ABSTRACT

QIAN, LEILEI. Evaluation of Antibiotic Resistance Profiles of Enteric Bacteria in Swine Feces Before and After Lagoon Treatment. (Under the direction of Dr. Alexandria Graves.)

Antibiotics are used in livestock production for the treatment of diseases and for improvement of feed efficiency and growth. However, agricultural use of antibiotics may be partly responsible for the emergence of antibiotic-resistant organisms. Large amounts of managed manure are land applied, which opens the door for the spread of antibiotic resistance in the environment. Thus, the goal of this project was to evaluate the effects of lagoon treatment on the persistence of antibiotic resistant enteric bacteria isolated from swine feces. Both cool season and warm season samples were collected from a swine farm located in Sampson County, NC. Each season samples included three nursery swine fecal samples, three nursery swine lagoon liquid samples, four finishing swine fecal samples, three finishing swine lagoon liquid samples, and four soil samples from both nursery and finishing swine spray field. A total of 4032 *E. coli* isolates and 4896 *Enterococcus* isolates were obtained from the samples. The antibiotic resistance profiles of the isolates were determined using a set of antibiotics at various concentrations. The antibiotic cephalothin, erythromycin, oxytetracycline, tetracycline, streptomycin, and neomycin were tested for both bacterial species, but different concentrations were applied. For *E. coli*, rifampicin was also tested; for *Enterococcus*, chlortetracycline, vancomycin, and amoxicillin were also tested. After antibiotic resistance analysis was achieved, 25 isolates were randomly selected from each sample for further evaluation by polymerase chain reaction test. Soil samples were collected; however, fecal indicator bacteria were not recovered. Additionally, *E. coli* was not

recovered from warm season nursery lagoon samples. All isolates displayed multiple antibiotic resistance, and for the isolates from the same source, the resistance patterns were similar for the antibiotics within the same antibiotic family. Percentages of resistant isolates were greater in nursery fecal samples than in finishing fecal samples for majority of antibiotic tests. For nursery samples, percentages of antibiotic resistant isolates decreased after lagoon treatment for majority of antibiotic tests. For finishing samples, no such trend was obvious. The results indicated that antibiotic resistant isolates still persist in the lagoon liquid, which may cause potential risk to human and environmental health. And because antibiotic resistance may affect later therapeutic and subtherapeutic value of these antibiotics, management strategies of agricultural antibiotic use may be improved. The antibiotic resistance patterns and molecular banding patterns of the isolates were not unique to a specific source. The results suggest that there is considerable overlap among nursery feces, nursery lagoon, finishing feces and finishing lagoon samples. However, if combine of the feces and the lagoon isolates together and only classify isolates from nursery to finishing swine, the percentages of correctly classified isolates became larger. The results suggest that ARA and PCR would best be used for identifying fecal contamination from swine sources based on broad categories (nursery versus finishing) instead of relying on these procedures for specific identification of lagoon and feces separately.

EVALUATION OF ANTIBIOTIC RESISTANCE PROFILES OF ENTERIC BACTERIA IN SWINE FECES BEFORE AND AFTER LAGOON TREATMENT

by

LEILEI QIAN

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

SOIL SCIENCE

Raleigh, North Carolina

2007

APPROVED BY:

_____	_____
Dr. Daniel W. Israel	Dr. Eric van Heugten
Soil Science	Animal Science

_____
Dr. Alexandria K. Graves (Chair)

Soil Science

DEDICATION

I dedicate this thesis to

my parents

SUCHENG GE and ZHONG QIAN

and my husband

YUNJIE DING

BIOGRAPHY

Leilei Qian was born in the province of Jiangsu in southeast China.   She was the only child of the family.   She spent 19 years living with her parents in the city of Yancheng. The author moved away from home and began her undergraduate studies at Nanjing University in August of 1999.   In 2003, she received her bachelor degree in Environmental Science and Engineering from Nanjing University and started her graduate studies at the same university.   After two years, she decided to withdraw and step forward to learn something new and to experience the life of studying in the US.   The application went smoothly and the author started her master degree studies in Soil Science at North Carolina State University in August of 2005.

ACKNOWLEDGEMENTS

I would first acknowledge my committee chair Dr. Alexandria Graves and committee members Dr. Daniel Israel and Dr. Eric van Heugten for their devotion and patience during my research.   Without their guidance and support, this thesis could not be achieved.   In particular I would like to thank Dr. Graves for her continued support and encouragement in the past two years.   I would like to thank Joy Smith for working with me on my statistical models.   Without her help, I may not have finished the output by now.   Many thanks go to Dr. Wei Shi and Emily Dell who guided me on how to use the UV machine for printing the fingerprint and the band analyzing software.   I would also like to thank Jay Sullivan and Emily Norris who helped me collecting samples.   Last but not least, I am indebted to my parents and my husband for their constant support, assistance and encouragement throughout the preparation of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ix

LITERATURE REVIEW

I. Introduction

An increasing number of antibiotics are used in US agriculture, especially in the swine industry.   Benefits of antibiotic use in swine industry include the therapeutic value in the treatment of diseases, and the subtherapeutic value for improvement of feed efficiency and growth.   These benefits have helped sustain intensive animal production to meet consumer demands for food products.   However, contrasting the above benefits are suggestions that agricultural use of antibiotics may be partly responsible for the emergence of antibiotic-resistant microorganisms.   The resistant bacteria can be passed on to people through the food supply or through direct contact with manure or livestock (Bicudo and Goyal, 2003).   Antibiotics also enter the environment through application of animal manure containing antibiotics on croplands, or the seepage of lagoon liquid containing antibiotics to ground water.   The predominant manure management for swine manure is the anaerobic lagoon system.   Lagoons are simple to manage and effective in reducing organic matter and nutrients when properly designed and operated.   However, lagoons are not designed to control pathogens (Sobsey et al., 1999).   As a result, there is a great need to determine the effect of manure management on antibiotic resistance of enteric bacteria, especially considering that there is a significant amount of treated manure applied to cropland, thus opening the door for the spread of antibiotic resistant organisms into the environment.

II. Problems Associated with the Occurrence of Antibiotic Resistance

A. The Mechanism and Transmission of Antibiotic Resistance

A.1. Emergence of Antibiotic Resistance

Antibiotics are widely used for treatment of both human and animals because appropriate use of these agents has many benefits.   These benefits include the therapeutic value in the treatment of bacterial infection and the subtherapeutic value for growth promotion and disease prevention.   However, much evidence has demonstrated that inappropriate use of antibiotics has contributed to the emergence of antibiotic resistance. One of the most popular explanations is that antibiotics can not completely kill all target bacteria within the complex microbial communities, especially in agricultural systems (McAllister et al., 2001).   Feeding antibiotics at subtherapeutic levels can accelerate the occurrence of antibiotic resistant bacteria, because more bacteria can survive at low levels of antibiotics.   The relationship between the use of antibiotics and the occurrence of antibiotic resistance is illustrated in Figure 1 (Barbosa and Levy, 2000).   Antibiotic use is the main factor which can lead to antibiotic resistance, but many other factors, although difficult to quantify, such as antibiotic residues, cross selection and travel of people and foodstuffs, can also influence emergence of antibiotic resistance.   Take antibiotic residues for example, animals may not take up all the antibiotics in the feed and the portion of antibiotics not assimilated by animals may be excreted in the animal feces.   Antibiotic residues may enter the environment through the application of the animal waste to croplands or leaching of lagoon liquid to ground water.   These antibiotic residues may cause the emergence of antibiotic resistant microorganisms in the environment.

The occurrence of antibiotic resistance in agricultural systems may have great impacts on human health because agricultural systems have important linkages with the human environment. The antibiotic-resistant bacteria that emerge in agricultural systems can enter human environment through multiple ways, leading to hazardous effects on human health. The emergence and transmission of antibiotic-resistant bacteria as well as the consequences to the environment, the food chain and human health is illustrated in Fig. 2 (Khachatourians, 1998). These bacteria can move and disseminate in the environment through a number of routes, and consequently, their presence may have tremendous negative effects on the environment and human health.

A.2. Mechanisms of Antibiotic Resistance

Bacteria possess a number of antibiotic resistance mechanisms. McAllister et al (2001) have explained and summarized some common mechanisms. One of the most common mechanisms of resistance in bacteria is production of enzymes that degrade the antibiotic (Davies, 1994; McAllister et al., 2001) . For instance, enzymes such as β-lactamases can destroy penicillins and cephalosporins (Levy and Marshall, 2004). Also, some bacteria can produce particular enzymes that inactivate the antibiotic by adding additional chemical structures onto the antibiotic. Bacteria may change their cell surface to reduce the affinity of the antibiotic to its target site. And some bacteria can rapidly pump the antibiotic out of the cell before it has the chance to interact within the cell. Additionally, bacteria can produce a large amount of metabolic products which the antibiotics target and overwhelm the amount of antibiotic. Furthermore, bacteria can develop other metabolic pathways that substitute for the cellular processes that the antibiotic

inhibits. Last but not least, formation of biofilms is also a mechanism associated with antibiotic resistance that is not completely understood. Bacteria may express more than one type of mechanism to resist one antibiotic. For instance, tetracycline resistance can result from either efflux or ribosome protection (Levy and Marshall, 2004). Because some bacteria are sensitive to several antibiotics, whereas others are insensitive, different bacterial species may develop different resistance mechanisms.

A.3. Transfer of Antibiotic Resistance

There are two modes by which antibiotic resistance can be transferred. The antibiotic resistant bacteria can be transferred from one animal to another animal, or from animals to humans through the food chain or contact or drinking the ground water which contains antibiotic resistant bacteria. The other mode is the transfer of antibiotic resistance genes. Bacteria can carry and transfer antibiotic resistance genes because the genetic material of bacteria can be exchanged and transferred to other bacteria. If the genetic material of one bacterium codes for a trait that causes antibiotic resistance, then it is highly possible that the recipient bacteria will become resistant to the same antibiotic. There are several ways that the resistance genes can be transferred among bacteria, such as plasmids, bacteriophages, naked DNA or transposons (Levy and Marshall, 2004). Plasmids are the most frequent way bacteria use to carry and transfer antibiotic resistance genes (McAllister et al., 2001). Bacteria can be infected by bacteriophages during which resistance genes are transferred. Bacteria can also receive resistance genes by scavenging DNA that code for antibiotic resistance from dead bacteria (McAllister et al., 2001). Antibiotic resistance

genes will confer resistance only if they are integrated into the host's chromosome when they are transferred by bacteriophages or dead bacteria (McAllister et al., 2001).

B. Development of Antibiotic Resistance within a Family of Antibiotics

Different antibiotics target different sites in the cell.   For instance, some antibiotics target the cell walls (e.g., cephalosporins, penicillins and glycopeptides), some target cell membranes, some inhibit the synthesis of proteins (e.g., tetracyclines, aminoglycosides and macrolides), some inhibit DNA synthesis, some inhibit RNA synthesis (e.g., rifamycins), or particular biochemical pathways such as folic acid synthesis (Khachatourians, 1998; Levy, 1998).   A description of 20 antibiotic families and their mechanisms of action are indicated in Table 1.

Antibiotics within the same family may have similar chemical structures, which means that they are likely to have similar antibiotic resistance patterns.   For example, oxytetracycline, tetracycline and chlortetracycline all belong to the family of tetracyclines; streptomycin and neomycin fall into the aminoglycosides family; erythromycin belongs to the macrolides family; cephalothin belongs to the cephalosporins family; rifampicin falls into the rifampin family; vancomycin is within the glycopeptides family and amoxicillin belongs to the penicillins family.   For the same bacterial species, these antibiotics within the same family may display similar antibiotic resistance patterns while the antibiotics that belong to different families may have different resistance patterns.

