria have been transmitted to humans is at best circumstantial, and based primarily on qualitative data from case reports (Khachatourians, 1998). Resistant bacteria have been shown to reach the environment via sewage (Harwood et al., 2001) and it should not be ignored or

overlooked that both food animals and humans may have acquired resistance from either environmental contamination or a common source exposure. Logically, humans contribute to the pool of resistant bacteria via human waste discharged into the environment (Harwood et al., 2001) and could possibly spread resistant bacteria directly to animals that come into contact with infected individuals (e.g., farm workers). Evidence exists that swine herds are 11 times more likely to have a high prevalence of *Salmonella* spp. when there is no toilet facility on the premises for human workers as opposed to when toilet facilities are provided (Funk et al., 2001).

AMR studies to date have included a multitude of case-control and cross-sectional designs. More than 100 case-control studies relating to AMR have been conducted with numerous conflicting results regarding which risk factors are important for AMR (Patterson, 2002). Some studies have indicated the importance of a particular risk factor, only to have subsequent studies find no significant relationship. This variation may be due to differences in study design, case definition, and control selection. A major limitation to the case-control approach for studying AMR is that the case-control study can only provide estimates of ratio measures of effect because it artificially inflates the disease/outcome prevalence in the source population. This flaw in the case-control study design is that only a portion of the population of interest is sampled based on disease status rather than following subjects based on exposure, which allows for calculation of "time at risk" (Rothman, 2002).

Numerous cross-sectional studies have focused on the prevalence characteristics of AMR in both humans and animals. Recent cross-sectional studies have focused on

the prevalence of AMR bacteria in swine, cattle, poultry, goat and human isolates collected over various periods of time (Dunlop et al., 1998b; White et al., 2002; Guerra et al., 2003; Lanz et al., 2003; Zhao et al., 2003; Larkin et al., 2004). All of these studies employed either phenotypic (e.g., MIC) or a combination of phenotypic and genotypic (PCR, PFGE) analyses to address the level of AMR in bacteria in their designated population of interest. Overall, these studies indicated that the level of AMR in host bacteria varies both within and between species and that genetic mechanisms such as the integron appear to play a significant role in the potential for transmission of AMR gene cassettes both within and between species (animal to human).

While these studies help to assess the level of AMR in many populations of interest and may serve as good proxies for longitudinal study designs (Rothman, 2002), they suffer from the inherent limitation of the cross-sectional study design. Because cross-sectional studies measure both exposure and outcome at the same time, causality can not be assessed. Indeed, in most of these studies direct exposure assessments of the humans to the animals or animal products were not even conducted. So, while these findings may be suggestive of a relationship between antimicrobial resistant bacteria in food animals and humans, they certainly do not provide evidence of a causal relationship. Moreover, all of these studies have explored AMR characteristics in an open population. Given the flow of animals, humans, food products, and the potential interactions between humans and animals in these populations it would be virtually impossible to make a quantitative statement of the risk of antimicrobial usage to human health as a result of transmission from animals to humans.

Studies such as the Hayes et al. (2003) assessment of resistance levels in retail meats attempt to bridge the gap between the AMR found in animals and humans by addressing the risk posed by consumption of animal products by human consumers. The authors purchased 981 packages of retail meat from 263 grocery stores during the study period to assess the prevalence of AMR bacteria, specifically *Enterococcus* species, in products destined for human consumption. They did not obtain any samples from human consumers or potential consumers of the products and as a result were not able to directly assess exposure due to consumption of animal products. This type of study also fails to address potential cross-contamination in the farrow-to-fork continuum such as transmission of resistance from human workers to food animals, contamination as a result of slaughter plant processing, retail packaging, transportation to retailers, and handling of the product in the retail setting. Humans have been found to transmit zoonotic pathogens to animals with which they have contact (Barber et al., 2003). Moreover, studies that have focused on food-related outbreaks may not accurately represent the "day-to-day" transmission which would theoretically account for most AMR in humans as a result of consumption of food animal products (Barber et al., 2003).

In order to implicate the use of antimicrobials in animal agriculture as the source of AMR in humans, treatment failures in human disease must include a characterization and accounting of the continuity of the bacterial resistance gene 'flow' from animals, through slaughter, food processing interventions, retail distribution (if applicable), food

preparation, human consumption, disease outbreak, antimicrobial treatment, and ultimately failure in the patient (Shryock, 1999).

Other studies have attempted to address the transmission dynamics of AMR by studying the resistance traits and characteristics in animal workers, their families, and their animals. Kariuki et al. (1999) employed genotypic methods to characterize the resistance traits of EC found in children and chickens living in close contact. While they found a high level of relatedness between the chicken isolates, there was no significant relationship between the patterns of resistance characteristics in children and chickens in linked households.

van de Bogaard et al. (2001) took this framework a step farther by assessing the resistance in EC of poultry, poultry workers, and poultry slaughterers in the Netherlands. The authors tested fecal samples from poultry, farm workers and slaughter-plant workers and correlated these data with antimicrobial usage history for a period of three months prior to the study. Using a combination of susceptibility testing and PFGE to determine the genetic relatedness of isolates, the authors found results that may be suggestive of spread of resistance from animals to humans. While this study incorporates historical data on antimicrobial usage, it suffers from the previously mentioned limitation of cross-sectional study designs (inability to address causality). In addition, the authors allowed the farmers to collect the poultry fecal samples as well as their own fecal sample. This could allow for contamination of one or both samples. The authors also received a fecal sample from a farmer with no broilers on his farm at the time of the study. This sample was noted, but the authors do not indicate if these results were used and this may

indicate a possible source of bias in the study. Overall, 27 different AMR patterns were found with only 5 showing similar traits between poultry and poultry workers, and only 3 of those were found in farmers and animals on the same farm (van de Bogaard et al., 2001). The authors do not indicate which farms these samples came from nor is there any analysis of farm factors that may play a role in these results (e.g., antimicrobial usage, housing and crowding of animals, animal handling practices). Further, the ability to extrapolate these findings to other populations is limited due to potential inability to generalize the results to the general population.

Although there is little doubt that cross-species (animal to human, human to animal) transmissions can occur, there are presently little or no quantitative longitudinal data available for reliable risk assessments (Isaacson and Torrence, 2001). There is a need for controlled epidemiological studies utilizing stable, integrated animal and human populations over time. Current research in AMR has largely been unable to achieve this goal due to lack of an integrated and closed study population, coupled with the difficulty of establishing a cause-and-effect relationship and assessing a true risk of transmission of AMR from animals to humans.

One of the greatest limitations in the study of AMR and transmission risk from animals to humans has been application of a suitable risk assessment framework. Both the Codex Alimentarius Commission (CAC) and the European Union Scientific Committee for Food have adopted a four-step risk assessment framework: hazard identification, exposure assessment, hazard characterization and risk characterization (Snary et al., 2004). In the United States, the Environmental Protection Agency (EPA)

and the U.S. FDA Center for Veterinary Medicine (CVM) have similar risk assessment methodologies: hazard identification, dose-response, exposure assessment, and risk characterization.

One of the problems with applying a risk assessment framework to AMR lies in the inability to move from qualitative to quantitative risk assessment. In order to quantify "risk", a researcher must be able to calculate a dose-response relationship. This requires valid longitudinal data, which to date are lacking in the epidemiological literature. In addition, the exposure assessment is likely to be difficult given the "open" status of the population. People may have a multitude of pathways of exposure each varying to some extent based on the geographic area, activities of the person or population, food sources and eating habits, and genetic characteristics of the individual and population. Characterization of the "risk" of transmission of AMR from animals to humans will continue to be difficult until the dose-response relationship and the exposure assessment/hazard characterization issues are addressed.

In conclusion, AMR is a complex ecological problem that requires quantitative data on both the characteristics (phenotypic and genotypic) and transmission dynamics within the farm-to-fork continuum before any definitive statement of risk of transmission from animals to humans can be definitively made. This research project and the continuing longitudinal project will for the first time attempt to address this need by working in a uniquely integrated population of humans and animals experiencing minimal outside influence via in- and out-migration and food sourcing.

# MATERIALS AND METHODS

## Study Population

During the study period, the study population was uniquely composed of groups of humans and swine in a semi-closed vertically integrated agri-food system. The population of both humans and swine were located across multiple sites in the state of Texas, with 13 of these sites housing both species. There was very limited movement of swine into the system and no movement of live animals or pork product out of the system. Almost all herd replacements were reared within the system, with purebred boars and gilts comprising the only outside seedstock purchased. Purchased boars were held in quarantine at a single, isolated swine unit. Swine moved vertically within the system from farrowing units to nurseries and finally to grower-finisher barns until slaughter. All swine raised on the farm units were slaughtered (at a single facility), processed, and consumed within the system (Figure 2).

In addition, all processing and transportation equipment was owned by the operation. Each swine unit relied on an operation-owned feed processing facility and cooking facility for human food waste. Properly cooked food waste was fed back into the swine operations at each site. In fiscal year 2000, the packing plant facility processed and shipped approximately 9.5 million pounds of pork products. These products fed a total human population of 180,000 individuals within the study system. The slaughter plant had 380 employees who worked 5 days per week (2 shifts/day) and the plant was inspected by the Texas Department of State Services. Approximately 50,000 hogs were slaughtered each year within the study system.

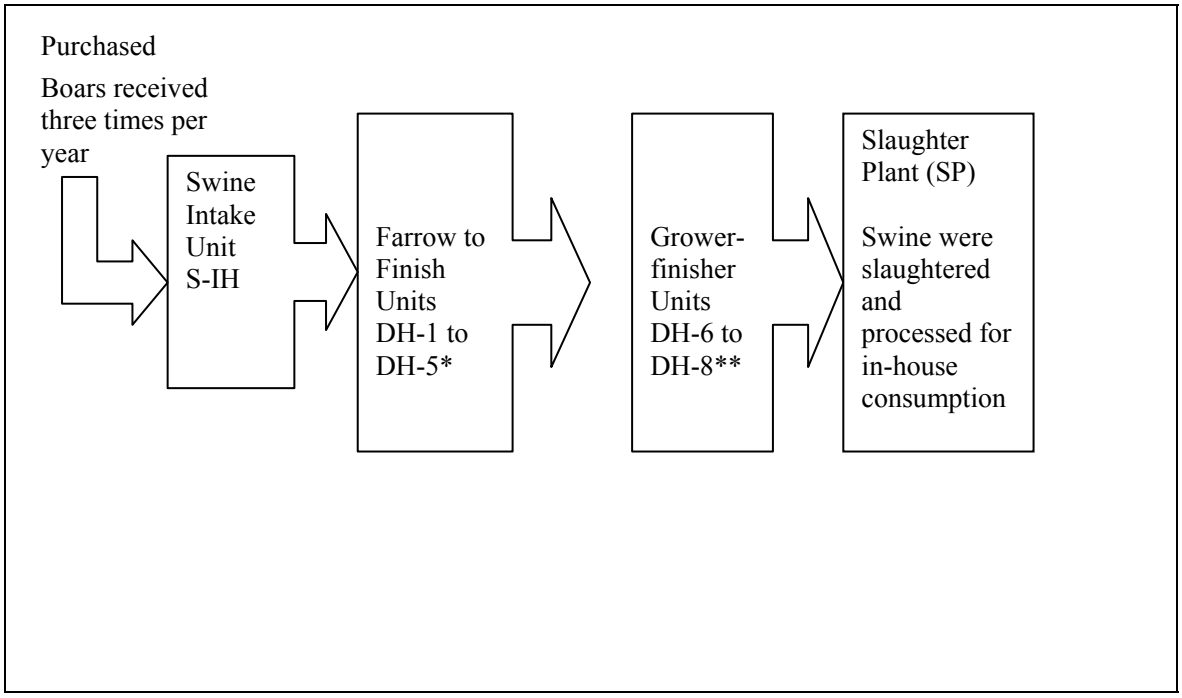


**Figure 2. Unidirectional flow of swine within the study population. Dual housing (DH) indicates integrated units which house humans and swine.**

* DH-5 provides replacement stock for units DH-1 to DH-4.
**Swine moved from any farrow-finish unit to any grower finisher unit. Eight of 13 swine production units in the system were sampled during the course of this study.

Movement of humans and swine was quite restricted both coming into and leaving the system. New residents to the system came through a central intake unit prior to being assigned to their permanent housing location. Swine purchased outside of the study system also came through a centralized intake unit prior to being transferred to the farrow-finish or grower-finish swine operations. Approximately 130,000 individuals were housed within the system and all meals were centrally prepared and consumed within each unit. Approximately 95% of the population was male and the average age was 36 years. A total of 62 human housing units comprised the study population with only 10 of the human housing units sampled for this study.

Criteria for unit inclusion into the study group included: presence of a swine operation, close proximity to Texas A&M University, and ease of access for sample collection. The selected units represented: 1 intake unit for new residents who entered into the system, 8 of the 13 human housing units with swine operations represented cohorts of swine and non-swine workers, and the single unit with human cohorts for non-swine workers, and packing plant workers (Figure 3). Human populations fluctuated, but approximately 1500 residents were housed at the human intake unit, 3000 residents comprised the swine worker cohort, and 18,000 comprised the non-swine worker (control) cohort across all 10 units (anonymous personal communication). Approximately 350 employees worked at the packing plant facility during the study period. The packing plant was composed of four main areas: kill floor, cooler, cut room, and sausage prep room. Swine were processed on the kill floor, and then moved into storage in the cooler for a 24-hour period. Upon removal from the cooler the carcasses were transferred to the cut room where fresh meat cuts (ham, loins, ribs, shoulders, pork roll, and party mix) were prepared and boxed. Sausage was prepared in a separate room within the packing plant, which was the final processing step.