Much research has been done to evaluate the antibiotic resistance patterns of the antibiotics which belong to the same antibiotic family or different families.   Adwan et al (1998) tested 24 *Enterobacteriaceae* isolates which were gentamicin resistant for resistance

to neomycin, kanamycin, tobramycin and amikacin or not.   These antibiotics all belong to aminoglycosides family.   Resistance percentages were 29.2% for neomycin, 58.3% for kanamycin, 45.8% for tobramycin and 8.3% for amikacin.   Fifty eight percent of the isolates were found to be multi-resistant.   Another study (Gautier-Bouchardon et al., 2002) tested the resistance of three avian Mycoplasma species to five antibiotics - enrofloxacin, erythromycin, tylosin, tiamulin, and oxytetracycline.   Enrofloxacin is a member of the fluoroquinolones family of antibiotics.   Erythromycin and tylosin both belong to the macrolides family.   Tiamulin is within the pleuromutilin family, and oxytetracycline belongs to the tetracyclines family.   High level resistance to erythromycin and tylosin developed quickly while resistance to enrofloxacin developed gradually.   No resistance to tiamulin and oxytetracycline could be detected in two of the three species.   Cross-sensitivity tests demonstrated that mycoplasmas which were resistant to tylosin were also resistant to erythromycin, but mutants resistant to erythromycin were not always resistant to tylosin. Some mutants resistant to tiamulin were also resistant to both antibiotics in the macrolides family, whereas enrofloxacin and oxytetracycline did not induce any cross-resistance to others (Gautier-Bouchardon et al., 2002).

C. Occurrence of Antibiotic Resistance in Fecal Indicator Organisms

Fecal coliform bacteria are bacteria found in feces, which usually reside in the intestinal tract of warm-blooded animals.   The presence of fecal coliforms is a reliable indicator of fecal contamination and of the potential presence of pathogens associated with wastewater or sewage sludge (http://en.wikipedia.org/wiki/Fecal_coliform).   However, one must be cautious because the absence of fecal coliforms does not equate to the absence of

fecal contamination.   Indicator organisms are selected according to three factors: (1) they are abundant in the monitored environment; (2) the method for testing for the presence of the indicators is convenient and accurate; (3) there is a reasonable correlation between the presence or absence of the indicators and some specific pathogens (http://www.des.state.nh.us/factsheets/wwt/inc/18.html).   The effectiveness and accuracy of the indicator is determined by the strength of the correlation.

E. coli and Enterococcus are widely used as indicator organisms to signal fecal contamination in water systems.   E. coli is the most reliable indicator of fecal contamination of surface waters in the United States according to water quality standards set by the EPA (www.uvm.edu/envnr/sal/ecoli/pages/waterqu.htm).   Therefore, to study the antibiotic resistance patterns in fecal indicator organisms - E. coli and Enterococcus – has great scientific and practical value.

E. coli

A number of research efforts have been conducted to evaluate the antibiotic resistance patterns of E. coli.   Sunde et al (1998) studied antibiotic resistance of twelve hundred E. coli isolates from swine.   E. coli were isolated from 10 herds of swine with different histories of exposure to antibiotics.   The strains were tested for phenotypic resistance against several antibiotics.   Resistance to streptomycin was found to be most common, followed by sulfonamides and tetracycline.   The highest number of resistant bacteria was found in herds where the use of antimicrobial agents was high.   This study indicates that nonpathogenic E. coli from swine may represent a considerable reservoir of antibiotic resistance genes that might be transferable to pathogens.

Mathew et al (1998) studied the resistance patterns of *E. coli* to five commonly used antibiotics: apramycin, carbadox, gentamicin, neomycin and oxytetracycline. Sows and pigs were sampled from 10 commercial swine farms. A total of 6296 *E. coli* isolates from 750 pig fecal samples and 462 *E. coli* isolates from 50 sow fecal samples were tested. Percentage of resistant organisms was compared between pigs at the different stages of growth, between sows and their respective pigs and between farms of high and low antibiotic use. Farms of high antibiotic use were those where subtherapeutic concentrations of feed-based antibiotics and injectable antibiotics were routinely used. And the farms of low antibiotic use were those where subtherapeutic concentrations of feed-based antiobiotics were not used and injectable antibiotics were not used except for short periods. It was found that oxytetracycline treatments had the greatest percentages of resistance for all samples, around 90%, but according to different ages of pigs and different levels of antibiotic use, percentages were different. For example, when pigs were 7 days old, oxytetracycline resistant percentages were 96.2% and 82.6% for high level antibiotic use and low level use respectively. For pigs which were 35 days old, resistant percentages were 94.4% and 89.9% for high level and low level use respectively. For 63-day-old pigs, oxytetracycline resistant percentages were 98.8% and 99.5% for high level and low level use respectively. This study indicates that patterns of antibiotic resistance are dependent on ages of pigs and levels of antibiotic use.

Enterococcus

Enterococcus is becoming more important due to its increasing prevalence in hospitals and associated multiple antibiotic resistance characteristics. The multiple antibiotic resistant *Enterococcus* strains have significant resistance to many antibiotics, such

as β-lactam-based antibiotics (some penicillins and virtually all cephalosporins) as well as many aminoglycosides, especially virulent strains which are resistant to vancomycin (Vancomycin-Resistant *Enterococcus*) have emerged in nosocomial infections of hospitalized patients (Huycke et al., 1998). Avoparcin is an antibiotic that is only used for animal feeds or veterinary practice but has a similar mode of action as vancomycin (Khachatourians, 1998). The use of avoparcin as a growth promoter in food animals has created a major reservoir of resistant strains of *Enterococcus faecium*.

A study was conducted to determine the correlation between the use of the glycopeptides antibiotic avoparcin as a growth promoter and the occurrence of high level vancomycin resistant *Enterococcus faecium* on poultry and pig farms (Bager et al., 1997). In poultry farms, the relationship was confounded by the use of broad-spectrum antibiotics. In pig farms, the association had a similar magnitude. Sixty-seven percent of pig samples fed with avoparcin contained vancomycin resistant *Enterococcus faecium* isolate, and only 20% of pig samples which did not use avoparcin contained vancomycin resistant *Enterococcus faecium*. This study provides evidence of a causal association between the use of avoparcin and the occurrence of vancomycin-resistant *Enterococcus faecium*, which is very important for the human and community health.

III. Antimicrobial Agents in the Swine Industry

A. The Status of Antimicrobial Agents in the Swine Industry

Antibiotics and chemotherapeutics are medications that have been added to feed of farm animals since the 1950's. These medicinal agents are used to improve the health and performance of farm animals. Chemotherapeutics are different from antibiotics in that they

are organic compounds which are chemically synthesized and also can inhibit the growth of some microorganisms (Carlson and Fangman, 2000).   The amount of these antimicrobial agents usage in animal feeds has increased rapidly, from 1 million kilograms a year around 1960's to approximately 3 million kilograms annually in mid-1980's (Cromwell, 2002).   A list of compounds and use levels that can be used for specific purposes such as growth promotion, prevention of disease, and treatment of a specific disease can be found by consulting the Feed Additive Compendium (Miller Publishing Co, 12400 Whitewater Drive, MN 55343, published annually).   These medications, usages and levels are determined by the Food and Drug Administration (FDA).   At present, there are about 12 antibiotics and 5 chemotherapeutics approved to use in swine feeds.   The antibiotics are apramycin, bacitracin methylene disalicylate, bacitracin zinc, bambermycins, chlortetracycline, lincomycin, neomycin, oxytetracycline, penicillin, tiamulin, tylosin, virginiamycin and the chemotherapeutics are arsanilic acid, carbadox, roxarsone, sulfamethazine, sulfathiazole (Cromwell, 2002; Animal Health Institute).   Some of these antimicrobial agents can be applied for combination usage, such as penicillin, chlortetracycline plus sulfamethazine or sulfathiazole, neomycin plus oxytetracycline (Cromwell, 2002).   The combination usage of penicillin, tetracycline and sulfamethazine or sulfathiazole can control or treat cervical abscesses, bacterial enteritis and atrophic rhinitis in swine, and they are also used to improve weight gain and feed efficiency of pigs, especially for 6 to 16 weeks old pigs (Burbee et al., 1985).   Tetracyclines are widely used in the swine industry as growth promoters, and are also used to control cervical abscesses, atrophic rhinitis, as well as to treat enteric and respiratory infection (Burbee et al., 1985).

B. Mechanisms of Growth Promoting Effects of Antimicrobial Agents

Burbee et al (1985) wrote that "antimicrobial agents are fed at subtherapeutic levels at all stages of the hog growth cycle, but are commonly used during the early pig growing stages".   Gaskins et al (2002) also mentioned that the improvement in growth and feed efficiency are greater for young pigs than for older ones.   Thus, mechanisms for growth promoter activity of antimicrobials should be consistent with their effects on growth, feed efficiency and with the relatively greater responses observed in younger animals than older ones.

It is generally accepted that the beneficial effects of antimicrobial compounds result from alteration of the bacterial population within the animal's digestive tract.   The actual mechanisms by which antibiotics and chemotherapeutics can promote growth are not completely understood throughout a long history of feeding these compounds.   Possible modes of action are: (1) disease control effects, (2) nutrient sparing effects, and (3) metabolic effects (Carlson and Fangman, 2000; Cromwell, 2002).

1   Disease control effects

Antibiotics can control the diseases by suppressing organisms which can cause diseases and produce toxin in the animals' environment (Carlson and Fangman, 2000). Evidence suggests that young pigs which are more vulnerable to diseases than the older ones respond more to antibiotics than the older ones, and that responses to antibiotics are greater in pigs having a high disease load compared with healthy pigs (Cromwell, 2002).

2   Nutrient sparing effects

Nutrient sparing effects of antibiotics are effects on the microbial population of the intestinal tract, which act in three ways (Carlson and Fangman, 2000).   Firstly, depress the

growth of organisms which compete with the host animal for nutrients and can degrade glucose and amino acids, such as *Clostridium difficile* and *Salmonella kedougou*. Secondly, improve the growth of microorganisms (e.g. *Bacteroides*) which synthesize nutrients required by the host animals, such as vitamins and amino acids. Thirdly, reduce the thickness of intestinal wall to improve absorption of nutrient.

3   Metabolic effects

Metabolic effects imply that antibiotics directly influence the metabolic processes, such as change the rate of certain biochemical process in the animals.

Furthermore, antibiotics have great reproductive benefits for sows. Although the mechanism is not fully understood, their role is probably associated with reducing the number of undesirable microorganisms in the reproductive tract which can potentially cause undetectable, subclinical disease (Cromwell, 2002). Subclinical disease is an illness that stays below the surface of clinical detection. Reducing the amount of these microorganisms can provide a healthier environment in the reproductive tract which is beneficial for the embryos to survive.

C. Antibiotic Resistant Bacteria in Swine

Even though antibiotic use in the swine industry has so many advantages, which indeed bring great benefits to both swine producers and consumers, it leads to a big issue for swine health as well as human health - antibiotic resistance. Most concerns about antibiotic resistance in bacteria in swine are directed towards: first, the potential reduction of therapeutic effects of antibiotics on the resistant bacteria in swine; second, the transmission of antibiotic resistant bacteria from swine to the environment and human body, and the

transfer of antibiotic resistant genes from swine enteric bacteria to swine pathogens or even to human pathogens.

There is considerable information on antibiotic resistance found in bacteria in swine. Antibiotic resistance in *E. coli* in swine has been reported by many studies through the long period of antibiotic use in feed (Belloc et al., 2005; Hart et al., 2004; Mathew et al., 1998; Schroeder et al., 2002) and the overall conclusion is that the resistance to antibiotics is very common in *E. coli*. *Enterococcus* did not attract people's interests in antibiotic resistance until vancomycin resistant *Enterococcus faecium* was detected in pigs which were fed with avoparcin (Bager et al., 1997). Antibiotic resistance in *Salmonella* isolates from swine was also detected (Delsol et al., 2005; Gebreyes and Altier, 2002; Gebreyes et al., 2000), which may cause problems in treating infections caused by these bacteria in animals.