**Sample Collection**

Initial wastewater grab samples (n=420) were collected by trained facility staff at each of the designated human housing units on a biweekly basis for four months (May – Aug. 2003). A final set of samples (n=105) were collected in the spring of 2004 (Feb.—Mar.). Samples were obtained by suspending a collection bucket into the center of the flow channel, rinsing 3 times before raising, and subsequently transferring a 100 ml

aliquot into a sterile pre-labeled 100 ml container. Each housing cohort was sampled at pre-designated manhole locations representing each housing cohort at 10:00, 12:00, and 14:00.

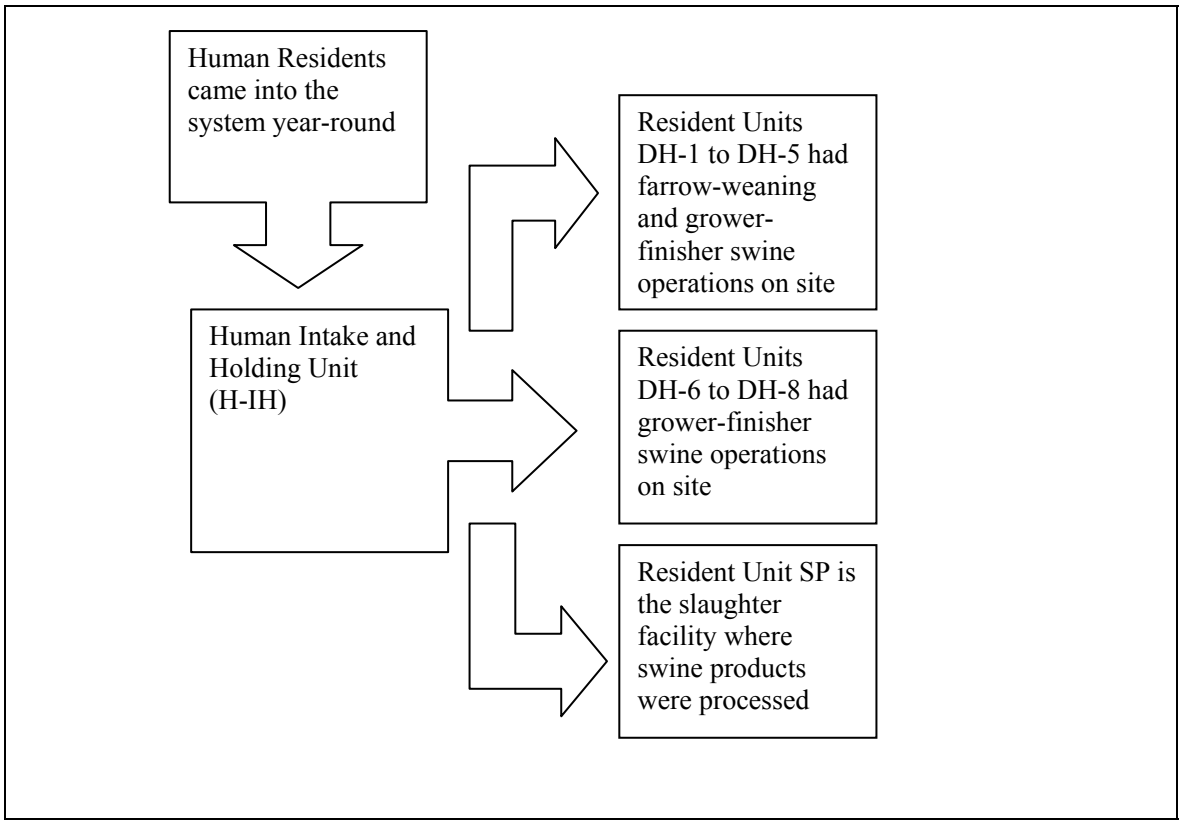


**Figure 3. Unidirectional flow of human residents in the study population. DH indicates integrated units which housed humans and swine.**

In addition, a wastewater plant influent sample was collected for each of the 10 sampled units by a privately licensed and contracted agency (Figure 4). During each bi-weekly sampling period, a total of 7 samples were collected from each of the 9 resident units containing 2 housing cohorts (swine worker, non-swine). Samples were stored on ice,

and within 48 hours of collection all samples were shipped to the USDA-ARS facility in College Station, TX for analysis.

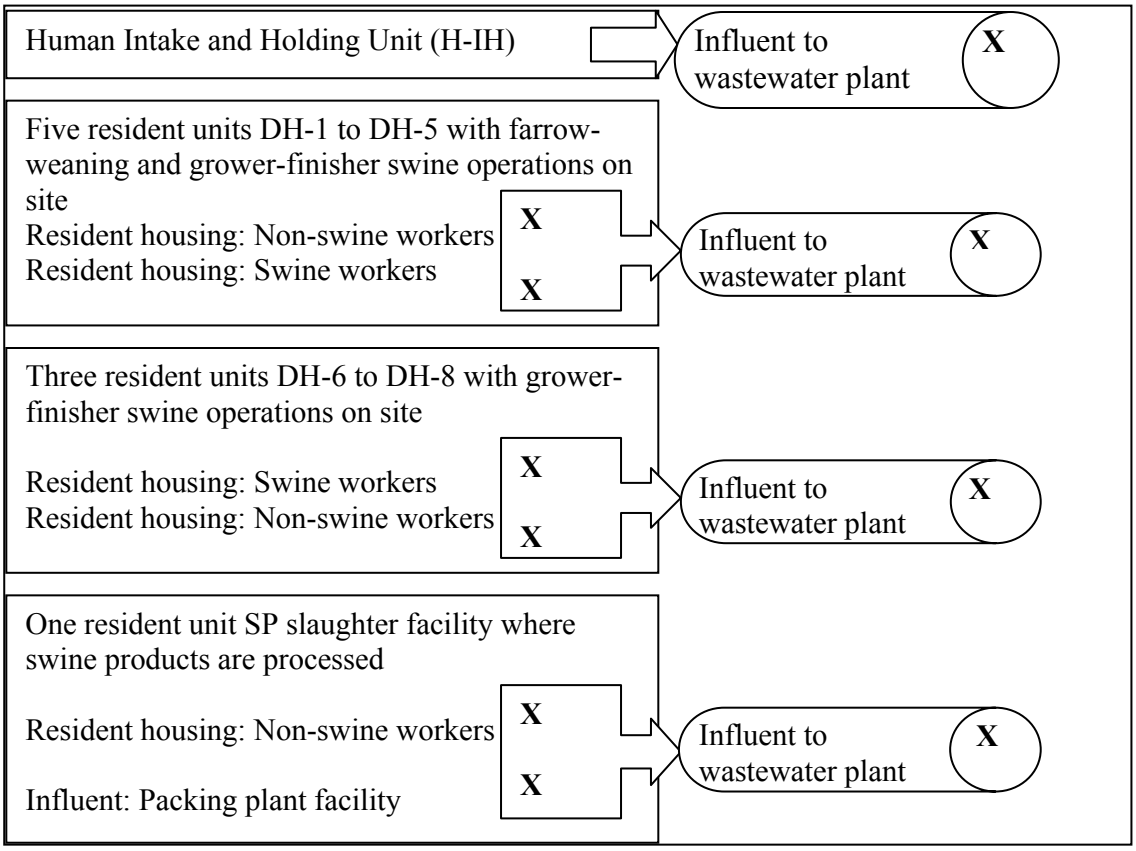


**Figure 4. Human housing sample collection location by unit type.**

**X** indicates a sampled location.

Initial swine fecal samples (n=61) were collected in the fall of 2002 (Sept. – Nov.). In the summer of 2003 additional swine fecal samples (n= 95) were collected (May – Sept.) using the following sampling methodology. Four composite swine fecal samples were collected from each of the 5 farrowing units along with a floor-wash sample (combined) from each of two houses and a single lagoon sample draining the

farm (Figure 5). Two composite fecal samples were collected from each of the 3 grower-finisher units along with a combined floor-wash and a lagoon sample (Figure 5). A final set of swine fecal samples was collected in the spring of 2004 (n=305). Individual fecal samples were collected from the swine quarantine facility whenever outside boars were purchased. No other outside purchases of swine were made during this study. Swine samples were collected by veterinarians in charge of swine health at the operation and transported to USDA-ARS, College Station, Texas.

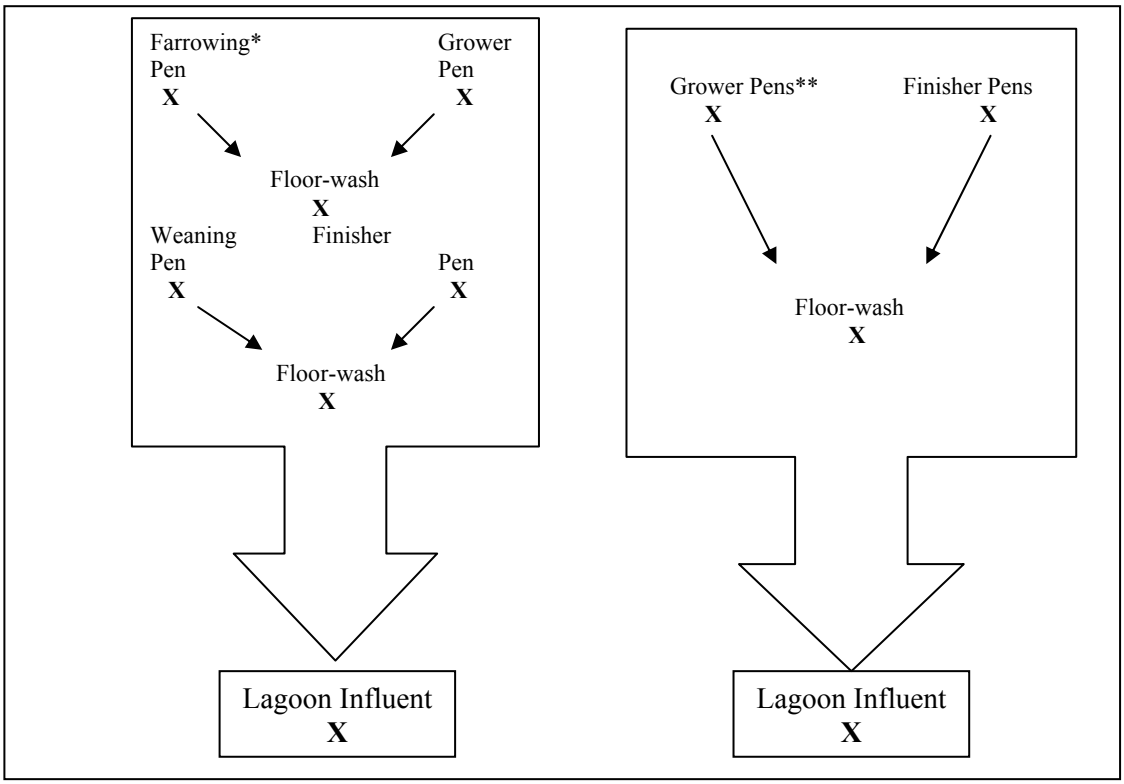


**Figure 5. Swine sample collection by location.**

*Sampling for DH units with farrow-finish swine operations
**Sampling for DH units with grower-finisher swine operations.

**Laboratory Analysis**

Upon arrival at the USDA facility, human wastewater samples were handled as follows: 32 ml of wastewater was transferred to a 50 ml conical tube that was labeled with the unit, sample location, date and time of collection. Eight ml (20% v/v) of sterile glycerol was added to each tube. Swine fecal samples arrived in labeled 50 ml conical tubes and were covered with glycerol prior to freezing. Both wastewater and fecal samples were frozen at -72$^{o}$C until processing, when the samples were removed and thawed in a 37$^{o}$C water bath. A loop of swine feces was collected from the thawed sample using a sterile inoculating loop and mixed with 1ml of sterile saline. The thawed wastewater samples were mixed using a sterile inoculating loop, and both swine fecal and wastewater samples were streaked onto CHROMagar-E.coli™ (DRG International, Mountainside, NJ) agar, as well as onto Difco™ mEnterococcus agar (ME) (Becton Dickinson, Sparks, MD) that contained 20 μg/ml of vancomycin. EC samples were incubated at 37$^{o}$C for 24 hours, and EF at 45$^{o}$C for 24-48 hours, upon which further microbial characterization for each species followed (Figure 6). A single EC isolate from each plate was selected based on morphology (Figure 6) and streaked on blood agar.

When present, a single VRE isolate from each plate was selected based on morphology (Figure 6) and streaked onto blood agar. All VRE isolates were verified using biochemical tests (API, bioMerieux, Hazelwood, MO) to ensure correct speciation prior to further testing. Some EC isolates were verified using the API Strep 20 biochemical test kit (API, bioMerieux, Hazelwood, MO) to ensure correct speciation prior to further testing. EC isolates were verified using the API 20 biochemical test kit (API, bioMerieux, Hazelwood, MO) only if the isolate produced unusual growth on 5% sheep blood agar (normal growth consisted of large, round, smooth colonies). Unusual growth was normally (but not limited to) large mucous colonies or large colonies with heavily ruffled edges which were dry in appearance. Upon confirmation of the bacterial identification, VRE samples were ribotyped using the RiboPrinter® system (Qualicon Inc., Wilmington, DE). EC isolates from swine and human samples were used to create a total genomic DNA template by the boiling lysis method for subsequent genotypic analysis. Both EC and EF (VRE) were screened for phenotypic characteristics as described in detail in the following sections. Pure culture isolates of both species were frozen in triplicate at -72$^{o}$C in a bacterial repository at the USDA-ARS facility.