IV. Swine Manure Management and Its Effects on Antibiotic Resistance

A. The Anaerobic Lagoon System

The anaerobic lagoon system has become one of the most common components of a swine manure management system from small livestock production units to large specialized operations because they are one of the simplest systems for storing and treating large quantities of swine waste. Even though they were originally viewed as a total disposal system, they are now recognized as a manure management or one pretreatment process in an overall manure management plan. Anaerobic lagoons are widely used in the swine manure management because: (1) flexibility for storage and treatment; (2) they need less land; (3) liquid recycling for waste removal from pits beneath houses; (4) liquid in lagoons can be used to irrigate crops, which is convenient and cost saving; (5) they need less labor and

operating costs (Barker, 1996). However, lagoons also have some disadvantages, such as loss of nutrient value, offensive odors, frequent sludge removal if undersized, and potential groundwater contamination (Barker, 1996).

The anaerobic lagoon is a system in which anaerobic bacteria break down organic matter in the waste. Because anaerobic bacteria decompose the organic matter in the absence of oxygen, there is no requirement to maintain dissolved oxygen in the anaerobic lagoon, which means that anaerobic lagoons can be constructed deeper under the ground and less surface area is needed. Anaerobic lagoons should be properly designed, such as the liquid capacity, the shape, as well as the site investigation, which are important for the function of lagoons. To keep the anaerobic lagoon working in a high efficacy, appropriate management and operation is also needed (Barker, 1996).

Anaerobic digestion processes in anaerobic lagoons can be divided into two stages. First, acid-forming bacteria, such as *Clostridium butyricum* and *Anaerofilum pentosovorans*, convert the manure waste into volatile fatty acids (VFAs). The VFAs are a group of compounds that include acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, hexanoic acid and other higher molecular weight compounds (http://www.valleyair.org/Workshops/postings/05-02-05/VFA%20tech%20assessment.pdf) and they are the primary source of the odor. Second, methanogenic bacteria convert the VFAs into acetic acid and finally into methane, also releasing carbon dioxide and hydrogen gases. The anaerobic digestion of the organic compounds also leads to the formation of inorganic compounds of nitrogen and phosphorus, which can be used to fertilize crops (Cochran, 2000). When the lagoon reaches the maximum wastewater storage level, lagoon

liquid should be applied to the land.   But the nutrient concentration in lagoons differs individually and seasonally.

There is another type of lagoon treatment, the aerobic lagoon, which is not as widely used as anaerobic lagoons in swine manure treatment.   Aerobic bacteria decompose the organic matter in aerobic lagoons which means that aerobic lagoons need some aerators or mechanical agitators to introduce oxygen into the lagoon liquid (Cochran, 2000).

B. Impacts of Lagoon System on Bacterial Loading and Antibiotic Resistance

Swine manure contains many microorganisms, among which some are nonpathogenic, such as nonpathogenic *E. coli*, and some can cause diseases, such as *E. coli* O157:H7, *Enterococcus faecium*, *Salmonella*, *Campylobacter*, *Cryptosporidium*, and *Yersinia* (Guan and Holley, 2003).   Swine lagoon liquid may also contain numerous microorganisms that are at concentrations risky to environment and human health and these pathogenic or nonpathogenic microorganisms may be transported to surface water or ground water through spray irrigation of lagoon liquid to croplands or seepage (Guan and Holley, 2003).   The World Health Organization recommended that the lagoon liquid that contained no more than 3 $\log_{10}$ fecal coliforms per 100 mL could be applied to crops which are likely to be eaten uncooked, and researchers recommended that the maximum concentration should be 5 $\log_{10}$ per 100 mL lagoon liquid which was applied for pasture, cereal crops and fodder crops (Hill and Sobsey, 2003).   Studies have been done to investigate the change in the amount of microorganisms in swine manure before and after lagoon treatment.   The study by Krieger et al (1975) reported that *Salmonella* can be reduced by swine lagoon treatment

from initial population counts of 10^5 - 10^6 per mL to non-recoverable levels in 24 days and fecal coliforms can be reduced from 20 organisms per mL to complete die-off in 10 days.

However, many other studies demonstrated that swine lagoons are not designed to control pathogens. Swine lagoon liquid which was applied to fields contained 100-10000 times more pathogens than human waste treated by municipal treatment systems (Sobsey et al., 1999). The study by Hill and Sobsey (2003) indicated that *E. coli*, *Enterococcus* and *Salmonella* in swine manure could be reduced from 7-8 $\log_{10}$ per 100 mL to 5-6 $\log_{10}$ per 100 mL in single–stage lagoon system and finally to 3-4 $\log_{10}$ per 100 mL in two–stage lagoon systems at four farms in North Carolina. A previous study by the same researchers reported that bacterial indicators were reduced by 3.8 $\log_{10}$ for *E. coli* and 3.3 $\log_{10}$ for *Enterococci* in a lagoon plus wetlands treatment for swine manure at a nursery swine farm in North Carolina (Hill and Sobsey, 1998). These two studies suggested that a secondary treatment system using either lagoon system or surface flow constructed wetlands could reduce microorganism in swine manure to a greater extent than a single-cell anaerobic lagoon. Another study assessed pathogen and indicator microorganism removal from liquid swine manure in multi-step biological and chemical treatment at the Swine Educational Unit at Lake Wheeler Rd. in Raleigh, NC (Vanotti et al., 2005). They analyzed *Salmonella* and fecal contamination indicators from each step of the treatment system. Their results demonstrated a consistent trend in reduction of pathogens and microbial indicators at different steps of treatment.

In a report by Centers for Disease Control and Prevention (CDC, 1998), the presence of microorganisms, antibiotic residues, and organic and inorganic constituents were evaluated in lagoon liquid samples as well as surface water and ground water samples.

Seven lagoon liquid samples, three surface water samples and thirteen ground water samples were collected from swine operations in Iowa. In seven lagoon liquid samples, tetracycline levels were 11, 66, 68, 110, 150, 250, and 540 μg/L, respectively. Sulfonamide levels were all larger than 20 μg/L. β-Lactam levels were 2.1, 2.1, 2.9 and 3.5 μg/L from four of the seven samples. Macrolide levels were 15, 60, 81, 227, 275 μg/L from five out of the seven samples. None of the three surface water samples had detectable levels of antibiotics. One sample out of the 13 ground water samples contained detectable sulfonamide and the level was 7.6 μg/L. Additionally, bacteria numbers and antibiotic resistance patterns were estimated. Samples from the lagoon liquid contained the greatest number of bacterial isolates for *E. coli*, *Enterococcus* and *Salmonella*. Out of the 23 samples, *E. coli* were isolated from 18 samples (78%), *Enterococci* were isolated from 20 samples (87%), and *Salmonella* were isolated from 2 samples (9%). Many of these isolates demonstrated resistance to a particular antibiotic or combination of antibiotics. The results of this study demonstrated the possibility that pathogens could be transported thus posing a threat to ground and surface waters.

Chee-Sanford et al (2001) assessed the occurrence and diversity of bacterial genes resistant to the antibiotic tetracycline in lagoons and migration to ground water. Samples were collected from two swine production sites in Illinois and both of two lagoons were unlined. Site 1 was a finishing swine operation which was located on glacial outwash and terrace deposits along a stream valley. Antibiotics were used in the operation. The average depth of liquid in the lagoon was 1.5 m. The topsoil which was silt (1.3-2 m thick) overlaid a 0.6-1.3 m thick upper layer of fluvial silty sand and gravel outwash. Twelve of the 16 monitoring wells were installed in the upper sand layer. Below this layer were 1.6-3

m of silt loam diamicton, and then a 1-2 m thick lower sand layer which was locally used as an aquifer with four monitoring wells installed in this lower sand layer.   Below this layer was more silt loam diamicton.   The multiple sand layers made the leachate more susceptible to migrate from the lagoon.   Site 2 was a farrowing and nursery operation located on a glacial till plain.   Chlortetracycline was added to the swine feed.   The waste in the lagoon was about 6 m deep.   The silt loam glacial diamicton was 3-15 m thick with a thin glacial gravelly loam layer (less than 30 cm thick).   There were seven wells at the site and two of them were in the thin loam layer.   Ground water samples were collected from 14 monitoring wells at site 1 and 6 monitoring wells at site 2 and lagoon liquid samples were collected from both the two lagoons.   Tetracycline resistant genes were found in DNA extracted from lagoon liquid and the ground water samples which were 250 m downstream from the lagoons.   No detectable tetracycline resistant genes were found from the samples from two of the shallow wells at site 1 and one well at site 2 which located close to the lagoon but vertical to the direction of ground water flow.   The tetracycline resistant gene *tet*(M) detected in the ground water samples was not dominant in the environment and was identical to *tet*(M) from the lagoon.   In addition, the results showed that this gene could be transmitted to some soil microbes.   This study displayed that the antibiotic resistance genes could persist in the swine lagoons and could migrate into ground water, which may be a potential risk to environment and human health.

V. Impacts of Agricultural Use of Antibiotics on Humans

Although inappropriate use of antibiotics in human medicine is the main reason of the occurrence of antibiotic resistance bacteria in the human body, agricultural use of antibiotics

may also have great impacts on human health. First, antibiotic residues may enter the environment and finally reach the human body through a series of food chains or contact or seepage from lagoon to ground water that was consumed by humans. If the animal manure waste or lagoon liquid was applied to croplands, antibiotic residues can also seep down through the soil and enter ground water, potentially drinking water. And unused antibiotics disposed into the sewage system which can not degrade or eliminate the antibiotics may reach surface water and ground water (Kummerer, 2003). However, little data show the occurrence, fate and risks associated with antibiotics entering the environment after being used in agriculture. Second, antibiotic resistant bacteria or antibiotic resistant genes can be transferred from animal microorganisms to human microorganisms. The transfer may occur through the food chain or through animal handlers (Levy and Marshall, 2004). So if the bacteria in animals possess antibiotic-resistance genes, then it is possible for antibiotic resistance to spread from animals to human.

Chee-Sanford's study (2001) that was talked about in the last chapter gave us a great example of the impacts of agricultural use of antibiotics to the environment and humans. The tetracycline resistant genes migrated from swine lagoons to groundwater and traveled about 250 m from the lagoons. In addition, people at the sampled sites were drinking the affected groundwater.

The antibiotic resistance of *E. coli* from humans involved in animal handling such as pig farmers was studied. People with a high level contact with farm animals have significant percentages of antibiotic-resistant *E. coli* in their intestinal microflora (Teuber, 1999). Hunter et al (1994) isolated apramycin-resistant *E. coli* from not only the pigs, but also a stockman, and it appeared that the human isolates carried the same plasmid as that

carried by the pig *E. coli*.   This is good evidence that antibiotic resistant microorganisms or

antibiotic resistance genes can be transferred from animals to humans because apramycin is

not used in human medicine.   Another similar study was conducted for nourseothricin, an

antibiotic growth promoter for animals, but not used in human medicine.   The resistance

was detected in *E. coli* isolated from pigs, pig farm employees and their families, and

residents of nearby villages (Hummel et al., 1986).

Schroeder et al (2002) studied antimicrobial resistance of *E. coli* O157 isolated from

human, swine, cattle and food.   Among a total of 361 *E. coli* O157 isolates, 220 (61%)

isolates were susceptible to all 13 antimicrobials, but 99 (27%) isolates were resistant to

tetracycline, 93 (26%) were resistant to sulfamethoxazole, 61 (17%) were resistant to

cephalothin, and 48 (13%) were resistant to ampicillin.   Swine isolates showed the highest

percentages of resistance.   Tetracycline was not used to treat human enteric infections

frequently, but there were a number of human *E. coli* isolates that showed resistance to

tetracycline.   One possibility was that tetracycline-resistant bacteria were transmitted from

animals to humans via the food chain or occupational exposure.   Other possibilities were

the selection via genetic linkage of resistance determinants or the use of tetracycline to treat

nonenteric bacterial infections in humans (Schroeder et al., 2002).

The other important antibiotic-resistance problem is vancomycin-resistant

Enterococcus in humans.   Accumulating evidence indicated a correlation between the use

of glycopeptides antibiotic avoparcin as a growth promoter for food animals and the

occurrence of vancomycin-resistant *Enterococcus faecium* (Bager et al., 1997;

Khachatourians, 1998).   Furthermore, vancomycin resistant strains as well as resistance

genes can be transferred to humans.   Because *Enterococcus faecium* can cause a wide range

of infections in humans, and they are resistant to a number of antibiotics, vancomycin is the only treatment drug that remains effective (Wegener et al., 1999).   That's why the occurrence of vancomycin-resistant *Enterococcus faecium* attracted so much concern.

Antibiotic resistance can lead to outright treatment failure in humans.   The number of infected people and infection frequency increases and there are more antibiotic-resistant infections that occur in humans.   The infections become more severe which prolongs the duration of infections because antibiotic resistance can be related to the occurrence of more virulent pathogens.   Furthermore, the outcomes will become worse through the choice of an ineffective drug (Barza et al., 2002).

VI. Methods to Identify and Characterize Antibiotic Resistance

A. Antibiotic Resistance Analysis (ARA)

Antibiotic resistance analysis is a phenotypic method to characterize antibiotic resistance patterns and it is widely used in the studies of bacterial source tracking.   The antibiotic resistance profiles of the bacteria are determined using a battery of antibiotics and several concentrations are chosen for each antibiotic so that minimum inhibitory concentration (MIC) can be established.   The antibiotic resistance patterns for *E. coli* and *Enterococcus* can be used to identify the fecal contamination sources.   The premise is that human fecal bacteria will have greater resistance to antibiotics than those from animal sources and fecal bacteria from different animals should have different resistance patterns to various antibiotics (Simpson et al., 2002).

B. PCR based on the 16S-23S rDNA Intergenic Spacer Region of Enteric Bacteria

Ribosomal DNA (rDNA) are sequences encoding ribosomal RNA (rRNA). The rRNA genetic locus, *rrn*, is sufficiently conservative and can be used in a universal organization of evolutionary relationships (Cedergren et al., 1989). PCR analysis of the 16S-23S rDNA intergenic spacer region (ISR) has been used to evaluate *E. coli* and *Enterococcus* isolates and is emerging as a tool in microbial source tracking research. The rDNA operons are typically arranged in the order 16S-ISR-23S-ISR-5S and are present in multiple copies. For example, *E. coli* has seven rrn operons (Condon et al., 1992). The *Enterococcus* spp. genome contains as many as six operons (Sechi and Daneomoore, 1993). *E. faecalis* is reported to have four operons (Acinas et al., 2004). The absence of selection pressures in the ISR region, as opposed to the highly conserved nature of the bordering rDNA, has proven useful as a target site for the molecular subtyping of a variety of pathogenic bacteria (Chun et al., 1999; Graham et al., 1996; Gurtler and Stanisich, 1996; Stubbs et al., 1999). Universal primers targeted to conserved sites in the 16S and 23S rDNA genes can be used to amplify the ISR (Jensen et al., 1993). Therefore, PCR based on the 16S-23S rDNA ISR can be performed on all microorganisms and yet can discriminate between species and strains (Anton et al., 1998).

VII. Objectives of Research

In conclusion, a perusal of the literature suggests that antibiotic resistant *E. coli* and *Enterococcus* are prevalent in the feces of swine and swine lagoon liquid. The resistance patterns of these enteric bacteria may be different based on the types and quantities of antibiotics administered to swine. The presence of antibiotic resistant bacteria may cause

potential risk to the environment and human health.   Limited research has been conducted

on the change of antibiotic resistance in enteric bacteria before and after lagoon treatment.

Therefore, the objectives of this study were: first, to use ARA to determine the effects of

lagoon treatment on the patterns of antibiotic resistance in *E. coli* and *Enterococcus*; second,

to use ARA to evaluate the patterns of antibiotic resistance in *E. coli* and *Enterococcus* in

different stages of swine growth.   The final objective was to evaluate the molecular banding

patterns of *E. coli* and *Enterococcus* in feces and lagoon liquid after treatment with the use of

PCR.

MATERIALS AND METHODS

I. Research Site Description

The study was conducted at a nursery and finishing swine facility located within Sampson County, NC.   The nursery facility housed 2200 nursery pigs ranging in age of 2 weeks to 8 weeks old.   Three separate swine houses held pigs at different stages of development.   The pigs entered the house at an age of around 2 weeks old and remained in this house until being moved to the finishing facility.   Pigs in house one were moved in the earliest so they were the oldest, and pigs in house three were the youngest.   Antibiotics added to the nursery swine feed were tilmicosin, chlortetracycline and lincomycin in cool season; tilmicosin, chlortetracycline, lincomycin, tylosin and tiamulin in warm season.

The finishing swine facility housed around 2500 swine.   The finishing facility had four separate swine houses.   The swine entered the finishing facility at approximately 8 to 9 weeks of age and were held in the house until being sent to slaughter.   The swine were sent to slaughter at approximately 26 weeks of age.   The ages of the swine in different houses were different.   Swine in house one were moved in first and they were the oldest; swine in house four were moved in last and were the youngest.   Antibiotics added to the finishing swine feed were chlortetracycline, lincomycin and tylosin in cool season; chlortetracycline, lincomycin and virginiamycin in warm season.

Each swine facility had an anaerobic lagoon to treat swine manure before spraying the effluent on the fields.   The lagoons were 12 to 15 years old and the sludge had never been removed.   The nursery lagoon was approximately 45 x 45 m in size and the finishing lagoon was approximately 118 x 45 m in size.   The nursery houses were flushed at least

once a week, maybe twice a week during summer months.   The finishing houses on the other hand were on a continuous flushing system and were flushed every 4 hours.   The frequency of spraying the fields depended on the need of the crops and on maintenance of liquid level below the maximum liquid level allowed by waste management regulations. Lagoon liquid applications averaged 2 times a year.   The cropping system in the fields during the time of the study was corn, and wheat double crop soybean.   Soybean was no tilled drilled into stubble after harvest of wheat.   The soil type of the spray fields at both the nursery swine facility and the finishing swine facility is a Norfolk series; a Fine-loamy Siliceous Thermic Typic Kandiudults.

II.   Sample Collection

There were two sampling events.   One was in December of 2005, which represented the cool season samples.   The other was in July of 2006, which represented the warm season samples.   Samples from the nursery and finishing facilities were collected during both sampling events.   Composite nursery fecal samples were collected from each of the three nursery swine houses, and finishing fecal samples were collected from each of four finishing swine houses.   Fecal samples were obtained randomly along the pens from each house and mixed to make one composite sample per house.   Wearing new disposable latex gloves and using a sterilized Whirl-Pac bag to pick up the feces, samples were placed in an individual sterilized Whirl-Pac bag.   The bags were labeled with date, source of sample, and other pertinent information before storing on ice in a cooler.   To eliminate cross contamination, a separate pair of fresh disposable gloves and Whirl-Pac bag was used for collecting each individual sample.   Three lagoon liquid samples were collected at three

different sites within the lagoon at both the nursery facility and finishing facility.   Samples

were collected from edge of the lagoon using a bottle attached to a long pole.   The sites

were randomly selected.   During sample collection, the bottle was not allowed to move into

the sludge layer.   Lagoon liquid samples were stored in sterile plastic bottles and kept on ice

in the cooler.   Four soil samples were collected from each of the spray fields adjacent to the

nursery facility and finishing facility.   Sampling locations from within the fields were

selected at random.   The upper 0-20 cm of soil were collected, stored on ice and transported

to the lab to be analyzed within 6 hours.

III. Isolation of *E. coli* and *Enterococcus*

E. coli

        Soil samples were subdivided into two portions, which were 0-5 cm from surface and

below the 5 cm layer.   All the fecal, liquid, and subdivided soil samples were diluted in

10-fold serial dilutions with sterile distilled water.   Dilutions were spread on m-FC agar

(Becton, Dickinson and Company, Sparks, MD) for culturing *E. coli*.   The m-FC plates

were incubated for 24 h at 44.5ºC in a water bath (Hurst and Crawford, 2002).   Dark blue

colonies were indicative of fecal coliforms.   Fecal coliforms were later confirmed as *E. coli*

with the use of the colilert system (IDEXX Laboratories Inc, Westbrook, ME) (Hurst and

Crawford, 2002).   Sterile toothpicks were used to transfer the isolates from m-FC plates to

96-microwell trays (pre-sterilized) filled with 200 μl colilert system.   Every colony was

selected randomly and equally from the m-FC plates.   The isolate was scraped from the

plates with the toothpick and thoroughly inoculated into one well of the 96-microwell trays.

After 24 h at 37ºC in an incubator, the presence of *E. coli* was confirmed if microwells turned fluorescent under ultraviolet light (Graves et al., 2002; Hurst and Crawford, 2002).

Enterococcus

Serial dilutions were spread on m-Enterococcus agar (Becton, Dickinson and Company, Sparks, MD) to culture *Enterococcus*.   The plates were incubated for 48 h at 37ºC in an incubator.   After incubation, red to burgundy colored colonies were indicative of *Enterococcus*.   Sterile toothpicks were used to transfer the isolates to 96-microwell trays (pre-sterilized) filled with 200 µL Enterococcosel broth (Becton, Dickinson and Company, Sparks, MD).   After the 48 h incubation at 37ºC, isolates were confirmed as *Enterococcus* when the Enterococcosel broth turned black.   The black color was a result of esculin hydrolysis by the bacteria (Hurst and Crawford, 2002).

IV. Antibiotic Resistance Analysis (ARA)

A. Stock solution preparation

One hundred milliliter of each antibiotic stock solution was prepared according to Table 2.   These antibiotics were chosen because they are commonly used in both animals and humans, and are very important for both animal and human health.   The antibiotic resistance study based on these antibiotics has scientific and practical value.

B.   Antibiotic Resistance Analysis (ARA) for *E. coli* and *Enterococcus*

The antibiotic resistance profiles of the isolates were determined using a battery of antibiotics.   Isolates were inoculated onto plates containing different concentrations of each antibiotic.   For *E. coli*, antibiotics were cephalothin, erythromycin, oxytetracycline,

tetracycline, streptomycin, neomycin, and rifampicin; for *Enterococcus*, antibiotics were cephalothin, erythromycin, oxytetracycline, tetracycline, streptomycin, neomycin, chlortetracycline, vancomycin, and amoxicillin.

Sets of ARA plates were prepared by adding different concentrations of antibiotics to Trypticase Soy Agar (TSA) (EMD Chemicals Inc, Darmstadt, Germany) before pouring plates. Four grams of TSA were added into 100 ml distilled water in flasks, autoclaved and cooled. Plates with different antibiotic concentrations were prepared by adding different volumes of stock solutions to TSA flasks which were poured into plates. Different antibiotic concentration tests were used for *E. coli* and *Enterococcus* (Table 3.). These concentrations were chosen because they were widely used in antibiotic resistance studies and bacterial source tracking research, which means that this research can also be used to expand the bacterial source tracking data library.

The isolates were transferred with a stainless steel 48-prong replica-plater from 96-microwell trays to a set of TSA plates containing various concentrations of antibiotics and to a control plate containing no antibiotic. Any isolate that did not grow on the control plates were not used in the analysis. The plates were incubated at 37°C (air incubator) for 24 h for *E. coli* and for 48 h for *Enterococcus*. After that, the growth of each isolate for each concentration of every antibiotic was determined. In this study, only the isolates which displayed whole, fulfilled, round circles in the control plates were analyzed by ARA or PCR. An isolate was considered to be resistant to a given concentration of antibiotic if growth on the antibiotic containing plate was comparable to the growth on control plate. For this result the isolate was recorded as "1". Any isolate that did not show comparable

growth in antibiotic containing plates as the control plates (i.e., that did not exhibit whole, fulfilled, round circles in the antibiotic containing plates) was recorded as "0".