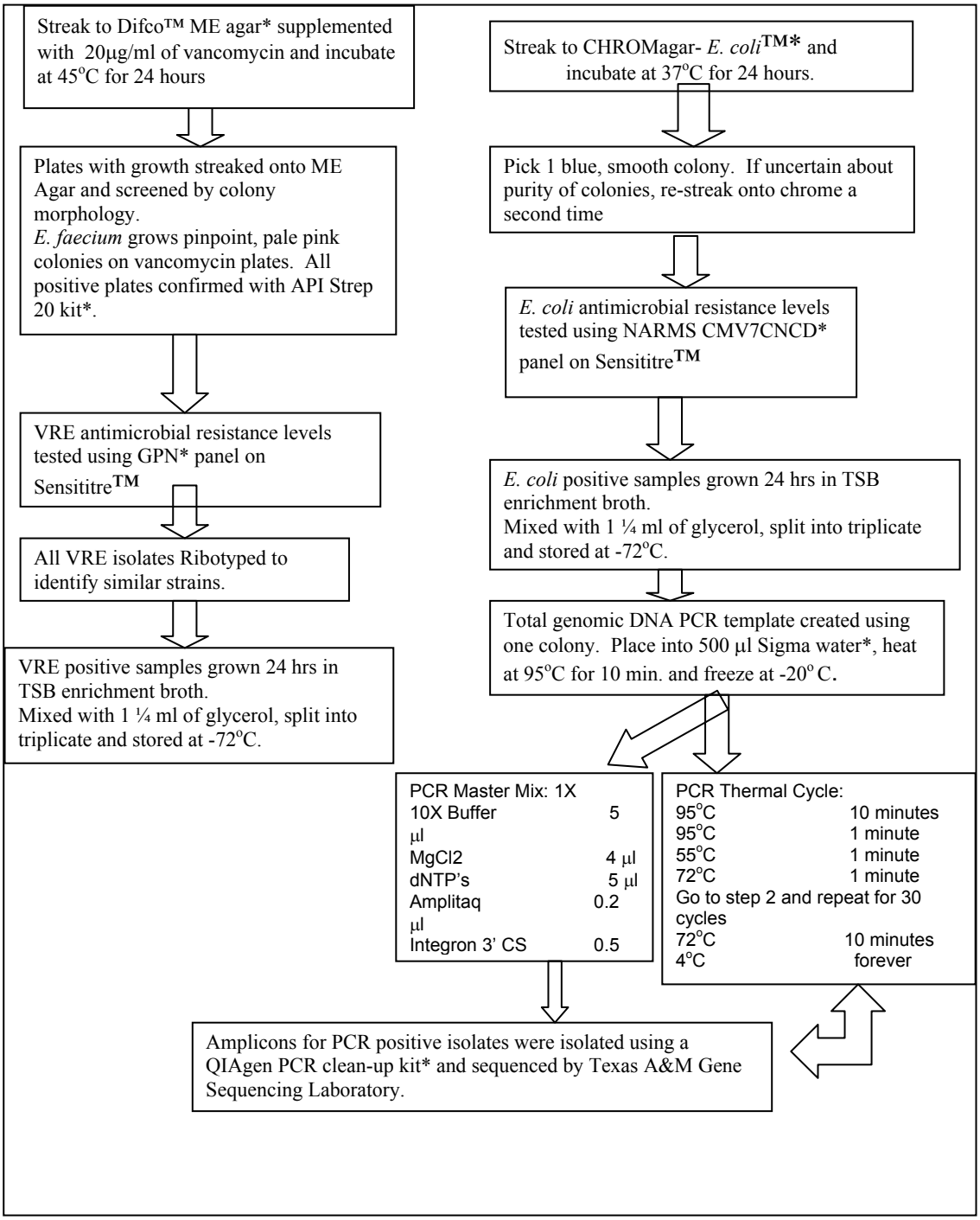


**Figure 6. Schematic flowchart of laboratory methods employed for *E. Coli* and *Enterococcus faecium* (VRE) analysis.**

Difco™ mEnterococcus agar (ME) (Becton Dickinson, Sparks, MD). CHROMagar-E.coli™ (DRG International, Mountainside, NJ). API Strep 20 biochemical test kit (API, bioMerieux, Hazelwood, MO). NARMS CMV7CNCD and GPN panels (Trek Diagnostics Inc., Cleveland, OH). Sigma water (Sigma-Aldrich, St. Louis, MO). QIAgen clean-up kit (Qiagen, Hilde, Germany)

**Phenotypic Characterization of Resistance**

Antimicrobial susceptibility was determined for EC and EF by measuring the minimum inhibitory concentration (MIC) to 16 and 19 antibiotics respectively (Tables 1 and 2). The micro-broth dilution method in Sensititre™ (Trek Diagnostics Inc., Cleveland, OH) test panels were used. Custom panels (CMV7CNCD), as designed by the National Antimicrobial Resistance Monitoring System (NARMS) were used for EC and the GPN panel (Trek Diagnostics Inc., Cleveland, OH) for EF (VRE). We utilized the NCCLS breakpoints for determination of resistance (National Committee for Clinical Laboratory Standards, 1999). Isolates were screened using both manual inspection and the Sensititre autoreader system (TREK Diagnostic Systems, Inc., Cleveland, OH). Isolates with intermediate MIC's were not considered resistant for our study. The dilution levels in the NARMS panels used in this study did not go as high as the recommended NCCLS breakpoint for classification as resistant for amikacin on the Gram-negative panel, and ampicillin, chloramphenicol, and quinupristin/dalfopristin on the Gram-positive panel. Therefore, we were not able to dichotomize and interpret amikacin results for the Gram-negative isolates. We were also not able to dichotomize and interpret ampicillin, chloramphenicol, and quinupristin/dalfopristin results for the Gram-positive isolates.

**Table 1. Interpretation criteria of 16 antimicrobial agents tested against *E. coli.***

| Antimicrobial | Range | Breakpoint |
|---|---|---|
| Amikacin** | 0.5 – 4 | ≥ 64 |
| Amoxicillin/Clavulanic Acid | 1 – 32 | ≥ 32 |
| Ampicillin | 1 – 32 | ≥ 32 |
| Ceftriaxone | 1 – 64 | ≥ 64 |
| Cephalothin | 2 – 32 | ≥ 32 |
| Chloramphenicol | 2 – 32 | ≥ 32 |
| Ciprofloxacin | 0.015 – 4 | ≥ 4 |
| Trimethoprim/Sulphamethoxazole | 0.12 – 4 | ≥ 4 |
| Cefoxitin | 0.5 – 16 | ≥ 32 |
| Gentamicin | 0.25 – 16 | ≥ 16 |
| Kanamycin | 8 – 64 | ≥ 64 |
| Nalidixic Acid | 0.5 – 32 | ≥ 32 |
| Ceftiofur* | 0.12 – 8 | ≥ 8 |
| Sulphamethoxazole | 16 – 512 | ≥ 512 |
| Tetracycline | 4 – 32 | ≥ 16 |
| Streptomycin* | 32 – 64 | ≥ 64 |

*No NCCLS interpretive standards exist for this antimicrobial. NARMS recommended breakpoint used.
** No interpretation was made for this antimicrobial due to the dilution level on the panel.

**Table 2. Interpretation criteria of 19 antimicrobial agents tested against *E. faecium.***

| Antimicrobial | | Range | Breakpoint |
|---|---|---|---|
| Ampicillin** | | 0.12 – 8 | ≥ 16 |
| Chloramphenicol** | | 2 – 6 | ≥ 32 |
| Ciprofloxacin | | 0.5-2 | ≥ 2 |
| Clarithromycin | | 0.12 – 4 | ≥ 4 |
| Clindamycin | | 0.25 – 2 | ≥ 2 |
| Erythromycin | | 0.12 – 4 | ≥ 4 |
| Gatifloxacin* | | 0.06 – 4 | ≥ 4 |
| Gentamicin | (2-16μg/ml) | 2 – 16 | ≥ 16 |
| Gentamicin | (500μg/ml) | 500 | ≥ 500 |
| Moxifloxacin* | | 0.25 – 4 | ≥ 4 |
| Levofloxacin | | 0.12 – 4 | ≥ 4 |
| Oxacillin + 2% NaCl | | 0.25 – 2 | ≥ 2 |
| Penicillin | | 0.03 – 8 | ≥ 1 |
| Rifampin | | 0.5 – 2 | ≥ 2 |
| Streptomycin | | 1000 | ≥ 1000 |
| Trimethoprim/Sulphamethoxazole | | 0.5 – 4 | ≥ 4 |
| Quinupristin/Dalfopristin*,** | | 0.12 – 4 | ≥ 8 |
| Tetracycline | | 4 – 32 | ≥ 32 |
| Vancomycin | | 0.5 – 16 | ≥ 16 |

*No NCCLS interpretive standards exist for this antimicrobial.
** No interpretation was made for this antimicrobial due to the dilution level on the panel.

Quality control strains EC 25922, EC 35218, EF 29212, and *Pseudomonas aeruginosa* 27853 obtained from the American Type Culture Collection (ATCC, Manassas, VA) were used for broth microdilution susceptibility testing for the EC isolates. ATCC *Enterococcus faecalis* 35218 served as the quality control strain for broth microdilution susceptibility testing for the EF isolates.

**Ribotyping**

Ribotyping matches the ribosomal RNA pattern of a bacteria to an identification library in order to assess genetic relationships between isolates. Patterns from each isolate are placed into common groups based on their similarity of band position and intensity (Hollis et al., 1999). Genetic relatedness of bacterial clones of VRE were determined using the RiboPrinter® Microbial Characterization System (Qualicon Inc., Wilmington, DE) using the standard EcoRI DNA prep kit. Isolates were compared to the DuPont identification library (Dupont, Wilmington, DE).

**Characterization of Integrons**

Total genomic DNA templates of EC isolates were created by suspending 1 colony of pure culture isolate in 500 µl of Sigma water (Sigma-Aldrich, St. Louis, MO), heating at 95$^{o}$C for 10 minutes to lyse the bacteria, centrifuging for 2 minutes to remove cell debris, and storing at -20$^{o}$C . Polymerase Chain Reaction (PCR) (cycles described in Figure 6) was used to amplify the Class 1 integron and subsequently any AMR gene cassettes which may be inserted between the integron variable regions (5'conserved

sequence and the 3'conserved sequence) as previously described by Levesque et al. (1995) and summarized in Table 3.

**Table 3. Oligonucleotide primers used for PCR amplification.**

| Gene | Primer | Sequence | Product size |
|---|---|---|---|
| Class 1 Integron | 5'CS | 5'-GGCATCCAAGCAGCAAG-3' | Variable |
| | 3'CS | 5'-AAGCAGACTTGACCTGA-3' | |

PCR products were subsequently electrophoresed on 1.0% agarose gels stained with ethidium bromide. A 100 base pair (bp) DNA ladder (Sigma-Aldrich, St. Louis, MO) provided a molecular size standard for all reactions. PCR products were purified using a QIAquick PCR clean-up kit (Qiagen, Hilde, Germany) and frozen at -20$^{o}$C. PCR products were submitted for DNA sequencing at the DNA Core Facility in the Department of Veterinary Pathobiology, Texas A&M University, College Station, TX. AMR genes were identified by sequence comparisons using the BLAST program available at the National Center for Biotechnology Information (Altschul et al, 1997). BLAST search results were screened for the presence of AMR gene cassettes based on 95% of the expected fragment size (600 bp) and a match of 95% or greater of the sequence of the fragment and the corresponding gene cassette(s).

**Statistical Analysis**

*Antimicrobial Resistance in EC*

MIC results for each of 15 antimicrobials were dichotomized for statistical analysis. Isolates with intermediate MIC's were categorized as sensitive. Basic descriptive statistics describing resistance to each of the 15 antimicrobials were cross-tabulated for each explanatory variable in SPSS 11.5 (SPSS Inc., Chicago, IL). Variables tested in the analyses included the following: proportion of EC with phenotypic resistance by: host species (human versus swine), unit, unit type, housing cohort by host species and time of day (human only).

Because responses within each geographic unit were not independent of each other due to collection of repeated samples at each unit, generalized estimating equations (GEE) were used to adjust for the within-unit dependence (Hardin and Hilbe, 2003) when there were sufficient observations to allow for modeling. Potential explanatory variables that were analyzed as categorical variables utilizing GEE in a GLM (generalized linear model) framework (McCullagh and Nelder, 1989) included: host species and time of day. GEE analysis was conducted using SAS PROC GENMOD (SAS Institute, Cary, NC) by regressing each antibiotic (dependent variable) on the independent variable in a GLM procedure using a binomial distribution, a logit link function, and an independent correlation structure repeating on unit as the cluster. Antibiotics that lacked model convergence were excluded from the analysis. The other potential explanatory variables: unit, unit type, and housing cohort by species were

analyzed using asymptotic likelihood tests in SPSS due to insufficient observations (resistance to each antimicrobial) across each category.

Unit was assessed as an 11-level categorical variable. Category H-IH corresponded to the human intake unit, category S-IH corresponded to swine intake unit, categories DH- 1 to DH- 8 corresponded to the units which housed both humans & swine and category SP corresponded to the slaughter plant.

Unit type was assessed as a 4-level categorical variable and was analyzed for only those antimicrobials found to have significant differences between humans and swine as modeled in the host species analysis. Category H-IH corresponded to the single human intake unit. Category DH corresponded to the 8 units housing both human and swine cohorts. Category SP corresponded to the workers at the slaughter plant where all swine are processed. Category S-IH corresponded to the swine intake unit (swine only) where newly purchased boars and replacement gilts came into the system.

Housing type was initially assessed as a categorical variable with 4 categories for human samples: swine worker corresponded to swine worker housing, non-swine worker corresponded to non-swine-worker housing, p-plant corresponded to packing plant influent wastewater samples and influent corresponded to mixed influent samples. A second analysis excluded human influent wastewater samples because they constituted a mixture of all of the cohorts and to allow direct assessment of differences among humans with and without direct swine contact. A third analysis looked specifically at differences in swine workers and non-swine workers only. Swine housing cohort was

assessed as an 8-level categorical variable: boars, dry sows, intake boars, lactating sows, lagoon, nursery, pig influent (wash water), and piglet.