V.   Polymerase Chain Reaction

In order to characterize the genetic banding patterns of antibiotic resistant isolates, 25 isolates were randomly selected from the ARA control plates for each sample for PCR analysis.   Three major steps were taken to achieve the banding patterns: PCR amplification, restriction fragment length polymorphism (RFLP) and gel electrophoresis.

A. PCR Amplification

Individual colony from the ARA control plates that was resuspended in sterile water was used as DNA template.   Each PCR reaction contained 10 µL 5X Green Go Taq Flexi Buffer, 5 µL $MgCl_2$ Solution, 1 µL Nucleotide Mix, 0.25 µL Go Taq Flexi DNA Polymerase, 30.75 µL Nuclease Free Water (Promega Corporation, Madison, WI), 1 µL Primers (Invitrogen Molecular Probes, Eugene, OR), and 1 µL DNA Template (Rademaker and de Bruijn, 1997).   Intergenic spacer regions located between the 16S and 23S rDNA regions of *E. coli* and *Enterococcus* were amplified.   Primers were designed manually that would anneal to highly conserved downstream 16S rDNA and upstream 23S rDNA sequences.   *E. coli* fingerprints were obtained by using 16S primer (5'-GGGAACCTGCGGTTGGATCA-3') and 23S primer (5'-CCGTGTACGCTTAGTCGCTTA-3').   *Enterococcus* fingerprints were obtained by using 16S primer (5'- GCCTAAGGTGGGATAGATGA-3') and 23S primer (5'-CCCGTCCTTCATCGGCTCCTA-3').   PCR amplification was performed with a thermocycler (Eppendorf AG, Hamburg, Germany).   The temperature program consisted of

94 ºC for 6 min, followed by 35 cycles consisting of 94 ºC for 1 min, 57 ºC for 1 min, and extension at 72 ºC for 1 min.   The reaction was terminated with a final extension step at 72 ºC for 7 min and was finally held at 4 ºC.

B. Restriction Fragment Length Polymorphism (RFLP)

Composition of digestion components was as follows: 0.5 μL restriction enzyme, 2 μL 10x buffer, 1 μL bovine serum albumin acetylated (Promega Corporation, Madison, WI), 5.5 μL sterile distilled water, and 10 μL PCR product.   Restriction enzymes used were *Rsa* I for *E. coli* and *Mbo* I for *Enterococcus*.   The tubes were centrifuged to ensure proper mixing and were incubated at 37 ºC for 3.5 hours.   The reaction was stopped by placing the tubes in the freezer.

C. Electrophoresis Protocols

Agarose gel was prepared by 3% Agarose low-melting agarose (Fisher, Fair Lawn, NJ).   One point five μL of marker (100 bp ladder, Promega Corporation, Madison, WI) and 9 μL 6x loading dye (Fermentas) were mixed on parafilm.   Three point five μL of the mixture was loaded into the first and last well of the gel.   Two to 2.4 μL of loading dye and 2 to 2.2 μL of each RFLP reaction product were mixed on parafilm and then loaded into each well of agarose gel.   The gels were electrophoresed at 100 V for 80 min and stained overnight in Sybr Green I (Invitrogen Molecular Probes, Eugene, OR) at a ratio of 10 μL per 200 mL water.   Gel images were captured in a UV machine (Syngene GeneGenius gel documentation system, Syngene, Frederick, MD) the next day.

VI. Statistical Analysis

Antibiotic Resistance Analysis data were analyzed using JMP (version 5.1; SAS Institute, Cary, NC), SAS (version 8; SAS Institute, Cary, NC) and Microsoft Office Excel (Microsoft Corporation, Redmond, WA).   Percentages of antibiotic resistant isolates were calculated by JMP and Excel.   The data were first sorted by season and bacteria, and the subsequent analysis was conducted separately for each classified group.   The percentages of antibiotic resistant isolates from the nursery and finishing fecal samples based on swine stages of development (nursery and finishing) and antibiotic tests (Table 3) were analyzed in SAS as a split-plot experimental design.   The growth stage of the swine was the whole plot variable, and the antibiotic tests was the split plot variable.   The significance level considered throughout the study was 0.05.   The SAS statistical code can be found in Appendix A.

Gel images were analyzed by Gel-Pro Analyzer software (version 6.0; Media Cybernetics, Silver Spring, MD).   The positions of fragments on each gel were normalized by using the 100 bp ladder from 100 to 1500 bp as an external reference standard, and the normalization with the same external standard helped to compare multiple gels.

Additionally, the discriminant analysis tool in JMP was used to evaluate the ARA and PCR binary data for their applicability to microbial source tracking.   Discriminant analysis is similar to cluster analysis, which can allow us to cluster data based on similarities. Discriminant analysis can produce a correct classification set for every known source isolate (nursery feces, nursery lagoon, finishing feces and finishing lagoon).   The rate of correct classification is determined by averaging the percentages of correctly classified isolates for

31

each source.   The purpose for analyzing ARA and PCR binary data by discriminant analysis

is to determine the amount of overlap between source categories.

RESULTS AND DISCUSSION

I. Numbers of *E. coli* and *Enterococcus* Recovered for Analysis

E. coli or *Enterococcus* isolates were not recovered from soil samples for both cool and warm sampling seasons.   There were no isolates grown on both m-FC and mEnterococcus media for all 10-fold serial soil sample dilutions.   The number of *E. coli* and *Enterococcus* isolated from feces and lagoon samples for both cool and warm seasons are indicated in Table 4.   A total amount of 2496 *E. coli* isolates and 2496 *Enterococcus* isolates were isolated from cool season samples, which were three nursery fecal samples, three nursery lagoon liquid samples, four finishing fecal samples, and three finishing lagoon liquid samples.   For the warm season samples, samples were collected from the same sources as cool season samples.   No *E. coli* isolates were recovered from nursery lagoon samples large populations of other fecal coliform overpopulated the m-FC agar plates.   For the same reason *E. coli* was isolated from only one finishing lagoon liquid sample out of three.   Other media, such as EMB, was used to culture *E. coli* from nursery lagoon samples, however, isolation was unsuccessful.   A total of 1536 *E. coli* isolates and 2400 *Enterococcus* isolates were isolated from warm season samples.

The numbers of *E. coli* and *Enterococcus* isolates analyzed by antibiotic resistance analysis (ARA) are indicated in Table 5.   Isolates that did not grow on the ARA control plates were not evaluated by antibiotic resistance analysis.   A total of 2491 *E. coli* isolates and 1220 *Enterococcus* isolates from cool season samples were analyzed by ARA.   A total of 1525 *E. coli* isolates and 769 *Enterococcus* isolates from warm season samples were analyzed by ARA.

The numbers of *E. coli* and *Enterococcus* isolates evaluated by PCR are indicated in Table 6. For majority of the samples, 25 isolates were randomly selected from each sample. These isolates were selected from the isolates that were analyzed by ARA. Some of samples had less than 25 isolates analyzed by PCR because the amount of the isolates grown on the ARA control plates was less than 25. A total of 325 *E. coli* isolates and 314 *Enterococcus* isolates from cool season were analyzed by PCR. A total of 175 *E. coli* isolates and 275 *Enterococcus* isolates from warm season were analyzed by PCR.

II. Antibiotic Resistance Analysis (ARA)

Three pairs of comparisons were conducted for antibiotic resistance analysis (ARA) data. These comparisons include evaluation of nursery fecal and nursery lagoon liquid samples, the comparison between finishing fecal and finishing lagoon liquid samples, and the comparison between nursery fecal and finishing fecal samples. The difference between the fecal and the lagoon liquid samples can indicate whether the antibiotic resistant bacteria persist after lagoon treatment. The difference between nursery fecal and finishing fecal samples may indicate whether swine development influence percentages of enteric bacteria resistant to antibiotics.

A. Cool Season *E. coli*

A.1. Nursery Fecal and Nursery Lagoon Liquid Samples

The percentages of antibiotic resistant *E. coli* isolates from nursery fecal and nursery lagoon liquid samples are illustrated in Figure 3. All antibiotic concentrations are indicated in Table 3. The results of resistance to rifamycin, cephalothin and erythromycin which belong to three different antibiotic families are shown in Figure 3 (a). Percentages of *E.*

coli isolates resistant to rifamycin and erythromycin were greater those from nursery fecal samples than those from nursery lagoon samples.   In addition, the percentages for nursery fecal samples did not change much according to the concentrations, around 35% for rifamycin and 80% for erythromycin; however, the percentages for nursery lagoon liquid samples displayed a sharp decrease with the increase of antibiotic concentration, from 29.7% at RIF60 to 4.4% at RIF90, and from 84.3% at ERY60 to 49% at ERY100.   For cephalothin test, the resistant isolate percentages increased from around 52% of the nursery feces to about 80% after lagoon treatment.

The percentages of resistant isolates to oxytetracycline and tetracycline, which belong to the same antibiotic family-the tetracycline family are illustrated in Figure 3 (b).   The percentages of *E. coli* isolates from both samples resistant to oxytetracycline and tetracycline were very large, close to 100% at all concentrations except at TET15.

The results for streptomycin and neomycin tests which belong to the aminoglycosides family are displayed in Figure 3 (c).   The percentages of resistant isolates were larger in nursery feces than nursery lagoon liquid at all streptomycin concentrations and NEO10. Furthermore, the percentages for nursery fecal samples did not change according to the concentrations for streptomycin except for STR15, but the percentages for nursery lagoon liquid samples decreased with increasing the antibiotic concentration.   However, almost 80% of the isolates from nursery lagoon were resistant to the STR15 level.

Overall, the percentages of *E. coli* resistant isolates from nursery feces were obviously larger than nursery lagoon liquid for rifamycin, erythromycin, streptomycin, and TET15 (Figure 3).   For cephalothin, the percentages of resistant isolates from nursery lagoon liquid were larger than isolates from feces.   All isolates were resistant to antibiotics

in the tetracycline family.

A.2. Finishing Fecal and Finishing Lagoon Liquid Samples

The percentages of antibiotic resistant *E. coli* isolates from finishing fecal and finishing lagoon liquid samples are illustrated in Figure 4.   The results of resistance to rifamycin, cephalothin and erythromycin which belong to three different antibiotic families are shown in Figure 4 (a).   At RIF60 and RIF75, the percentages of *E. coli* isolates from finishing lagoon liquid samples were slightly greater than those from finishing fecal samples, but at RIF90, the percentages of finishing fecal samples were larger than those from finishing lagoon liquid samples.   For cephalothin and erythromycin, resistant-isolate percentages displayed an obvious increase after lagoon treatment.   The percentages of resistant isolates to these three antibiotics showed a decrease when the concentration of antibiotic increased.

The percentages of resistant *E. coli* isolates to oxytetracycline and tetracycline which belong to the same antibiotic family are illustrated in Figure 4 (b).   All the percentages were very large for finishing fecal samples, close to 100%.   The percentages of oxytetracycline resistant isolates from finishing lagoon samples were very large too, close to 100% for all the concentrations.   Tetracycline resistant isolates displayed decreasing percentages after lagoon treatment to around 86%.

The percentages of resistant *E. coli* isolates to streptomycin and neomycin isolated from finishing fecal and lagoon liquid samples are shown in Figure 4 (c).   At STR2.5, STR5.0 and NEO10, percentages decreased after lagoon treatment.   At STR7.5, STR10, and STR15, percentages increased after lagoon treatment.   For streptomycin, the percentages displayed a decreasing trend with an increase of the antibiotic concentration.

To sum up, finishing lagoon liquid had smaller percentages of resistant *E. coli* isolates than finishing fecal samples for RIF90, and tetracycline. The lagoon liquid had greater percentages of resistant isolates for cephalothin and erythromycin.