Time of day was assessed as a categorical variable for human wastewater samples only. Category A corresponded to samples collected at 10:00, category B to samples taken at 12:00, and category C to samples taken at 14:00. Samples for each time of day were collected repeatedly from the same sample location. Influent samples were not collected at any given time of day and were excluded from this category.

*Pan-susceptibility in EC*

Pan-susceptible outcomes (i.e., susceptible to all 15 antimicrobials) in EC were dichotomized and compared by host species utilizing GEE in a GLM framework in SAS. The other potential explanatory variables: unit, unit type, housing cohort by species and time of day (human only) were assessed using asymptotic likelihood methods in SPSS due to model instability in GEE.

*Multiple Resistance in EC*

Multiple resistance outcomes in EC (potential range 0-15; isolates with resistance to 7 or more antimicrobials were grouped into a single upper category) were compared by: host species, unit, unit type, housing cohort by species, and time of day (human only) in a GLM framework utilizing a multinomial distribution and a cumulative logit link function (McCullagh and Nelder, 1989; Hardin and Hilbe, 2003). GEE utilizing the repeat statement (by unit) and an independent correlation structure was used to adjust for within unit dependence for all potential explanatory variables.

*Phenotypic Resistance Patterns within EC*

To further assess the pattern(s) of resistance within the study population, the total number of possible and actual phenotypes was calculated by resistance category (0-10) for each observation (n=895) using SPSS. A new variable was created and each observation was assigned to the corresponding phenotype. Unique phenotypes were identified and contrasted by species and within each unit to assess differences which require further study.

*Genotypic Resistance in EC*

Potential genotypic resistance, as assessed by both the presence of a gene cassette coding for resistance and a Class 1 integron, in EC, was compared by host species utilizing GEE in a GLM framework. Unit, unit type, time of day, and housing cohort by species were compared by asymptotic likelihood methods in SPSS due to model instability in GEE. Isolates which were positive for the presence of an integron (n=51) were further assessed for the presence of a gene cassette by host species using a $\chi^2$ test in Epi-Info 2000 (CDC, Atlanta, GA). Isolates containing AMR gene cassettes (n=31) were then assessed for differences by host-species using a Fischer's exact test in Epi-Info 2000.

*Antimicrobial Resistance in EF (VRE)*

MIC results were dichotomized for statistical analysis. Isolates with intermediate MIC's were categorized as sensitive. Basic descriptive statistics describing resistance to each of the 15 antimicrobials were cross-tabulated for each potential explanatory variable in SPSS. VRE (human only) were assessed by: unit, unit type, housing cohort,

and time of day utilizing either a Fischer's exact test or asymptotic likelihood method in SPSS. Unique phenotypes were identified and contrasted within each unit to assess differences which require further study.

# RESULTS

A total of 957 (525 human and 432 swine) samples were collected from September 2002 to March 2004 over 14 collection cycles. Due to lack of uniformity at the beginning of the study and as a result of developing sampling methodologies, not all units and sample cohorts were represented equally within the dataset. Early problems included inconsistent sample collection dates (every week instead of bi-weekly) and inconsistent labeling of the sample vials. These problems were quickly addressed and sampling remained consistent throughout the remainder of the study. Not all of the 957 samples received during the study period were positive for EC or EF (VRE) growth. As a result, a total of 895 observations (isolates) were used for statistical analysis of EC. A total of 17 observations were used for statistical analysis of EF (VRE).

*Descriptive Statistics for Multi-resistance in EC*

Multi-resistance was assessed as a categorical outcome variable with 7 categories due to insufficient results in the highest categories (8-10) and in order to avoid statistical model instability during analysis (Tables 4.1. and 4.2.).

**Table 4.1. Initial categories for multi-resistance.**

| Multi-Resistance Category | Frequency | Percent |
|---|---|---|
| 0 | 313 | 35.0 |
| 1 | 257 | 28.7 |
| 2 | 111 | 12.4 |
| 3 | 77 | 8.6 |
| 4 | 57 | 6.4 |
| 5 | 40 | 4.5 |
| 6 | 23 | 2.6 |
| 7 | 3 | .3 |
| 8 | 6 | .7 |
| 9 | 6 | .7 |
| 10 | 2 | .2 |
| **Total** | 895 | 100.0 |

**Table 4.2. Re-classified categories of multi-resistance.**

| Multi-Resistance Category | Frequency | Percent |
|---|---|---|
| 0 | 316 | 35.3 |
| 1 | 260 | 29.1 |
| 2 | 109 | 12.2 |
| 3 | 74 | 8.3 |
| 4 | 56 | 6.3 |
| 5 | 40 | 4.5 |
| 6 | 23 | 2.5 |
| 7 | 17 | 2.0 |
| **Total** | 895 | 100.0 |

*Phenotypic Resistance of EC by Species*

Significant differences in resistance were observed between humans and swine (see Table 5) with swine at a higher risk for resistance to kanamycin (OR=30.3), streptomycin (OR=3.70), sulphamethoxazole (OR=2.44) and tetracycline (OR=22.22). Only one human isolate and no swine isolates displayed resistance to ciprofloxacin.

**Table 5. Phenotypic resistance in EC assessed by host species: swine (n=379), human (n=516).**

| Antimicrobial | Percentage of resistant EC: swine | Percentage of resistant EC: human | $\chi^2$ Value | Odds Ratio (human as referent) |
|---|---|---|---|---|
| Amoxicillin/Clavulanic Acid | 2.4 | 1.7 | 0.19 | 1.37 |
| Ampicillin | 22.7 | 14.3 | 3.27 | 1.75 |
| Cefoxitin | 3.7 | 2.1 | 0.67 | 1.76 |
| Ceftiofur | 2.1 | 1.0 | 0.62 | 2.20 |
| Ceftriaxone | 2.9 | 0.0039 | 2.33 | 7.68 |
| Cephalothin | 26.9 | 29.1 | 0.72 | 0.89 |
| Chloramphenicol | 4.7 | 2.9 | 1.70 | 1.67 |
| Ciprofloxacin | 0.0 | 0.0019 | . | . |
| Gentamicin | 3.4 | 0.006 | 1.75 | 6.25 |
| Kanamycin | 19.3 | 0.008 | 6.44 | 30.3* |
| Nalidixic Acid | 2.4 | 5.0 | 3.44 | 0.46 |
| Streptomycin | 32.2 | 11.4 | 6.81 | 3.70* |
| Sulphamethoxazole | 23.7 | 11.4 | 5.08 | 2.44* |
| Tetracycline | 81.5 | 16.5 | 10.30 | 22.22** |
| Trimethoprim/Sulphamethoxazole | 2.4 | 8.3 | 3.59 | 0.27 |

**.** denotes model that did not converge due to lack of resistance
*p<0.05 **p<0.001 based on GEE score statistic

*Phenotypic Resistance of EC by Unit within Species*

Significant differences ($p<0.05$) in resistance were observed across unit for each host species. Resistance in human wastewater samples varied across unit for cefoxitin, ceftiofur, tetracycline, and trimethoprim/sulphamethoxazole (Table 6). Significant differences ($p<0.05$) in resistance were observed in swine across geographic unit for amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, gentamicin, kanamycin, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim/sulphamethoxazole (Table 7).

**Table 6. Phenotypic resistance in EC isolates by unit in human wastewater samples (n=516).**

| Antimicrobial | Facility* and sample size (n) within facility Percentage of Resistant EC | | | | | | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-IH (n=11) | DH-1 (n=57) | DH-2 (n=57) | DH-3 (n=57) | DH-4 (n=59) | DH-5 (n=53) | DH-6 (n=48) | DH-7 (n=44) | DH-8 (n=71) | SP (n=59) | S-IH (n=0) | |
| Amoxicillin / Clavulanic Acid | 0.0 | 8.8 | 1.8 | 1.8 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | - | 0.059 |
| Ampicillin | 9.1 | 21.1 | 14.0 | 17.5 | 10.2 | 11.3 | 10.4 | 13.6 | 19.7 | 10.2 | - | 0.632 |
| Cefoxitin | 0.0 | 8.8 | 5.3 | 1.8 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | - | 0.028 |
| Ceftiofur | 0.0 | 7.1 | 0.0 | 0.0 | 0.0 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.042 |
| Ceftriaxone | 0.0 | 3.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.449 |
| Cephalothin | 36.7 | 35.1 | 21.1 | 31.6 | 30.5 | 28.3 | 29.2 | 36.4 | 31.0 | 18.6 | - | 0.536 |
| Chloramphenicol | 0.0 | 8.8 | 0.0 | 0.0 | 3.4 | 0.0 | 2.1 | 4.5 | 4.2 | 3.4 | - | 0.066 |
| Ciprofloxacin | 9.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.556 |
| Gentamicin | 9.1 | 0.0 | 1.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | - | 0.354 |
| Kanamycin | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.9 | 2.1 | 4.5 | 0.0 | 0.0 | - | 0.280 |
| Nalidixic Acid | 18.2 | 8.8 | 10.5 | 1.8 | 3.4 | 1.9 | 2.1 | 2.3 | 4.2 | 6.8 | - | 0.185 |
| Streptomycin | 0.0 | 14.0 | 3.5 | 10.5 | 8.5 | 16.9 | 16.7 | 9.1 | 14.1 | 11.9 | - | 0.234 |
| Sulfamethoxazole | 9.1 | 16.9 | 5.3 | 8.7 | 6.8 | 9.4 | 6.3 | 15.9 | 19.7 | 11.9 | - | 0.163 |
| Tetracycline | 0.0 | 14.0 | 12.3 | 7.0 | 18.6 | 18.9 | 35.4 | 11.4 | 14.1 | 22.0 | - | 0.007 |
| Trimethoprim / Sulfamethoxazole | 9.1 | 12.3 | 0.0 | 8.8 | 3.4 | 3.8 | 4.2 | 13.6 | 19.7 | 6.8 | - | 0.001 |

* H-IH denotes the human intake unit. DH- 1 to DH- 8 denotes units housing both humans and swine. S-IH denotes the swine intake unit. SP denotes the slaughter plant.

**Table 7. Phenotypic resistance in EC isolates by unit in swine fecal samples (n=379).**

| Antimicrobial | Facility* and sample size (n) within facility<br>Frequency of Resistant EC (%) | | | | | | | | | | | p-*value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-IH (n=0) | DH-1 (n=47) | DH-2 (n=44) | DH-3 (n=28) | DH-4 (n=48) | DH-5 (n=50) | DH-6 (n=52) | DH-7 (n=24) | DH-8 (n=33) | SP (n=0) | S-IH (n=53) | |
| Amoxicillin / Clavulanic Acid | - | 8.5 | 0.0 | 3.6 | 8.3 | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.006 |
| Ampicillin | - | 27.7 | 15.9 | 14.3 | 35.4 | 6.0 | 21.2 | 12.9 | 24.2 | - | 37.6 | 0.001 |
| Cefoxitin | - | 12.8 | 0.0 | 7.1 | 8.3 | 4.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.001 |
| Ceftiofur | - | 6.4 | 0.0 | 0.0 | 8.3 | 0.0 | 0.0 | 0.0 | 3.0 | - | 0.0 | 0.016 |
| Ceftriaxone | - | 10.6 | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 | - | 9.4 | 0.002 |
| Cephalothin | - | 29.8 | 15.9 | 32.1 | 39.6 | 24.0 | 25.0 | 16.7 | 24.2 | - | 30.2 | 0.314 |
| Chloramphenicol | - | 10.6 | 0.0 | 3.6 | 6.3 | 2.0 | 1.9 | 0.0 | 9.1 | - | 7.5 | 0.089 |
| Ciprofloxacin | - | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - | 0.0 | - |
| Gentamicin | - | 0.0 | 4.5 | 0.0 | 0.0 | 0.0 | 15.4 | 0.0 | 0.0 | - | 5.7 | <0.0001 |
| Kanamycin | - | 10.6 | 15.9 | 10.7 | 14.6 | 10.0 | 32.7 | 12.5 | 6.1 | - | 45.3 | <0.0001 |
| Nalidixic Acid | - | 6.4 | 2.3 | 3.6 | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 | - | 5.7 | 0.162 |
| Streptomycin | - | 36.2 | 43.2 | 28.6 | 47.9 | 22.0 | 32.7 | 12.5 | 12.1 | - | 37.7 | 0.003 |
| Sulfamethoxazole | - | 14.9 | 18.2 | 14.3 | 33.3 | 26.0 | 19.2 | 8.3 | 27.3 | - | 39.6 | 0.019 |
| Tetracycline | - | 85.1 | 75.0 | 78.6 | 93.8 | 64.0 | 84.6 | 79.2 | 69.7 | - | 96.2 | <0.0001 |
| Trimethoprim / Sulfamethoxazole | - | 0.0 | 0.0 | 3.6 | 2.1 | 2.0 | 11.5 | 0.0 | 0.0 | - | 0.0 | 0.011 |

* H-IH denotes the human intake unit. DH- 1 to DH- 8 denotes units housing both humans and swine. S-IH denotes the swine intake unit. SP denotes the slaughter plant.

*Phenotypic Resistance by Unit type*

Differences in resistance by unit type were modeled for the four antimicrobials which exhibited overall risk differences between host species. There were significant differences in resistance by unit type (Table 8) in swine for kanamycin, sulphamethoxazole, and tetracycline. There were no significant differences ($p>0.05$) across unit type for humans.

*Phenotypic Resistance of EC by Housing Type*

Modeling indicated there were no significant differences in resistance by human housing cohorts for any of the 15 antimicrobials when influent samples (mixed) were included in the analysis (Table 9). There were significant differences in housing type when influent (to the wastewater plant) was excluded from the analysis (packing plant, swine worker, non-swine worker only) for resistance to cephalothin ($p=0.033$) only (data not shown in Table 9). There were also significant differences in resistance to cephalothin when packing plant influent samples and influent samples were excluded from the analysis ($p=0.02$) with non-swine workers 1.89 times more likely to have resistance to cephalothin ($OR=1.89$) than swine workers (data not shown in Table 9).

**Table 8. EC phenotypic resistance by unit type to each of 4 antimicrobials that exhibited significant risk differences (p < 0.05) between humans (n=516) and swine (n-379).**

| | | | Unit-type* Frequency of resistant EC | | | | |
|---|---|---|---|---|---|---|---|
| Antimicrobial | Resistant | Host Species (Frequency of EC) | H-IH | DH | SP | S-IH | p-value |
| Kanamycin | Yes | Human (n=4) | 0 | 4 | 0 | - | 0.557 |
| | No | Human (n=512) | 11 | 442 | 59 | - | |
| | Yes | Swine (n=73) | - | 49 | - | 24 | <0.0001 |
| | No | Swine (n= 306) | - | 277 | - | 29 | |
| Streptomycin | Yes | Human (n=59) | 0 | 52 | 7 | - | 0.259 |
| | No | Human (n=457) | 11 | 394 | 52 | - | |
| | Yes | Swine (n=122) | - | 102 | - | 20 | 0.357 |
| | No | Swine (n=257) | - | 224 | - | 33 | |
| Sulphamethoxazole | Yes | Human (n=59) | 1 | 51 | 7 | - | 0.964 |
| | No | Human (n=457) | 10 | 395 | 52 | - | |
| | Yes | Swine (n=90) | - | 69 | - | 21 | 0.005 |
| | No | Swine (n=289) | - | 257 | - | 32 | |
| Tetracycline | Yes | Human (n=85) | 0 | 72 | 13 | - | 0.074 |
| | No | Human (n=431) | 11 | 374 | 46 | - | |
| | Yes | Swine (n=309) | - | 258 | - | 51 | 0.001 |
| | No | Swine (n=70) | - | 68 | - | 2 | |

* H-IH denotes the human intake unit. DH denotes units housing both humans and swine. S-IH denotes the swine intake unit. SP denotes the slaughter plant.

**Table 9. Phenotypic resistance among human EC wastewater isolates by housing type (n=491)*.**

| Antimicrobial | Influent (n=157) | Non-swine worker ( n=98) | Packing-plant (n=36 ) | Swine-worker ( n=200) | p-value* |
|---|---|---|---|---|---|
| Amoxicillin / Clavulanic Acid | 2.5 | 1.0 | 2.8 | 1.5 | 0.774 |
| Ampicillin | 11.5 | 18.4 | 8.3 | 14.5 | 0.327 |
| Cefoxitin | 2.5 | 4.1 | 2.8 | 1.0 | 0.377 |
| Ceftiofur | 1.3 | 0.0 | 0.0 | 1.0 | 0.454 |
| Ceftriaxone | 1.3 | 0.0 | 0.0 | 0.0 | 0.205 |
| Cephalothin | 30.6 | 36.7 | 19.4 | 23.5 | 0.057 |
| Chloramphenicol | 4.5 | 1.0 | 2.8 | 3.0 | 0.429 |
| Ciprofloxacin | 0.0 | 1.0 | 0.0 | 0.0 | 0.357 |
| Gentamicin | 0.6 | 1.0 | 2.8 | 0.0 | 0.244 |
| Kanamycin | 1.9 | 1.0 | 0.0 | 0.0 | 0.133 |
| Nalidixic Acid | 5.1 | 4.1 | 8.3 | 4.5 | 0.803 |
| Streptomycin | 12.7 | 16.3 | 11.1 | 8.5 | 0.244 |
| Sulphamethoxazole | 13.4 | 13.3 | 13.9 | 9.0 | 0.511 |
| Tetracycline | 13.4 | 21.4 | 22.2 | 14.5 | 0.250 |
| Trimethoprim/Sulphamethoxazole | 10.8 | 7.1 | 8.3 | 7.0 | 0.602 |

*A total of 25 samples were not identified to a specific housing type.

There were significant differences across swine housing types (Table 10) for resistance to amoxicillin, ampicillin, cefoxitin, gentamicin, kanamycin, streptomycin, sulphamethoxazole, and tetracycline.

*Phenotypic Resistance by Time of Day*

Modeling showed there were no significant differences ($p>0.05$) in resistance across time of day for human wastewater samples.

*Pan-susceptibility in EC*

Overall, 84% of human EC isolates were pan-susceptible compared with only 15% in swine (Figure 7). There were significant differences in pan-susceptibility between host species with humans being 7.76 times more likely to exhibit pan-susceptible EC isolates (OR=7.76, $p=0.0014$). There were no significant differences ($p>0.05$) in pan-susceptibility among human EC for unit, unit type, housing type or time of day. There were significant differences within swine EC by unit ($p=0.004$) and unit type (OR= 8.3, $p=0.011$) with the DH units more likely to have pan-susceptible isolates than the swine intake and holding unit. There were significant differences within swine EC by swine production groups ($p=0.001$) with finisher pigs having the most pan-susceptible isolates.

**Table 10. Phenotypic resistance among swine composite fecal samples by housing type (n=364)*.**

| Antimicrobial | Swine Housing Type Frequency (%) | | | | | | | | | | p-value* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Boar (n=11) | Dry Sow (n=20) | Finisher (n=73) | Grower (n=50) | Intake Boar (n=43) | Lactating Sow (n=28) | Lagoon (n=18) | Nursery (n=27) | Influent (n=64) | Piglet (n=30) | |
| Amoxicillin / Clavulanic Acid | 0.0 | 5.0 | 0.0 | 0.0 | 0.0 | 3.6 | 0.0 | 7.4 | 1.6 | 13.3 | 0.020 |
| Ampicillin | 36.4 | 15.0 | 15.1 | 24.0 | 46.5 | 21.4 | 11.1 | 18.5 | 23.4 | 26.7 | 0.032 |
| Cefoxitin | 0.0 | 5.0 | 0.0 | 2.0 | 0.0 | 7.1 | 0.0 | 11.1 | 3.1 | 13.3 | 0.019 |
| Ceftiofur | 0.0 | 5.0 | 0.0 | 2.0 | 0.0 | 3.6 | 0.0 | 7.4 | 0.0 | 10.0 | 0.052 |
| Ceftriaxone | 0.0 | 0.0 | 0.0 | 2.0 | 11.6 | 7.1 | 0.0 | 3.7 | 1.6 | 3.3 | 0.069 |
| Cephalothin | 36.4 | 20.0 | 27.4 | 20.0 | 32.6 | 35.7 | 16.7 | 25.9 | 25.0 | 36.7 | 0.685 |
| Chloramphenicol | 0.0 | 0.0 | 2.7 | 6.0 | 9.3 | 3.6 | 0.0 | 7.4 | 4.7 | 6.7 | 0.542 |
| Ciprofloxacin | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - |
| Gentamicin | 0.0 | 5.0 | 0.0 | 0.0 | 7.0 | 0.0 | 0.0 | 18.5 | 1.6 | 10.0 | 0.002 |
| Kanamycin | 0.0 | 15.0 | 11.0 | 10.0 | 53.5 | 14.3 | 16.7 | 25.9 | 15.6 | 33.3 | <0.0001 |
| Nalidixic Acid | 0.0 | 0.0 | 0.0 | 2.0 | 7.0 | 3.6 | 11.1 | 0.0 | 1.6 | 3.3 | 0.184 |
| Streptomycin | 9.1 | 35.0 | 20.5 | 30.0 | 44.2 | 28.6 | 11.1 | 59.3 | 28.1 | 63.3 | <0.0001 |
| Sulfamethoxazole | 27.3 | 15.0 | 13.7 | 10.0 | 44.2 | 17.9 | 16.7 | 44.4 | 18.8 | 53.3 | <0.0001 |
| Tetracycline | 100.0 | 70.0 | 75.3 | 92.0 | 95.3 | 75.0 | 50.0 | 81.5 | 79.7 | 93.3 | <0.0001 |
| Trimethoprim / Sulfamethoxazole | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 3.6 | 5.6 | 11.1 | 3.1 | 3.3 | 0.171 |

* A total of 15 samples were not identified to a specific housing type.

*Multiple Resistance in EC*

In contrast, 65% of swine isolates were in multi-resistance category 3 (resistance to 3 or more antimicrobials) or higher compared with only 35% of human isolates. There were significant differences for multiple resistance between host species with swine being at 4.81 times greater odds (OR=4.81, $p<0.0023$) of belonging to a higher category of multiple resistance across all units, and housing cohorts (Figure 7).

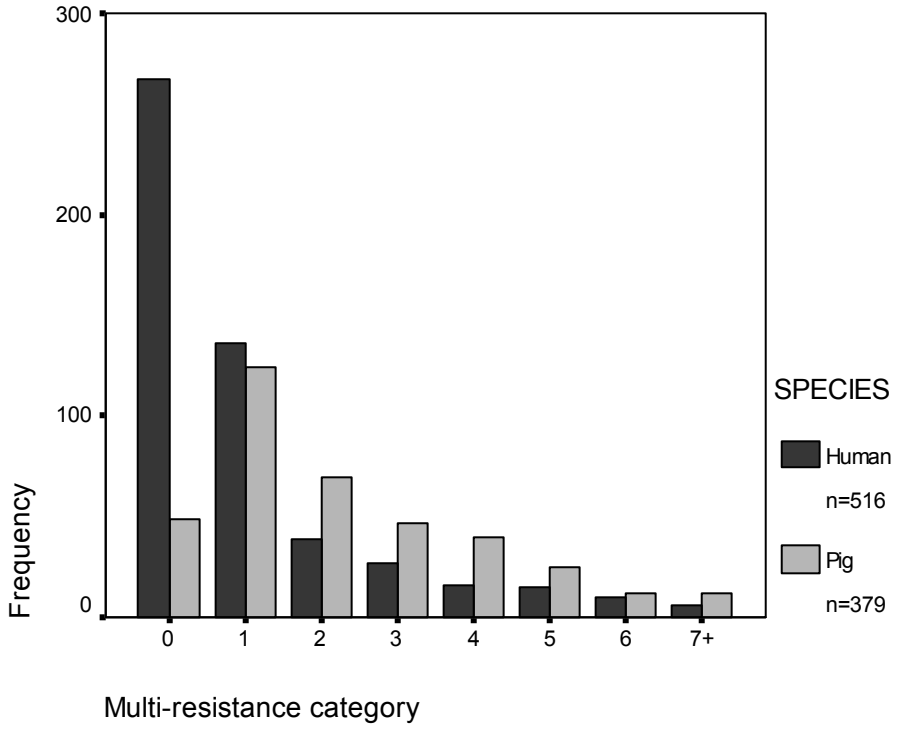


**Figure 7. Frequency of multi-resistance* in EC by species**

* Multi-resistance category represents the actual number of antimicrobials except category 7 which encompasses 7 through 10.

There were no significant ($p>0.05$) differences in multiple resistance outcomes for each host species by unit. There were no significant ($p>0.05$) differences in multiple resistance outcomes for each host species across unit type. There were no significant ($p>0.05$) differences in multiple resistance outcomes for each host species across housing cohort. There were no significant ($p>0.05$) differences in multiple resistance outcomes for humans across time of day (Figure 8).

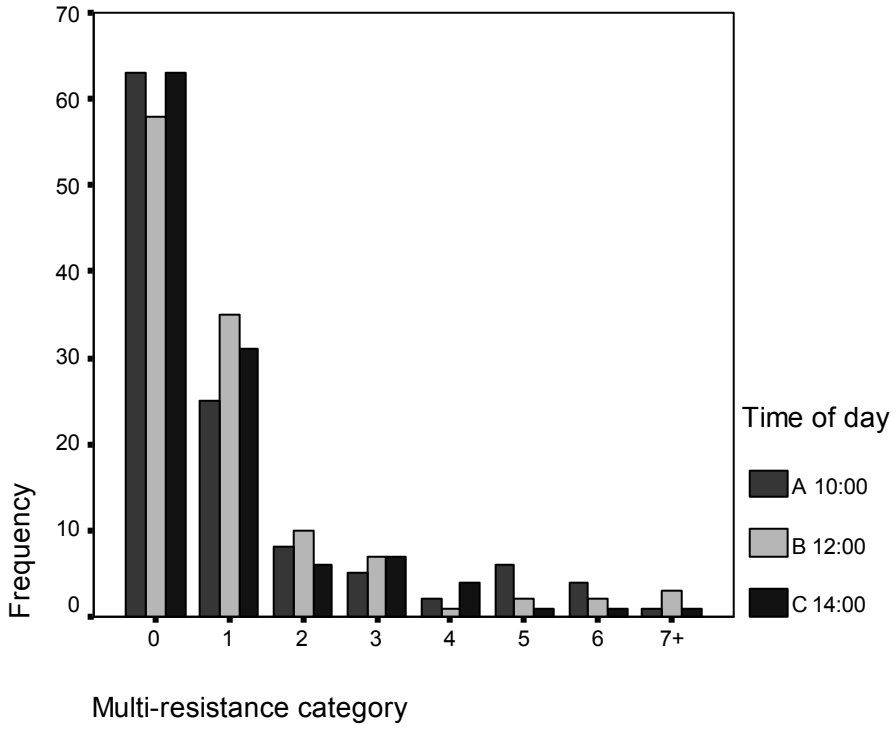


**Figure 8. Frequency of multi-resistance* in EC by time of day.**

* Multi-resistance category represents the actual number of antimicrobials except category 7 which encompasses 7 through 10.