A.3. Nursery Fecal and Finishing Fecal Samples

The percentages of resistant *E. coli* isolates of nursery fecal and finishing fecal samples are illustrated in Figure 5. Percentages of *E. coli* isolates resistant to rifamycin, cephalothin, and erythromycin are shown in Figure 5 (a). For these antibiotics, nursery fecal samples had greater percentages of resistant isolates than finishing fecal samples.

From Figure 5 (b), it is apparent that both nursery and finishing fecal samples close to 100% of *E. coli* isolates were resistant to tetracycline.

Nursery fecal samples had greater percentages of resistant isolates for streptomycin and neomycin than finishing fecal samples (Figure 5 (c)). And with the increase of antibiotic concentration, percentages of resistant isolates from nursery fecal samples did not change much, from 100% to 95%. But percentages for finishing fecal samples displayed an obvious decrease, from 100% to 44.3%. Percentages of isolates from both nursery and finishing fecal samples were large, close to 100% and did not decrease with increasing antibiotic concentrations.

Overall, for most antibiotic test, nursery fecal samples had larger percentages of resistant *E. coli* isolates than finishing fecal samples.

A statistical model was used to analyze the significance of antibiotic tests and the swine stages of development (nursery or finishing). The data was first classified by season (cool and warm) and bacteria (*E. coli* and *Enterococcus*), and the subsequent analysis was

conducted separately for each classified group.   The percentages of antibiotic resistant

isolates from the nursery and finishing fecal samples based on the swine stages of

development and antibiotic tests were analyzed in SAS as a split-plot experimental design.

The stages of development of the swine was the whole plot variable, and the antibiotic test

was the split plot variable.   The significance level considered throughout the study was p <

0.05.   The P-values for the variables are indicated in Table 7.   The antibiotic test was

statistically significant for all the isolates.   Swine development was statistically significant

for *Enterococcus* in cool season and *E. coli* in warm season.   Stages of Development and

antibiotic test interaction was statistically significant for *Enterococcus* in cool season and *E.

coli* in warm season, which means that for these two classified group, the application of a

certain type of antibiotics at a certain level was different for nursery and finishing swine.

The output of the SAS model for significance tests of percentage means of resistant

isolates from nursery and finishing swine fecal samples for different antibiotic tests for *E.

coli* in cool season are indicated in Appendix B (p-values can be found in Table 2 of

Appendix B).   The significance level considered throughout the study was p < 0.05.   For

the antibiotic tests of CEP25, CEP35, ERY90, ERY100, STR10, and STR15, the percentage

means of nursery and finishing fecal samples were significantly different.   In addition, for

each sample source, the significance test of the percentage means based on antibiotic

concentrations can also be found from the tables in Appendix B.   For nursery swine fecal

samples, percentage means were not significant based on the levels for all the antibiotics.

For finishing swine fecal samples, percentage means were not significant based on levels for

rifamycin, cephalothin, oxytetracycline, tetracycline, and neomycin.   For erythromycin,

percentage means were not significantly different for ERY60 and ERY70; percentage means

were not significantly different for ERY90 and ERY100, either; but percentage means at ERY60 and ERY70 were significantly different from that at ERY90 and ERY100. For streptomycin, there were no significant difference between adjacent concentrations, but were significantly different for other concentrations.

B. Cool Season *Enterococcus*

B.1. Nursery Fecal and Nursery Lagoon Liquid Samples

Percentages of resistant *Enterococcus* isolates from nursery fecal and nursery lagoon liquid samples are illustrated in Figure 6. All antibiotic levels are indicated in Table 3. Percentages of resistant isolates decreased after lagoon treatment for all the antibiotic levels except vancomycin. For nursery fecal samples, percentages were all greater than 80%. After lagoon treatment, the percentages of isolates resistant to cephalothin dropped to 27.4% at CEP10, and 2.9% at CEP50. For erythromycin, the percentages of resistant isolates decreased to around 60% after lagoon treatment. For amoxicillin, percentages of resistant isolates decreased from 81.9% in the feces to 12.1% in the lagoon liquid. However, for vancomycin, 0.2% of the isolates displayed resistance in the nursery fecal samples; but there were about 11.3% isolates resistant to vancomycin in the lagoon liquid.

For chlorotetracycline, the percentages were about 5% less for the lagoon samples than for fecal samples. For oxytetracycline, percentages were 50% less after lagoon treatment at OTC20 and 70% less at OTC100; for tetracycline, percentages were 44% less at TET10 and 78% less at TET100. In addition, percentages of fecal samples did not change much with increasing antibiotic concentrations, but percentages of resistant isolates from lagoon liquid samples decreased as the antibiotic concentration increased. In addition, the

three antibiotics which belong to the same antibiotic family had similar resistant patterns within the same source.

Percentages of *Enterococcus* isolates resistant to streptomycin and neomycin decreased after lagoon treatment. The percentages of resistant isolates from fecal samples were around 90%, but they decreased to around 57% in the lagoon liquid samples. Additionally, two antibiotics which were in the same antibiotic family had similar resistant patterns within the same sample source.

To summarize, for majority of the antibiotic tests, percentages of resistant *Enterococcus* isolates were lower in lagoon samples than fecal samples, except for vancomycin. Antibiotics which belong to the same antibiotic family had similar resistant patterns within the same source.

B.2. Finishing Fecal and Finishing Lagoon Liquid Samples

Percentages of resistant *Enterococcus* isolates from finishing fecal and finishing lagoon liquid samples are illustrated in Figure 7. Percentages of resistant isolates from finishing fecal samples were less than those of the lagoon samples for majority of antibiotic tests except vancomycin. For vancomycin, the percentage decreased 4% after lagoon treatment. For cephalothin and amoxicillin, percentages were around 22% higher in samples of lagoon liquid. For erythromycin, percentages of resistant isolates were 5% higher in lagoon samples than in fecal samples.

For three tetracycline family antibiotics, percentages of resistant isolates were 20% higher in lagoon samples than in fecal samples. The percentages of resistant isolates decreased with increasing of antibiotic concentrations for both fecal and lagoon samples and

the three antibiotics displayed similar resistant patterns.

Percentages of resistant isolates were 20% higher in lagoon samples than in fecal samples for streptomycin and neomycin.   *Enterococcus* resistant patterns were similar for these two antibiotics.

Overall, percentages of resistant isolates were greater in lagoon liquid samples than in fecal samples for the majority of antibiotic tests.   In addition, antibiotics which belong to the same antibiotic family had similar resistant patterns.

B.3. Nursery Fecal and Finishing Fecal Samples

Percentages of resistant *Enterococcus* isolates from nursery fecal and finishing fecal samples are illustrated in Figure 8.   Nursery fecal samples had much greater percentages of resistant isolates than finishing fecal samples for all antibiotic tests except vancomycin.   All percentages of resistant isolates from nursery fecal samples except vancomycin were very high, in the range of 81.9% to 99.3%.   But the percentages of finishing fecal samples were in the range of 8.8% to 77.2%.

The output of the SAS model for significance tests of percentage means of resistant *Enterococcus* isolates from nursery and finishing swine fecal samples for different antibiotic tests in cool season are indicated in Appendix C (p-values can be found in Table 2 of Appendix C).   The significance level considered throughout the study was $p < 0.05$.   For all antibiotic test levels except CTC60 and CTC80, the percentage means of resistant isolates from nursery and finishing fecal samples were significantly different.   Additionally, for nursery samples, percentage means were not significant based on the concentrations of all the antibiotic tests.   For finishing swine samples, percentage means were not significant

based on the levels by cephalothin, erythromycin, streptomycin and neomycin.   For

chlorotetracycline, percentage means were not significantly different between CTC60 and

CTC80, CTC80 and CTC100, but were significantly different for CTC60 and CTC100.   For

oxytetracycline and tetracycline, there were no significant difference between adjacent

concentrations, but were significantly different for other concentrations.

C. Warm Season *E. coli*

C.1. Finishing Fecal and Finishing Lagoon Liquid Samples

Percentages of resistant *E. coli* isolates from finishing fecal and finishing lagoon

liquid samples collected in warm season are illustrated in Figure 9.   For rifamycin and

erythromycin, percentages of resistant isolates were obviously greater in lagoon samples than

in fecal samples.   For neomycin, percentages were lower in lagoon samples.   No obvious

trend was found for other antibiotics.   Also, antibiotics within the same family displayed

similar resistance patterns for the isolates from the same source.

C.2. Nursery Fecal and Finishing Fecal Samples

Percentages of resistant *E. coli* isolates from nursery fecal and finishing fecal samples

are illustrated in Figure 10.   For majority of antibiotic tests, nursery fecal samples had

larger resistant percentages than finishing fecal samples.   For NEO2.5, finishing fecal

samples had larger percentages of resistant isolates than nursery fecal samples.   Percentages

were smallest for the rifamycin test, all less than 16%.

The output for the significance tests of percentage means of resistant *E. coli* isolates

from nursery and finishing swine fecal samples by different antibiotic tests in warm season

was indicated in Appendix D (p-values can be found in Table 2 of Appendix D). The significance level considered throughout the study was $p < 0.05$. The percentage means of resistant isolates from nursery and finishing fecal samples were significantly different for the test of ERY60, ERY70, ERY90, ERY100, STR10, STR15, and NEO2.5. Additionally, for nursery samples, percentage means were not significant based on the levels for all the antibiotic tests. For finishing swine samples, percentage means were not significant based on the levels for riphamycin, cephalothin, oxytetracycline, and tetracycline. For erythromycin, percentage means were not significantly different among ERY60, ERY70, and ERY90, but these three were significantly different from ERY100. For streptomycin, percentage means of STR2.5 and STR5.0 were significantly different from STR7.5 and STR10. Percentage mean of STR10 was not significantly different from STR15, but percentage mean of STR7.5 was significantly different from STR15. For neomycin, percentage means of NEO2.5 was significantly different from NEO5 and NEO10.

D. Warm Season *Enterococcus*

D.1. Nursery Fecal and Nursery Lagoon Liquid Samples

Percentages of resistant *Enterococcus* isolates from warm season nursery fecal and nursery lagoon liquid samples are illustrated in Figure 11. Nursery fecal samples showed larger percentages of resistant isolates than lagoon liquid samples for most of the antibiotic tests except VAN2.5 and TET100. For cephalothin, erythromycin, chlorotetracycline, oxytetracycline, tetracycline, and neomycin, the percentages decreased as antibiotic concentrations increased for both fecal and lagoon liquid samples. For streptomycin, percentages did not change much with the increase of antibiotic levels for fecal samples.

Percentages of vancomycin resistant isolates were very low for both fecal and lagoon liquid samples, 2.6% in the fecal samples and 3.5% in the lagoon samples.   Percentages of amoxicillin resistant isolates displayed a large decrease after lagoon treatment, from 31.7% in the fecal samples to 3.9% in the lagoon samples.   In addition, chlorotetracycline, oxytetracycline, and tetracycline showed similar trends, as percentages of resistant isolates decreased with the increasing of antibiotic concentrations.

D.2. Finishing Fecal and Finishing Lagoon Liquid Samples

The percentages of resistant *Enterococcus* isolates from warm season finishing fecal and finishing lagoon liquid samples are compared in Figure 12.   Percentages of resistant isolates from fecal samples were larger than for lagoon samples for the majority of antibiotic tests except vancomycin, streptomycin and neomycin.   In addition, it was very obvious that the antibiotics within the same antibiotic family had similar resistant patterns.   Isolates resistant to chlorotetracycline, oxytetracycline and tetracycline all displayed an obvious decreased trend with increasing of antibiotic levels.   For both streptomycin and neomycin percentages of resistant isolates did not decrease much as antibiotic levels increased.

D.3. Nursery Fecal and Finishing Fecal Samples

Percentages of resistant *Enterococcus* isolates from warm season nursery fecal and finishing fecal samples are illustrated in Figure 13.   For cephalothin, percentages of resistant isolates were larger in finishing fecal samples than in nursery feces.   Percentages of resistant isolates in nursery feces were larger than in finishing feces for all erythromycin tests.   Both cephalothin and erythromycin tests, percentages displayed a decreased trend

with increasing levels of antibiotics. Percentages of vancomycin resistant *Enterococcus* were very low for both nursery and finishing fecal samples, which was 2.6%. Percentage of amoxicillin resistant *Enterococcus* from nursery feces was larger, which was 31.7% in the nursery feces and 8.5% in the finishing feces.

Percentages of resistant isolates in nursery feces were larger than in finishing fecal samples for majority of tests except CTC60 and CTC80. In addition, the resistant patterns were similar for the three antibiotics; all displayed a similar trend with percentages decreasing with increasing of antibiotic levels.

Percentages of *Enterococcus* resistant to streptomycin and neomycin from nursery fecal samples were larger than for finishing fecal samples for all the antibiotic levels. And the trends of the change of percentages with the increase of the levels were similar for these two antibiotics, which was that the changes were not much as the antibiotic levels increased.

Overall, for majority antibiotic test, percentages of resistant *Enterococcus* from nursery fecal samples were larger than that from finishing fecal samples. The output for the significance tests of percentage means of resistant *Enterococcus* isolates from nursery and finishing swine fecal samples by different antibiotic tests in warm season are indicated in Appendix E (p-values can be found in Table 2 of Appendix E). The significance level considered throughout the study was $p < 0.05$. The percentage means of resistant isolates from nursery and finishing swine fecal samples were significantly different at all the levels of streptomycin and neomycin; means were not significantly different for other antibiotic tests. In addition, for nursery swine fecal samples, the percentage means were not statistically significant based on levels for erythromycin, chlorotetracycline, streptomycin and neomycin. For cephalothin, percentage means were statistically significant except for

two adjacent concentrations.   For oxytetracycline, percentage means of OTC20 were significantly different from OTC60, OTC80 and OTC100; percentage means of OTC40 were significantly different from OTC80 and OTC100.   For tetracycline, percentage means of TET100 was significantly different from other levels; and percentage means of TET50 was significantly different from TET10 and TET15.   For finishing swine samples, the percentage means were not statistically significant based on levels for streptomycin and neomycin.   For cephalothin, percentage means of CEP10 and CEP15 were significantly different from CEP50.   For erythromycin, percentage means of ERY10 was significantly different from ERY50.   For chlorotetracycline, percentage means of CTC60 was significantly different from CTC100.   For oxytetracycline, percentage means of OTC20 was significantly different from other levels; and percentage means of OTC100 was significantly different from OTC40 and OTC60.   For tetracycline, there were no significant difference between adjacent concentrations, but were significantly different for other concentrations.

E. Summary of ARA

E. coli or *Enterococcus* isolates were not recovered from any of the soil samples in both cool and warm season.   *E. coli* were not isolated from nursery lagoon liquid samples collected in the warm season because plates were overgrown with too many other species of bacteria.   *E. coli* and *Enterococcus* isolates from all the fecal and lagoon liquid samples displayed multiple antibiotic resistance.   All the isolates showed resistance to more than one antibiotic.   For the isolates from the same source, the resistance patterns were similar for the antibiotics within the same antibiotic family, because the antibiotics from the same antibiotic family had similar chemical structure to which the bacteria may have similar resistance

mechanisms.   *E. coli* showed higher percentages of resistance to antibiotics within

tetracycline family compared to other antibiotic tests.   Tetracycline family antibiotics have

widely been used as growth promoter for animals for a long period of time, which may have

caused the enteric bacteria to be highly resistant to these antibiotics.   Similar results were

found from other research (Langlois and Dawson, 1986; Mathew et al., 1998).

Additionally, vancomycin resistant *Enterococcus* was detected in this study.   Even though

the percentages of resistance were not great, this is relevant because vancomycin is an

important antibiotic to cure the disease caused by *Enterococcus*, therefore, emergence of

vancomycin resistant *Enterococcus* should attract people's attention.

No apparent seasonal variation was found by comparing the ARA data of cool season

and warm season.   It was important to compare the fecal samples and the lagoon liquid

samples because lagoons are widely used as a means to treat manure.   After lagoon

treatment, the effluent is applied to neighboring fields; as a result, antibiotic resistant bacteria

that persist after lagoon treatment may contribute to potential environmental health risks.   It

was also important to compare the bacteria antibiotic resistance from nursery swine sites and

finishing swine sites.   The comparison between these two stages of development can show

the effect of swine growth on the enteric bacteria antibiotic resistance.   According to the

results, for nursery swine site samples, percentages of antibiotic resistant isolates of both *E.

coli* and *Enterococcus* decreased after lagoon treatment for majority of antibiotic tests.   For

finishing swine site samples, no such trend was obvious.   Percentages of resistant *E. coli*

and *Enterococcus* isolates were greater in nursery fecal samples than in finishing fecal

samples for majority of antibiotic tests and some percentage means were significantly

different.   Similar results were observed by others which reported that higher percentages of

resistant bacteria were found in young animals than the old ones (Langlois and Dawson, 1986; Linton et al., 1972; Mathew et al., 1998).   Explanations for this may be that bacteria can more easily colonize the intestinal tract of younger swine than the old ones, or higher potential for resistance transfer between bacteria in intestinal tract of young swine (Langlois and Dawson, 1986; Mathew et al., 1998).

Furthermore, the levels of the antibiotics in this study were chosen according to the bacterial source tracking research so that the results of this study could be added to the database for future bacterial source tracking work.   Several concentrations were used for each antibiotic in order that minimum inhibitory concentration (MIC) can be reached.   The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (i.e. colonies on a plate or turbidity in broth culture) under standard conditions.   Because determination of MIC tests of the isolates was not conducted in this study, we can not verify this.   Bager er al (Bager et al., 1997) found that *Enterococcus faecium* displayed high-level resistance to vancomycin with MIC > 64 µg/mL on pig farms.   Delsol et al (Delsol et al., 2005) found out that the MIC profiles of *Enterococcus* isolated from avilamycin prior treated pigs had the MIC profiles of tetracycline ranging between 0.25 to 32 mg/L and vancomycin of 0.5 to 2 mg/L.   Similar test were done for Enteric *E. coli*.   The MIC profiles were erythromycin 32-64 mg/L, and tetracycline ranging from 0.25 to 256 mg/L.   Therefore, the MIC value of the isolates may vary according to samples and isolates.   Interestingly, even isolates recovered from the same sample source, may have different MIC values.

The results in this study suggest that populations of *E. coli* isolates were reduced from 6.9 $\log_{10}$ per 100 mL to 5.3 $\log_{10}$ per 100 mL in the lagoon, which is similar to results reported by Hill and Sobsey (2003).   To the contrary, the results for *Enterococcus* did not

show much difference before and after lagoon treatment, both populations were around 4.7 $\log_{10}$ per 100 mL.

Because most swine remained healthy and pathogenic tests were not conducted in this study, we can not explain how antibiotic resistance affected the health of the animals or effects on the efficiency of potential therapeutic value.   But it might be suggested that performance benefits associated with subtherapeutic use may be compromised by large-scale resistance.

III.   PCR

Figure 14 and 15 showed the typical fingerprints of *E. coli* and *Enterococcus* isolates generated by PCR.   The banding patterns of isolates from different sample sources were similar, but not always identical.   Molecular weight of each band was analyzed and the percentages of isolates having bands at specific molecular weight regions are illustrated in Figure 16 to 26.   The molecular weight was divided into 16 regions, 50 bp increments from 100 bp fragments to 800 bp fragments.   This was done because there were very few bands smaller than 100 bp and larger than 800 bp, and most 50 bp regions within 100 to 800 bp had only one band within them.   For *E. coli* bands in the region of < 200 bp and for *Enterococcus* in the region of < 100 bp were fuzzy and probably were the fingerprints of the primers.   Band lengths were quantified and converted to binary data in each region.

A. Cool Season *E. coli*

A.1. Nursery Fecal and Nursery Lagoon Liquid Samples

The banding patterns of *E. coli* isolated from nursery fecal and nursery lagoon liquid samples in cool season are illustrated in Figure 16.   Very small percentages of *E. coli*

isolates had bands at < 150 bp, 201-250bp, and > 700 bp regions for nursery fecal and lagoon liquid samples, less than 10%.   Most of the bands fell in the region of 251 to 700 bp. Nursery fecal samples had higher percentages of isolates with bands in the region of 251-300 bp and 351-400 bp than nursery lagoon liquid samples.   While nursery lagoon liquid samples had higher percentages of isolates with bands in the region of 401 to 700 bp.

A.2. Finishing Fecal and Finishing Lagoon Liquid Samples

Banding patterns of *E. coli* isolated from four finishing fecal and three finishing lagoon liquid samples are indicated in Figure 17.   The banding patterns of the two source samples were similar.   Finishing fecal samples had higher percentages of *E. coli* isolates with bands in the region of 251 to 600 bp and 701-750 bp.   While finishing lagoon liquid samples had higher percentages of isolates having band in the region of 601-650 bp and 751-800 bp.

A.3. Nursery Fecal and Finishing Fecal Samples

Banding patterns of *E. coli* isolated from nursery fecal and finishing fecal sample are illustrated in Figure 18.   Both had around 80% of the isolates with bands in the region of 251-300 bp.   Finishing fecal samples had higher percentages of isolates having bands in the majority of molecular weight regions except 351-400 bp and 601-650 bp.

B. Cool Season *Enterococcus*

B.1. Nursery Fecal and Nursery Lagoon Liquid Samples

Figure 19 displayed the banding patterns of *Enterococcus* isolated from nursery fecal and nursery lagoon liquid samples in cool season. *Enterococcus* had different banding patterns from *E. coli*. The bands mostly fell in the region <500 bp. The banding patterns of isolates from nursery fecal and nursery lagoon liquid were similar. Close to 100% *Enterococcus* isolates have bands in the region of 151 to 200 bp. Nursery fecal samples had higher percentages of isolates found bands in the region of 201 to 250 bp than nursery lagoon liquid samples, which was around 90% for the fecal samples and 40% for the lagoon liquid samples. Nursery lagoon had about 10% more isolates having bands in the regions of 251-300 bp and 451-500 bp and 10% less isolates having bands in the region of 301-350 bp than nursery fecal samples. They had similar percentages in the region of 351-450 bp.

B.2. Finishing Fecal and Finishing Lagoon Liquid Samples

Percentages of *Enterococcus* isolated from finishing fecal and finishing lagoon liquid samples in cool season with bands at specific molecular weight regions were illustrated in Figure 20. Finishing fecal samples had higher percentages of isolates with band in the regions of 100-200 bp and 401-450 bp. While finishing lagoon had higher percentages of isolates with bands in the region of 201-400bp.

B.3. Nursery Fecal and Finishing Fecal Samples

Banding patterns of *Enterococcus* isolated from nursery fecal and finishing fecal samples in cool season are presented in Figure 21. Nursery fecal samples had higher

percentages of *Enterococcus* isolates with bands in the majority of regions except 100-150 bp.   Nursery fecal samples had greater than 90% of the isolates having bands in the region of 151-250 bp and around 90% of the isolates with bands in the region of 301-350 bp.

C. Warm Season *E. coli*

C.1. Finishing Fecal and Finishing Lagoon Liquid Samples

Percentages of *E. coli* isolated from finishing fecal and finishing lagoon liquid samples in warm season with bands at specific molecular weight regions are illustrated in Figure 22.   Finishing fecal samples had about 30% more isolates with bands in the region of 351 to 400 bp than finishing lagoon liquid samples.   While finishing lagoon liquid samples had higher percentages of isolates with bands in the other regions.