*Phenotypic Resistance Patterns in EC by Species*

To aid in presentation of the phenotypic results Table 11 defines abbreviations for antimicrobials in subsequent tables. A total of 116 distinct phenotypes were observed during the study period with 20 of these accounting for 81.5% of all isolates (n=895) in humans and swine (Table 12). The most commonly observed phenotypes by host species, excluding pan-susceptibility, were resistance to cephalothin (16%) in human isolates and tetracycline in swine (27%). The highest level of multi-resistance found was to 10 antimicrobials: amo + amp + cefox + ceftio + ceftri + cephal + chlor + strep + sulpha + tet (See Table 11), in two human wastewater influent samples. Both were taken from the same dual-housing (DH) unit on the same date and displayed identical phenotypes. Of the isolates with resistance to 9 antimicrobials, three distinct phenotypes were observed with the predominant phenotype: amo + amp + cefox + ceftio + cephal + chlor + strep + sulpha + tet, found in four isolates; first in a human swine worker sample in Aug. 2003 and subsequently in Feb. 2004 in 3 swine isolates from the swine farm where the swine workers from the aforementioned DH unit work.

**Table 11. Antimicrobial abbreviations used for phenotype.**

| Antimicrobial | Abbreviation |
|---|---|
| Amikacin | ami |
| Amoxicillin/Clavulanic Acid | amo |
| Ampicillin | amp |
| Cefoxitin | cefox |
| Ceftiofur | ceftio |
| Ceftriaxone | ceftri |
| Cephalothin | cephal |
| Chloramphenicol | chlor |
| Ciprofloxacin | cipro |
| Clarithromycin | clarith |
| Clindamycin | clinda |
| Erythromycin | erythro |
| Gatifloxacin | gatiflox |
| Gentamicin | gen |
| Kanamycin | kan |
| Levofloxacin | levo |
| Moxifloxacin | mox |
| Nalidixic Acid | nal |
| Oxacillin + 2% NaCl | oxacil |
| Penicillin | pen |
| Quinupristin/Dalfopristin | quin |
| Rifampin | rifam |
| Streptomycin | strep |
| Sulphamethoxazole | sulpha |
| Tetracycline | tet |
| Trimethoprim/Sulphamethoxazole | tri |
| Vancomycin | vanco |

Among isolates with resistance to 8 antimicrobials, 3 phenotypes were observed: amox + amp + cefox + ceftio + cephal + strep + sulpha + tet was the only one having more than one observation. This phenotype was first observed in a piglet in Feb. 2004 and subsequently isolated from the same unit in a piglet and nursery sample in Mar. 2004. The multi-resistance phenotypes for resistance to 8, 9, or 10 antimicrobials appear to be nested in that each higher level phenotype differs from the next lower one by the addition of only one antimicrobial.

**Table 12. Predominant phenotypes of both human and swine EC isolates.**

| Phenotype | Frequency | Percent |
|---|---|---|
| Pansusceptible | 313 | 34.97 |
| Tet | 128 | 14.30 |
| Cephal | 90 | 10.06 |
| Cephal + Tet | 33 | 3.69 |
| Strep + Tet | 21 | 2.35 |
| Strep + Sulpha + Tet | 19 | 2.12 |
| Amp | 18 | 2.01 |
| Amp + Cephal+ Strep + Tet | 14 | 1.56 |
| Nal | 12 | 1.34 |
| Sulpha + Tet | 10 | 1.12 |
| Amox + Cephal | 9 | 1.01 |
| Kan + Tet | 9 | 1.01 |
| Amp + Strep + Tet | 9 | 1.01 |
| Amp + Cephal + Tet | 8 | 0.89 |
| Kan + Strep+ Sulpha + Tet | 8 | 0.89 |
| Amp + Tet | 7 | 0.78 |
| Amp + Cephal + Tri+ Sulpha+ Strep | 7 | 0.78 |
| Gen + Kan + Strep+ Tet | 5 | 0.56 |
| Amp + Kan + Strep+ Sulpha + Tet | 5 | 0.56 |
| Cephal + Nal | 4 | 0.45 |

The only EC resistant to ciprofloxacin during the study period was isolated from a Feb. 2004 human intake sample and it was resistant to a total of 7 antimicrobials: amp + cephal + cipro + gen + nal + sul + tri collected. In addition to being the only isolate resistant to ciprofloxacin during the study period, this isolate was also rare in that it exhibited resistance to gentamicin, nalidixic acid and trimethoprim/sulphamethoxazole. Only a very few isolates exhibited single resistance to these antimicrobials (gen <2%, nal <4%, and tri <6%). No other isolates showed multi-resistance to this combination of antimicrobials (gen, nal and tri).

*Genotypic Resistance in EC*

PCR was run on a total of 729 EC isolates (n=420 human, n=372 swine) to detect the presence of Class 1 integrons. Initial swine samples received in the fall of 2002

(n=61) were not tested using PCR, nor were human samples received in the spring of 2004 (n=105). Of the 420 human isolates tested, a total of 56 (13%) were positive on initial screening for any Class 1 integrons. A total of 23/56 (41%) remained positive on confirmatory screening. Nine of these 23 (39%) were positive for AMR gene cassettes. The other 14 had DNA inserted into the integron (range 525-1000 bp) but did not match any known resistance genes in the Genbank database. Seven of 9 samples (78%) were positive for the *aadA* gene which encodes resistance to spectinomycin and streptomycin. Variants of *dfr* encoding resistance to trimethoprim were present in 3 out of the 9 samples (33%). The *ant-3* gene that also encodes resistance to spectinomycin and streptomycin was found in only 1 isolate. Of the 372 swine isolates tested, a total of 51 (14%) were positive on initial screening for any Class 1 integrons. Of these 36/51 (71%) remained positive on confirmatory screening. Five of these 36 (14%) were positive for AMR gene cassettes with all 5 containing the *aadA* gene which encodes resistance to spectinomycin and streptomycin. The other 31 had DNA inserted into the integron (range 200-990bp) but did not match any known resistance genes in the Genbank database.

Statistically, there were significant differences between host species with swine isolates having greater odds for the presence of integrons (OR=2.33, p=0.0487). No significant differences were found ($p>0.05$) for facility location, facility type, human housing cohort or time of day. There were significant differences (p=0.006) between swine production groups (boars, dry sows, finisher, grower, intake boars, lactating sows, lagoon, nursery piglets, influent and piglets) with the grower group having lower odds

for the presence of a Class 1 integron than the nursery group (nursery as referent, OR=0.22, p=0.04). Among all isolates with an integron present, human isolates were at higher risk for the presence of an AMR gene cassette (OR=6.36, p=0.003). Comparing the isolates bearing gene cassettes coding for specific resistance to antimicrobials, there were no significant differences between host species (p>0.05) though there were few data to compare.

*Phenotypic Resistance in VRE*

A total of 17 (3%) human wastewater samples were positive for VRE during the study period. There were significant differences in VRE recovery by unit (p=0.001) with samples (n=17) taken from four different geographic locations representing all isolates positive for VRE. Three of these were sampled from influent (mixed) human housing (swine workers and non-swine workers) and 14 were sampled from swine worker housing locations. Statistically, swine workers were at higher risk for VRE than the other housing cohorts (OR=6.8, p=0.001). There were no significant differences in VRE recovery (p>0.05) by unit type or time of day. No VRE were isolated from swine samples. From the 17 isolates, a total of 12 unique AMR phenotypes were observed with only four phenotypes found in multiple isolates (Table 13). Of the four phenotypes with more than one isolate, none were observed in the same geographic unit. Of the three isolates observed from mixed housing, 2 were from the same DH unit and collected on the same date. The other isolate was collected from a DH unit on the same date that VRE were isolated in swine worker housing samples within that unit.

**Table 13. Phenotypes of human VRE isolates (n=17).**

| Phenotype | Frequency |
|---|---|
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 2 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 1 |
| Cipro + Clarith + Clinda + Erythro + Gatiflox + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 1 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Strep +Tri + Vanco | 3 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 2 |
| Erythro + Gen500 + Pen + Rifam + Strep + Tet + Vanco | 2 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 1 |
| Erythro + Gen500 + Pen + Strep +Tet + Vanco | 1 |
| Cipro+ Clarith + Clinda + Erythro + Levo + Oxacil + Pen + Rifam + Strep +Tet +Tri + Vanco | 1 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Strep +Tri + Vanco | 1 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Pen + Rifam + Tet +Tri + Vanco | 1 |
| Cipro+ Clarith + Clinda + Erythro + Gen + Gen500 + Levo + Oxacil + Rifam + Tet +Tri + Vanco | 1 |

## DISCUSSION AND CONCLUSION

In the present study, conducted from September 2002-March 2004, there were significant ($p<0.05$) differences in EC AMR between host species isolates (humans and swine) within the integrated study population. Swine isolates were at higher risk for single and multiple resistance than human isolates. In addition, pan-susceptibility was much more common in human EC isolates than in swine. These findings are consistent with other studies which found AMR prevalence differences between humans and animals (Dunlop et al., 1998b; White et al., 2002; Guerra et al., 2003; Lanz et al., 2003; Zhao et al., 2003; and Larkin et al., 2004. Dunlop et al. (1998b) studied resistance prevalence among fecal EC of swine taken from 34 farrow-finish farms in Canada. Observed resistance was predominantly to ampicillin, spectinomycin, and tetracycline with resistance varying more between farms than within. The present study demonstrated similar results with resistance to each of the aforementioned antimicrobials varying across geographic location (unit). The results of the Dunlop et al. (1998b) study also indicated that samples collected within a farm were more likely to be similar than those from different farms. This underscores the need to adjust for the effects of clustering in similar study designs such as the present study. White et al. (2002) looked at clinical *Escherichia coli* 0:111 isolates collected over a 24 year period to evaluate the prevalence of resistance in humans, swine, cattle, and poultry. Their results indicated that swine isolates showed higher resistance to one or more antimicrobials than human isolates, but lower prevalence of Class 1 integrons. The present study also found that swine isolates showed higher resistance to one or more antimicrobials, but found that

swine had a higher prevalence of Class 1 integrons while swine were more likely to have a AMR gene cassette present. Guerra et al. (2003) looked at resistance prevalence in cattle, poultry and swine isolates collected directly from live animals (health status of the animal was not indicated). Those authors found swine and poultry to have higher overall resistance prevalence than cattle. Additionally, swine were found to exhibit significant resistance (31%) to spectinomycin. While the present study did not assay spectinomycin it did look at streptomycin and gentamicin (also in the aminoglycoside drug class). We found swine to have higher levels of resistance to streptomycin than humans and the only AMR gene cassettes found within swine were encoding resistance to both spectinomycin and streptomycin. A study by Lanz et al. (2003) conducted in Switzerland addressed the resistance levels in dairy cattle, dogs, cats and swine. The authors found higher levels of resistance in swine for streptomycin, spectinomycin, and gentamicin than the other animal species. These findings are also similar to the present study which found high resistance in swine isolates to streptomycin. While the present study did not indicate significant differences between humans and swine for gentamicin resistance, there were significant differences among swine housing types. Zhao et al. (2003) studied the prevalence of *Salmonella enterica* serotype Newport in isolates from human, dairy cattle, poultry and swine. Interestingly, the authors found a high prevalence of Class 1 integrons (68%), but did not indicate how many were found from isolates of each species. This is in contrast to the present study which did not find a high prevalence of Class 1 integrons or AMR gene cassettes in humans or swine. Larkin et al. (2004) also looked at resistance in *Salmonella* isolated from hogs, cattle and poultry.

Higher levels of resistance were again found to streptomycin and tetracycline in swine. Additionally, swine isolates showed higher levels of multi-resistance than isolates from the other species. Those findings are similar to the present study which found high levels of resistance to both streptomycin and tetracycline within swine isolates and a significantly higher risk of multi-resistance in swine isolates versus humans.

Within host species of the present study, there were significant ($p<0.05$) differences in resistance of EC by geographic location (unit) for both human and swine isolates. Documentation of antimicrobial usage was variable across geographic location as indicated by antimicrobial feed records for swine (generally tylosin and chlortetracycline used in feed rations) and based on the differences in hospital and clinic types across locations (data not shown) so these results may or may not be indicative of the role of antimicrobial usage in AMR. After an extensive search of the literature, it appears that there is little information available about differences in resistance across multiple locations within an integrated population. van den Bogaard et al. (2001) compared AMR in the Netherlands across chickens, poultry workers, and slaughter house workers and found similar resistance patterns in the three groups. This was the most similar study in design and objective to the present study. Employing a cross-sectional study design the authors attempted to address the transmission risk from animal-to-human contact. This is similar to the present study in the desire to address the potential transmission risk from animals to human or *vice versa*. However, it differed significantly in approach. The present study worked within a semi-closed integrated population consisting of swine, swine workers, and non-contact swine consumers,

whereas, the van den Bogaard study worked in an open population. The study did find differential prevalence of resistance within the poultry population (broilers, and laying hens) which is similar to the present study's finding of differential prevalence across swine housing types.

When comparing human housing cohort (swine workers versus non-swine workers), there were no significant ($p>0.05$) differences in resistance of EC (except to cephalothin), multi-resistance, presence of a Class 1 integron, or VRE. The lack of similarity in resistance between swine and humans (swine workers and consumers) supports the findings of Kariuki et al. (1999) who found no significant similarity in resistance and PFGE patterns in isolates from chickens and humans living in close contact. This finding is in contrast to van den Bogaard et al. (2001) who found similarities in resistance patterns between humans and poultry with which they have close contact especially given that the present study found non-swine workers at higher risk for cephalothin resistance (OR=1.89). However, van den Bogaard et al. do not indicate which farms these samples came from nor is there any analysis of farm factors that may play a role in these results (e.g., antimicrobial usage, housing and crowding of animals, animal handling practices).

Swine isolates varied by housing for resistance to 8 of the antimicrobials with intake boars (received twice during the study), nursery piglets, weaned piglets, and lactating sows generally having more observed resistance than the other housing types. Piglets and lactating sows have been show to receive higher levels of antimicrobial treatment than other production groups (Akinna and Johnson, 1999). Because data on

individual treatment of the animals with antimicrobials were not available, we were not able to assess the role of individual level factors in the observed AMR in the swine housing types. However, the high level of resistance observed in the intake boars suggests that the resistance may have been introduced as swine moved into the study population. A study by Dunlop et al. (1998c) found that individual level treatments showed more variability than did group treatments for swine that were similar in age to the ones in our study. However, swine samples collected for the present study were composite in nature and were collected across multiple pens, which suggests that some group-level factor (e.g., antimicrobials in the feed) could be playing a role rather than individual level factors. Nonetheless, the aggregate nature of the data made this impossible to assess.

Multi-resistance to up to 10 antimicrobials was observed in the human isolates and resistance up to 9 antimicrobials was observed in swine isolates. Additionally, several unique phenotypes were found with both swine and human isolates in the present study. Only one isolate resistant to ciprofloxacin was found and was sampled from the human intake and holding unit. This indicates the possibility for the introduction of unique phenotypes into the system from incoming human residents. A swine worker EC isolate with resistance to 9 antimicrobials was first identified in 2003 and later found in 2 swine isolates from the same unit. These findings point to the possibility of resistance traits being passed from humans to swine within the study population. Similarly, the work of Barber et al. (2003) showed transmission of zoonotic pathogens from humans to animals with which they had contact, and that of Funk et al. (2001) which found higher

prevalence of *Salmonella* in swine when human toilet facilities were lacking. However, our data are not comprehensive since we selected only one colony per plate. As a result, temporal relationships could not be established. In our study, a swine isolate with resistance to 9 antimicrobials was initially discovered and was subsequently found in two other swine in the same herd. This would support the theory of horizontal transmission of resistance characteristics within swine housed in close proximity to each other.

Human EC isolates were more likely to have an integron encoded AMR gene cassette than swine isolates, even though swine isolates were more likely to have a Class 1 integron than human isolates. Overall, a low prevalence of Class 1 integrons and AMR gene cassettes were observed in both human and swine isolates in this study, which is in contrast to recent studies by Winokur et al. (2001) and Leverstein-van Hall et al. (2002), in which a substantial portion of AMR genes in Gram-negative bacteria were located within the Class 1 integron.

VRE were isolated only in human wastewater samples, and these findings are unique in that these represent to the author's knowledge the first environmentally isolated human VRE in the United States. VRE has been isolated from both dog feed and chicken feed in the U.S. in 1996 and 1999 respectively. Typically, vancomycin is administered to patients within a hospital setting; for example, persons on dialysis, in the intensive care unit (ICU), or immuno-compromised patients all may be candidates for treatment. Virtually all VRE in the United States to date have been directly attributed to hospital cases and have been isolated in clinical samples or isolated from hospital

sewers. The VRE in our study were predominantly isolated from swine worker samples. To find vancomycin resistance in healthy humans outside the hospital setting may indicate an important role of human carriers of VRE in its transmission. The only other VRE (n=3) were found in mixed influent samples which would have been taken 'downstream' from the housing cohorts and represented a combination of swine worker and non-swine worker wastewater. The survival of VRE as they move downstream and encounter an environment rich with other organisms may indicate that they are able to compete with other micro-organisms and survive. Given this capability, it is also possible that they may be able to move into the environment in the event of leaks in the sewage system. While the available evidence in Europe suggests that VRE are transmitted from animals to humans (Harwood et al., 2001) and is attributed largely to the use of growth promoters such as avoparcin and virginiamycin in food animals (Phillips et al., 2004), we found no evidence of VRE in the animal population in our study which may suggest that humans are the primary reservoir for VRE in the United States. While the general consensus is that VRE in humans in Europe is a result of animal to human transmission, there are several studies with findings to the contrary. Experimental studies appear to support some level of host-specificity in EF and an ingestion study conducted with human volunteers found no colonization as a result of consumption of animal strains of VRE (Phillips et al., 2004). Due to the cross-sectional nature of our study, further studies will need to be conducted before any definitive statement can be made about the causality of our present findings.

Use of GEE (generalized estimating equations) for the statistical analysis of the aforementioned risk factors provided a good estimate of the population parameters under study while adjusting for potential dependence of response variables by unit. GEE specifies only the marginal distribution of the outcome variable which produces estimates of the population parameters rather than cluster-specific values (Edwards, 2000). GEE methods can be used for both discrete and continuous outcome variables, but is most often used for correlated binary data. GEE methods assume an s x 1 vector for the correlation matrix. The estimate of the s x 1 vector is inserted into the equation and estimation proceeds to achieve a working correlation matrix. The working correlation matrix does not always approximate the true correlation matrix, but good estimates of the population parameters are still achieved due to the robust nature of GEE methods (Edwards, 2000).

To the author's knowledge this is the first study to have looked at resistance within an integrated population of humans and swine. The unique characteristics of the study population: 1) limited in and out migration of humans and swine; 2) raising, processing and consumption of swine products "in house"; and 3) the arrangement of cohorts of swine workers, slaughter plant workers, and non-swine contact consumers allowed for the first assessment of AMR within a "controlled" and semi-closed study population. Using a combination of phenotypic and genotypic methods, this study relied on an integrated approach to examining potential risk factors for AMR which should eventually lead to the type of data desperately needed to use in risk assessment modeling of AMR. Moreover, this study is distinctive in that we used non-clinical,

environmentally isolated EC and EF rather than human clinical isolates or retail meat samples. This reflects a more realistic approach of the "day to day" transmission potential and risk from animals to humans or *vice versa*. While these findings are suggestive and thought-provoking, they can not be judged in any way to suggest causality at this time. As an initial cross-sectional analysis, these findings will need to be followed and verified during an ongoing longitudinal study before causality can be addressed.

**Study Limitations**

Working within a semi-closed population represented a unique opportunity to study AMR within an integrated population of humans and swine. Therefore, it will also have resulted in a decreased ability to generalize these results to the human and swine populations at large due to differences in population characteristics and the sampling strategy of the study. Population groups chosen for inclusion into the study were selected as a convenience sample based on their proximity to our lab facilities and the ability to establish a working relationship with that particular administrative unit. Therefore, generalization of the findings beyond these particular groups should be cautioned. Data on antimicrobial usage within both the human and swine population were not evaluated during this study. Therefore the role of antimicrobial usage in the observed phenotypic and genotypic resistance could not be judged.

The selection of only one colony of EC per plate may have resulted in a bias in the estimation of prevalence if multiple phenotypes and/or genotypes were present and not considered due to the aforementioned selection methodology. Recognizing that

there will have been other AMR genotypes and phenotypes present on the media, but for financial reasons, we chose to analyze fewer isolates across a higher number of samples. Berge et al. (2003) studied EC resistance in dairy calves and sampled 5 colonies per plate. Those authors' noted a mean of 1.8 distinct AMR phenotypes per plate in their study, which considered upwards of five thousand isolates. This finding supports the selection of fewer isolates per sample. Any potential bias associated with this selection methodology would be non-differential unless the isolate selection process was knowingly associated with the risk factors under study. There could also have been bias if AMR phenotype was associated with a fitness disadvantage on the selective *in vitro* media used in this study. These potential biases are common to all *in vitro* cultivation studies, not just ours.

Due to the aggregate nature of the sampling procedure, it is possible that this study may suffer from aggregate bias, also known as the ecological fallacy. In the ecological fallacy, associations observed at the group or aggregate-level do not necessarily reflect an association at the individual level. In many cases, studies done at the population level are considered imperfect surrogates for individual level data, or initial studies which can suggest avenues of research to be conducted at the individual level. While this may be true, and widely applicable to studies concerned with disease etiology, there are situations in which population level studies are the more appropriate approach. If the variability within the population of interest is low but between-population variability is high, then important associations may be missed by individual level study designs. Dunlop et al. (1998b) studied the prevalence of AMR across 34

farrow-finish swine operations in Canada, and found that there was higher variability in resistance between farms than within. These findings underscore the applicability of the aggregate approach to studies of AMR within populations. The second situation in which a population level study may be more appropriate is if the level of prevention or intervention would be directed at the population level even if the preferred level of inference is to the individual. AMR reduction and risk analysis strategies would likely be focused on both group-level and individual-level risk factors.

The detection limit of the PCR assay (sensitivity) was not determined during the course of the study and subsequently our ability to consider an isolate truly negative was unknown. As such, we may have misclassified isolates that were below the detectable limit as negative when they were indeed positive. Also, integrons may be quite large in size, and may have been missed under the PCR parameters used in this study. Further, we did not assess the role of inhibitory compounds in the ability of the DNA to be amplified. This may have been a significant issue given that the samples were environmental in nature. One problem that presented itself late in this study was poor gene sequencing results in the last set of swine DNA samples (n=2) due to large amounts of primer dimer in the reaction.

**Recommendations for Future Studies**

The ability to quantitatively measure AMR is a key component to addressing the risk of transmission from animals to humans or vise-versa. In order to achieve that goal, future studies should look at community DNA samples for specific genotypic resistance characteristics over a longer period of time. That coupled with the use of real-time PCR,

would allow for better quantification of the samples under study. In addition, collection of individual level samples from within the human cohorts will allow for more accurate assessment of the resistance characteristics within the population and how they transfer over time. Further assessment of antimicrobial usage both within the swine and human populations will be necessary to address the role of drug usage in AMR characteristics and transmission dynamics. Tracking of new human residents and swine as they move into and through the system could aid in identifying unique AMR characteristics which are being introduced to the population from the outside. This would allow for characterization of movement or lack of movement through the study system.

**Summary/Conclusions**

To the author's knowledge this is the first study to address AMR characteristics within an integrated population of humans and swine across multiple sites in the farrow-to-fork continuum. Our study agrees with previously published work, further suggesting that: 1) the level of AMR to one or multiple antimicrobials is higher in swine than humans and 2) there are differences in resistance based on unit location, unit-type, and housing cohort within both humans and swine. Unlike other studies, our study found no significant differences in resistance between swine workers and non-swine workers with the sole exception of resistance to cephalothin. Further studies are necessary to quantitatively measure AMR and address the risk of transmission from animals to humans and *vice-versa*.

## REFERENCES

Aarestrup, F.M. 2000. Characterization of glycopeptide-resistant *Enterococcus faecium* (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides. J. Clin. Microbiol. 38:2774-2777.

Akkina, J.E. and R. Johnson. 1999. *Antimicrobial Resistance Issues in Animal Agriculture*. USDA: APHIS: CEAH, Fort Collins, CO.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

Anderson, AD, J. McClellan, S. Rossiter, and F.J. Angulo. 2003. Appendix A: Public Health Consequences of Use of Antimicrobial Agents in Agriculture. In: *The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment -- Workshop Summary*. S.L. Knobler, S.M. Lemon, M. Najafi, and T. Burroughs (eds.), pp. 231-243. Institute of Medicine of the National Academies Press, Washington, D.C.

Bailar, III, J.C., and K. Travers 2002. Review of assessments of the human health risk associated with the use of antimicrobial agents in agriculture. Clin Infect Dis. 34 (Suppl 3):S135-43.

Bakken, J.S. 2004. The fluoroquinolones: how long will their utility last? Scand. J. Inf. Dis. 36:85-92.

Barber, D.A., G. Y. Miller, and P. E. McNamara. 2003. Models of antimicrobial resistance and foodborne illness: examining assumptions and practical applications. J. Food Prot. 66:700-709.

Bartoloni, A., B. Filippo, A. Mantella, E. Dell'Amico, M. Roselli, et al. 2004. High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption. J. Inf. Dis. 189:1291-1294.

Barza, M. 2002. Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. Clin. Inf. Dis. 34 (Supp.3):S123-125.

Bass, L, C. Liebert, M. Lee, A.O. Summers, D.G. White, et al. 1999. Incidence and characterization of integrons and genetic elements mediating multiple-drug resistance in avian *Escherichia coli.* Antimicrob. Agents Chemother. 43:2925-2929.

Berge, A.C.B., E.R. Atwill and W.M. Sischo. 2003. Assessing antibiotic resistance in fecal *Escherischia coli* in young calves using cluster analysis techniques. Prev. Vet. Med. 61:91-102.

Brenner, D.J. 1984. Facultatively anaerobic Gram-negative rods: Family I. Enterobacteriaceae In: *Bergey's Manual of Systematic Bacteriology Vol. 1*. Krieg, N.R., and J.G. Holt (eds.), pp. 408-420. Williams & Wilkins, Baltimore.

Catry, B., H. Laevens, L. A. Devriese, G. Opsomer and A. De Kruif. 2003. Antimicrobial resistance in livestock. J. Vet. Pharmacol. Therap. 26:81-93.

Centers for Disease Control. (CDC) 2002. *CDC's Campaign to Prevent Antimicrobial Resistance in Healthcare Settings.* Atlanta, GA. Centers for Disease Control

Website. Available at: http://www.cdc.gov/drugresistance/healthcare/default.htm. Accessed 31 Aug 2004.

Centers for Disease Control. (CDC) 2004. *National Center for Infectious Diseases Antimicrobial Resistance Glossary.* Atlanta, GA. Centers for Disease Control Website. Available at: http://www.cdc.gov/drugresistance/miscellaneous/glossary.htm. Accessed 31 Aug 2004.

Chopra, I. and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. and Mol. Bio. Reviews. 65:232-260.

Cizman, M. 2003. The use and resistance to antibiotics in the community. Int. J. Antimicrob. Agents 21:297-307.

Collier, L., A. Barrows, and M. Sussman. 1998. *Topley and Wilson's Microbiology and Microbial Infections, 9th ed.* Arnold Press, Sydney, Australia.

Davies, J.D. and G. D.Wright. 1997. Bacterial resistance to aminoglycoside antibiotics. Trends Microbiol. 5:234-240.

Dean, W.R., and H. M. Scott. 2004. Antagonistic synergy? Process and paradox in the development of new agricultural antimicrobial regulations. Agric. Human Values. In press.

Diekema, D.J., B. J. BootsMiller, T. E. Vaughn, R.F. Woolson, J.W. Yankey, et al. 2004. Antimicrobial resistance trends and outbreak frequency in United States hospitals**.** Clin Infect Dis. 38:78-85.

Dunlop, R.H., S.A. McEwen, A.H. Meek, W.D. Black, R.C. Clarke and R.M. Friendship. 1998a. Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada. Prev. Vet. Med. 34:247-264.

Dunlop, R.H., S.A. McEwen, A.H. Meek, W.D. Black, R.M. Friendship, and R.C. Clarke. 1998b. Prevalences of resistance to seven antimicrobials among fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev. Vet. Med. 34:265-282.

Dunlop, R.H., S.A. McEwen, A.H. Meek, R.C. Clarke, W.D. Black and R.M. Friendship. 1998c. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev. Vet. Med. 34:283-305.

Edwards, L.J. 2000. Modern statistical techniques for the analysis of longitudinal data in biomedical research. Pediatr. Pulmonol. 30:330-344.

Fluit, A.C., and F. J. Schmitz. 1999. Class 1 integrons, gene cassettes, mobility and epidemiology. Eur. J. Clin. Microbiol. Infect. Dis. 18:761-770.

Funk, J.A., P.R. Davies, and W. Gebreyes. 2001. Risk factors associated with *Salmonella enterica* prevalence in three-site swine productions systems in North Carolina, USA. Berl. Munch. Tierarztl. 114:335-338.

General Accounting Office. (GAO) 1999. *Food Safety: The Agricultural Use of Antibiotics and Its Implications for Human Health.* GAO/RCED-99-74. Washington, D.C. General Accounting Office Website. Available at: http://www.gao.gov/archive/1999/rc99074.pdf Accessed 31 Aug 2004.

Goldstein, C., M.D. Lee, S. Sanchez, C. Hudson, B. Phillips, et al. 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. 45:723-726.

Guerra, B., E. Junker, A. Schroeter, B. Malorny, S. Lehmann, et al. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine, and poultry J. Antimicrob. Ther. 52: 489-492.

Hall, R.M. and C.M. Collis 1995. Mobile gene cassettes and integrons: capture and spread of genes by site specific recombination. Mol. Microbiol.15:593-600.

Hall, R.M. and C.M. Collis 1998. Antibiotic resistance in Gram-negative bacteria: the role of gene cassettes and integrons. Drug Resist. Updates 1:109-119.

Halling-Sorenson, B., S. Nors Nielsen, P.F. Lansky, F. Ingerslev, H.C. Holten Lutzhoft, and S.E. Jorgensen. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere. 36:357-393.

Hardin, J.W. and J.M. Hilbe. 2003. *Generalized Estimating Equations*. Chapman and Hall/CRC, Boca Raton, FL.

Harwood, V.J., M. Brownell, and W. Perusek. 2001. Vancomycin-resistant Enterococcus spp. isolated from wastewater and chicken feces in the United States. Appl. Environ. Microbiol. 67:4930-4933.

Hasman, H. and F. M. Aarestrup. 2002. *TcrB*, a gene conferring transferable copper resistance in *Enterococcus faecium*: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. Antimicr. Agents Chemother. 46:1410-1416.

Hayes, J.R., L. L. English, P. J. Carter, T. Proescholdt, K.Y. Lee, et al. 2003. Prevalence and antimicrobial resistance of *Enterococcus* species isolated from retail meats. Appl. Env. Microbiol. 69(12):7153-7160.

Heinemann, J.A. 1999. How antibiotics cause antimicrobial resistance. Drug Discovery Today 4:72-79.

Hollis, R.J., J.L. Bruce, S.J. Fritschel, and M.A. Pfaller. 1999. Comparative evaluation of an automated ribotyping instrument versus pulsed-field gel electrophoresis for epidemiological investigation of clinical isolates of bacteria. Diagn. Microbiol. Inf. Dis. 34:263-268.

Houghton, D. 2002. Antimicrobial resistance in the intensive care unit: understanding the problem. AACN Clin. Issues 13:410-420.

Institute of Medicine. *Antimicrobial Drug Resistance: Issues and Options*. Workshop report. Washington: National Academy Press, 1998. Online. Available at: http://www.nap.edu/books/0309060842/html/index.html. Accessed 31 August 2004.

Isaacson, R.E. and M.E. Torrence. 2002. *The Role of Antibiotics in Agriculture*. American Academy of Microbiology, Washington, D.C.

Kariuki, S., C.F. Gilks, J. Kimari, J. Muyodi, P. Waiyaki, and C.A. Hart. 1997. Plasmid diversity of multi-drug resistant *Escherichia coli* isolated from children with diarrhea in a poultry-farming area in Kenya. Annals Trop. Med. Parasitol. 91:87-94.

Kariuki, S., C.F. Gilks, J. Kimari, A. Obanda, J. Muyodi, et al. 1999. Genotype analysis of *Escherichia coli* strains isolated from children and chickens living in close contact. Appl. Env. Microbiol. 65:472-476.

Kaye, K. S., H. S. Fraimow, and E. Abrutyn. 2000. Pathogens resistant to antimicrobial agents. Epidemiology, molecular mechanisms, and clinical management. Infect Dis Clin North Am. 14:293-319.

Khachatourians, G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Med. Assoc. J. 159:1129-1136.

Klare, I., C. Konstabel, D. Badstubner, G. Werner, and W. Witte. 2003. Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. Int. J. Food Microbiol.88:269-290.

Lanz, R., P. Kuhner, and P. Boerlin. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91:73-84.

Larkin, C., C. Poppe, B. McNab, B. McEwen, A. Mahdi and J. Odumeru. 2004. Antibiotic resistance of Salmonella isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. J. Food Prot. 67:448-455.

Leverstein-van Hall, M.A., A. Paaus, T.A. Box, H.E.M. Blok, J. Verhoef and A.C. Fluit. 2002. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. J. Clin. Microbiol. 40(8):3038-3040.

Levesque, C., L. Piche, C. Larose, and P.H. Roy. 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. 39:185-191.

Levin, B.R., M. Lipstich, and V. Perrot. 1997. The population genetics of antibiotic resistance. Clin. Inf. Dis. 24 (Supp.1):S9-S16.

Levy, S. 1998. The challenge of antibiotic resistance. Scientific American, March, 46-53.

Low, D.E., N. Keller, A. Barth, and R. Jones. 2001. Clincial prevalence, antimicrobial susceptibility, and geographic resistance patterns of *enterococci*: results from the SENTRY antimicrobial surveillance program, 1997-1999. Clin. Inf. Dis. 32 (Supp. 2):S133-145.

Maguire, A.J., D.F.J. Brown, J.J. Gray, and U. Desselberger. 2001. Rapid screening technique for class 1 integrons in *Enterobacteriaceae* and nonfermenting Gram-negative bacteria and its use in molecular epidemiology. Antimicrob. Agents Chemother. 45:1022-1029.

Martel, A., L. A. Devriese, A. Decostere and F. Haesebrouck. 2002. Presence of macrolide resistance genes in s*treptococci* and e*nterococci* isolated from pigs and pork carcasses. Int. J. Food Microbiol. 84:27-32.

Martinez, J.A., R. Ruthazer, K. Hansjosten, L. Barefoot, and D.R. Snydman. 2003. Role of environmental contamination as a risk factor for acquisition of vancomycin-resistant e*nterococci* in patients treated in a medical intensive care unit. Arch. Intern. Med. 163:1905-1912.

Mathai, E., G. Malin, and G. Kronvall. 2004. Integrons and multidrug resistance among *Escherichia coli* causing community-acquired urinary tract infection in southern India. APMIS 112:159-164.

McCullagh, P. and J. Nelder. 1989. *Generalized Linear Models*. Chapman and Hall/CRC, Boca Raton, FL.

McEwen, S.A. and P.J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. Clin. Inf. Dis. 34 (Supp. 3):S93-106.

McManus, M.C. 1997. Mechanisms of bacterial resistance to antimicrobial agents. American J. Health-Systems Pharmacol. 54:1420-1433.

Meyer, M., D.W. Kolphin, J.E. Bumgarner, J.L. Varns and J.V. Daughtridge. 2000. Occurrence of antibiotics in surface and groundwater near confined animal feeding operations and wastewater treatment plants using radio-immunoassay and liquid chromatography/electrospray mass spectrometry. Paper presented at 219th meeting of the American Chemical Society; March 26-30, 2000; San Francisco, CA (abstr 1:106).

Naas, T., Y. Mikami, T. Imai, L. Poirel and P. Nordmann. 2001. Characterization of In53, a class 1 plasmid and composite transposon-located integron of *Escherichia coli* which carries an usual array of genes. J. Bacteriol. 183:235-249.

National Committee for Clinical Laboratory Standards. 1999. *Performance Standards for Antimicrobial Susceptibility Testing Ninth International Supplement, vol. 19 number 1*. National Committee for Clinical Laboratory Standards, Wayne, PA.

O'Brien, T.F. 2002. Emergence, spread and environmental effect of antimicrobial resistance: how use of antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin. Inf. Dis. 34 (Supp. 3):S78-84.

Patterson, D.L. 2002. Looking for risk factors for the acquisition of antibiotic resistance: a 21st century approach. Clin. Inf. Dis. 34:1564-1567.

Phillips, I., M. Caswell, T. Cox, B. DeGroot, C. Friis, et al. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of the published data. J. Antimicrob. Chemother. 53:28-52.

Roe, M.T., A. Byrd, P. Smith, and S.D. Pillai. 2003a. Class 1 and Class 2 integrons in poultry carcasses from broiler house and poultry processing environments. J. Food Prot. 6:1426-1431.

Roe, M.T., E. Vega, and S. D. Pillai. 2003b. Antimicrobial resistance markers of class 1 and class 2 integron-bearing *Escherichia coli* from irrigation water and sediments. Emerging Inf. Dis. 9:22-826.

Rothman, K.J. 2002. *Epidemiology an introduction*. Oxford University Press, New York, p.91.

Salisbury, J.G., T.J. Nicholls, A.M. Lammerding, J. Turnidge, and M.J. Nunn. 2002. A risk analysis framework for the long-term management of antibiotic resistance in food-producing animals. Int. J. Antimicrob. Agents 20:153-164.

Shea, K.M. 2003. Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health? Pediatrics 112:253-258.

Shryock, T. 1999. Relationship between the usage of antibiotics in food-producing animals and the appearance of antibiotic resistant bacteria. Int. J. Antimicrob. Agents 12:275-278.

Snary, E.L., L.A. Kelly, H.C. Davison, C.J. Teale, and M. Wooldridge. 2004. Antimicrobial resistance: a microbial risk assessment perspective. J. Antimicrob. Chemother. 53:906-917.

Stohr, K. 2002. Problems from antimicrobial use in farming. Essential Drugs Monitor 28:10-11.

Summers, A.O. 2002. Generally overlooked fundamentals of bacterial genetics and ecology. Clin. Inf. Dis. 34 (Supp.3):S85-92.

Swann, M.M. 1969. Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. Cmnd. 4190. Her Majesty's Stationary Office, London, UK.

Tollefson, L. and W. T. Flynn. 2002. Impact of antimicrobial resistance on regulatory policies in veterinary medicine: status report. Pharm. Sci. 4:1-10.

Turnidge, J. 2004. Antibiotic use in animals-prejudices, perceptions, and realities. J. Antimicrob. Chemother. 53:2-27.

U. S. Food and Drug Administration. (U.S. FDA) 1999. *Veterinary Medicine Advisory Committee Transcript* (*VMAC*). Washington, D.C.: U.S.F.D.A. Center for Veterinary Medicine Website. Available at: http://www.U.S. FDA.gov/ohrms/dockets/ac/cvm99.htm Accessed 31 Aug 2004.

U. S. Food and Drug Administration. (U.S. FDA) 2002. *Discussion of Draft Guidance for Industry: Evaluating the Safety of Antimicrobial New Animal Drugs With Regards to their Microbiological Effects on Bacteria of Human Health Concern.* Washington, D. C.: U.S.F.D.A. Center for Veterinary Medicine Website. Available at: http://www.U.S. FDA.gov/cvm/antimicrobial/eval_safety.htm Accessed 31 August 2004.

van den Bogaard, A.E., N. London, C. Driessen, and E.E. Stobberingh. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers, and poultry slaughterers. J. Antimicrob. Chemother. 47:763-761.

van den Braak,N, E. Power, R. Anthony, H.P. Endtz, H.A. Verbrugh, and A. van Belkum. 2000. Random amplification of polymorphic DNA versus pulsed field gel electrophoresis of *Sma*I DNA macrorestriction fragments for typing strains of vancomycin-resistant enterococci. FEMS Microbiol. Letters 192:45-52.

Werner, G., R. Willems, B. Hildebrandt, I. Klare, and W. Witte. 2003. Influence of transferable genetic determinants on the outcome of typing methods commonly used for *Enterococcus faecium.* J. Clin. Microbiol. 1499-1506.

White, D.G., S. Zhao, P.F. McDermott, S. Ayers, S. Gaines, et al. 2002. Characterization of antimicrobial resistance among *Escherichia coli* 0111 isolates of animal and human origin. Microbial Drug Resis. 8:139-146.

White, P.A., C.J. McIver, and W. D. Rawlinson. 2001. Integrons and gene cassettes in the *Enterobacteriaceae.* Antimicrob. Agents Chemother. 45:2658-2661.

Winokur, P.L., D.L. Vonstein, L.J. Hoffman, E.K. Uhlenhopp, and G.V. Doern. 2001. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob. Agents Chemother. 45:2716-2722.

Witte, W. 2000. Selective pressure by antibiotic use in livestock. Int. J. Antimicrob. Agents. 16: S19-S24.

Zhao, S., S. Qaiyumi, S. Friedman, R. Singh, S.L. Foley, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol. 41(12):5366-5371.

Zhao, S., D. G. White, B. Ge, S. Ayers, S. Friedman, et al. 2001. Identification and characterization of integron mediated antibiotic resistance among shiga toxin producing *Escherichia coli* isolates. Appl. Env. Microbiol. 67:1558-1564.

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## PUBLICATIONS

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