C.2. Nursery Fecal and Finishing Fecal Samples

Banding patterns of *E. coli* isolated from nursery fecal and finishing fecal samples in warm season are indicated in Figure 23.   Isolates from the two samples displayed different banding patterns.   Finishing fecal samples had 10% to 20% more isolates with bands in the regions of 201 to 300 bp, and about 50% more isolates had bands within 351 to 400 bp. Nursery fecal samples had higher percentages of isolates with bands in the other molecular weight regions.

D. Warm Season *Enterococcus*

D.1. Nursery Fecal and Nursery Lagoon Liquid Samples

Banding patterns of *Enterococcus* isolated from nursery fecal and nursery lagoon liquid samples in warm season are illustrated in Figure 24.   Banding patterns of the isolates from two samples were similar except in the region of 351 to 450 bp.   In the region of 351 to 400 bp, nursery fecal samples had about 50% more isolates having bands than nursery lagoon liquid samples.   But in the region of 401 to 450 bp, nursery lagoon liquid samples had 20% more *Enterococcus* isolates with bands than nursery fecal samples.

D.2. Finishing Fecal and Finishing Lagoon Liquid Samples

Banding patterns of *Enterococcus* isolated from finishing fecal and finishing lagoon liquid samples in warm season are illustrated in Figure 25.   Isolates from the two samples displayed similar banding patterns.   With much as 80% of the isolates have bands in 151 to 200 bp region for both two samples.   Around 50% of the isolates have bands in the region of 201 to 300 bp and 70% of the isolates have bands in the region of 301 to 350 bp.   Ten percent to 20% more isolates from finishing fecal samples have bands in the 351 to 450 bp region than isolates from finishing lagoon liquid samples.

D.3. Nursery Fecal and Finishing Fecal Samples

Small differences in banding patterns of *Enterococcus* isolated from nursery fecal and finishing fecal samples in warm season were observed (Figure 26).   Nursery fecal samples had higher percentages of isolates having bands in the regions of 151 to 250 bp and 351 to 400 bp than finishing fecal samples, which were about 10% more in 151 to 200 bp, 28%

more in 201 to 250 bp, and 40% more in 351 to 400 bp.   On the other hand, around 20% more isolates from finishing fecal samples have bands in the regions of 251 to 350 bp, and 401 to 500 bp.

IV. Discriminant Analysis of ARA and PCR Data

A. Discriminant Analysis of ARA Data

Cool Season *E. coli*

Results of discriminant analysis for ARA data of cool season *E. coli* isolates from four source categories are illustrated in Table 8.   The isolates analyzed by discriminant analysis were the ones that were also selected by PCR evaluation.   The bold data mean the percentages of isolates that are correctly classified in the source category.   Take finishing feces for example, 100 isolates were recovered from finishing fecal samples.   73% of these 100 isolates were clustered correctly as the finishing feces; 5% of them were closely related to finishing lagoon; 6% of the isolates were closely related to nursery feces; and 16% of the isolates were similar to nursery lagoon.   For finishing lagoon samples, the percentage of correct classification is 52%; for nursery fecal samples, the percentage of correct classification is 66.67%; and for nursery lagoon samples, the percentage of correct classification is 80%.   This indicates much overlap of antibiotic resistance patterns among sources.   If finishing feces and finishing lagoon were combined as a finishing source, and nursery feces and nursery lagoon were combined as a nursery source, the percentages of correct classification by these two source categories became higher.   The percentages of correct classification were 74.86% for the finishing source and 82% for the nursery source (Table 9).

Cool Season *Enterococcus*

Results of discriminant analysis for ARA data of cool season *Enterococcus* isolates from four source categories are shown in Table 10.   Also, the bold data mean the percentages of isolates that are correctly classified in the source category.   For finishing fecal samples, the percentage of correct classified isolates is 72.34%; for finishing lagoon samples, the percentage of correct classification is 56%; for nursery fecal samples, the percentage of correct classification is 97.33%, which is very high; and for nursery lagoon samples, the percentage of correct classification is 39.73%.   There are also a lot of overlaps of antibiotic resistance patterns among sources.   If feces and lagoon were combined, the percentages of correct classification by these two source categories were 78.7% for finishing source and 68.24% for nursery source (Table 11).

Warm Season *E. coli*

Results of discriminant analysis for ARA data of warm season *E. coli* isolates from four source categories are shown in Table 12.   Also, the bold data mean the percentages of isolates that are correctly classified in the source category.   For finishing fecal samples, the percentage of correct classified isolates is 89%; for finishing lagoon samples, the percentage of correct classification is 52%; for nursery fecal samples, the percentage of correct classification is 92%, which is very high.   There are some overlaps of antibiotic resistance patterns among sources.   If feces and lagoon were combined, the percentages of correct classification by these two source categories increased, which were 92.8% for finishing source and 92% for nursery source (Table 13).

Warm Season *Enterococcus*

Results of discriminant analysis for ARA data of warm season *Enterococcus* isolates from four source categories are illustrated in Table 14. Also, the bold data mean the percentages of isolates that are correctly classified in the source category. For finishing fecal samples, the percentage of correct classified isolates is 60%; for finishing lagoon samples, the percentage of correct classification is 73.85%; for nursery fecal samples, the percentage of correct classification is 70.67%; and for nursery lagoon samples, the percentage of correct classification is 40%. There are also many overlaps of antibiotic resistance patterns among sources. If feces and lagoon were combined, the percentages of correct classification by these two source categories were 81.43% for finishing source and 64.44% for nursery source (Table 15).

B. Discriminant Analysis of PCR Data

Cool Season *E. coli*

Discriminant analysis results of the percentages and number for PCR data of cool season *E. coli* isolates from four sources are indicated in Table 16. Considerable overlap between sources was observed. The percentage of isolates which was correctly classified as finishing feces was high, 89%. But the percentages of the other three sources were low, all less than 70%. However, the results of classification of isolates by nursery and finishing source categories were better (Table 17). Both the percentages of correct classification were larger than 70%.

Cool Season *Enterococcus*

The percentages and number of cool season *Enterococcus* isolates which were correctly classified for PCR banding patterns by four sources are displayed in Table 18. There was also considerable overlap among sources. The percentage of correct classification for nursery feces was large, close to 90%; the percentages of correct classification for other three sources were low, especially for the finishing lagoon, which was only 38.67%. The percentages of correct classification became larger if combine feces and lagoon together, which were around 80% for both nursery and finishing source category (Table 19).

Warm Season *E. coli*

The percentages and number of warm season *E. coli* isolates which were correctly classified for PCR banding patterns by three source categories are displayed in Table 20. The percentages of correct classification for finishing feces and nursery feces were both high, no less than 80%; but the percentage of correct classification for finishing lagoon was very low, 44%. The results for classifying isolates by nursery and finishing swine were better, around 80% (Table 21).

Warm Season *Enterococcus*

Discriminant analysis results of the percentages and number for PCR banding patterns of warm season *Enterococcus* isolates correctly classified by four sources are indicated in Table 22. There was considerable overlap among the four sources. The percentages of correctly classified isolates by four sources were low except for nursery feces.

If combining feces and lagoon together, the percentages of correct classification became

larger, which were 56.97% for finishing source and 71.72% for nursery source (Table 23).

Discriminant analysis has been widely used in bacterial source tracking research

(Dickerson et al., 2007; Graves et al., 2007; Graves et al., 2002).   Even though source

tracking research was not conducted in this study, analyzing ARA and PCR data in this

method can give us a clear picture of how much overlap is between different source

categories.   In addition, this method can help us to determine what data is valuable to add to

the bacterial source tracking database.   Antibiotic resistance patterns and molecular banding

patterns of the isolates were not unique to a specific source.   The results of the discriminant

analysis displayed that there was considerable overlap among nursery feces, nursery lagoon,

finishing feces and finishing lagoon sources.   If combining feces and lagoon together, the

percentages of correct classification became larger.   The results suggest that both antibiotic

resistance analysis and PCR would best be used for identifying fecal contamination from

swine sources based on broad categories (nursery versus finishing) instead of relying on

these procedures for specific identification of lagoon and feces separately.

CONCLUSIONS

The objectives of this study were to compare the antibiotic resistance patterns and molecular fingerprint patterns of enteric bacteria *E. coli* and *Enterococcus*: 1) before and after lagoon treatment; 2) in two different stages of swine development (nursery and finishing); 3) in two seasons (warm and cool). *E. coli* and *Enterococcus* isolates from all the fecal and lagoon liquid samples displayed multiple antibiotic resistance. Isolates recovered from the same source had resistance patterns similar for the antibiotics within the same antibiotic family. *E. coli* showed higher percentages of resistance to antibiotics within the tetracycline family compared to other antibiotic tests. Vancomycin resistant *Enterococcus* was detected and 9% of the isolates were resistant to vancomycin. The ARA results of cool season and warm season were not statistically different. Percentages of resistant *E. coli* and *Enterococcus* isolates were greater in nursery fecal samples than in finishing fecal samples for the majority of antibiotic tests and some percentage means were significantly different. For nursery swine samples, percentages of antibiotic resistant isolates of both *E. coli* and *Enterococcus* decreased after lagoon treatment for the majority of antibiotic tests. For finishing swine site samples, no such trend was obvious. Even though percentages of antibiotic resistant isolates decreased after lagoon treatment for nursery swine site, some antibiotic resistant isolates still survived in the lagoon liquid for both sampling sites, which may cause potential risk to human and environmental health. Because antibiotic resistance may affect later therapeutic and subtherapeutic value of these antibiotics, new management strategies for use of agricultural antibiotics may need to be developed.

The antibiotic resistance patterns and molecular banding patterns of the isolates were not unique to a specific source. The results displayed substantial overlap among nursery feces, nursery lagoon, finishing feces and finishing lagoon samples. However, when the feces and the lagoon isolates were combined and classified as nursery and finishing swine source, the percentages of correctly classified isolates were close to 80%. The results suggest that ARA and PCR would best be used for identifying fecal contamination from swine sources based on broad categories (nursery versus finishing) instead of relying on these procedures for specific identification of lagoon and feces separately.

Because most swine remained healthy and pathogenic tests were not conducted in this study, how antibiotic resistance affected the health of the animals or effect on the efficiency of potential therapeutic value can not be determined. However, performance benefits associated with subtherapeutic use may be compromised by development of large-scale resistance. In the future work, pathogenic tests should be conducted to determine whether the antibiotic resistance can influence the therapeutic value of the antibiotics. Since specific primers were not designed and antibiotic resistance genes were not sequenced in this study, whether the PCR fingerprints represent the antibiotic resistance genes can not be determined. In the future work, specific primers should be designed to amplify specific antibiotic resistance genes and the genes should be sequenced to determine whether the isolates contain specific antibiotic resistance genes.

REFERENCES

Acinas, S.G., L.A. Marcelino, V. Klepac-Ceraj, and M.F. Polz. 2004. Divergence and
  redundancy of 16S rRNA sequences in genomes with multiple rrn operons. Journal of
  Bacteriology 186:2629-2635.

Adwan, K., N. Abu-Hasan, and H. Al-Asmar. 1998. Analysis of neomycin, kanamycin,
  tobramycin and amikacin resistance mechanisms in gentamicin-resistant isolates of
  Enterobacteriaceae. Journal of Medical Microbiology 47:1019-1021.

Animal Health Institute. Feed additive compendium. Minnapolis. Miller Publishing
  Company.

Anton, A.I., A.J. Martinez-Murcia, and F. Rodriguez-Valera. 1998. Sequence diversity in the
  16S-23S intergenic spacer region (ISR) of the rRNA operons in representatives of the
  Escherichia coli ECOR collection. Journal of Molecular Evolution 47:62-72.

Bager, F., M. Madsen, J. Christensen, and F.M. Aarestrup. 1997. Avoparcin used as a growth
  promoter is associated with the occurrence of vancomycin-resistant Enterococcus
  faecium on Danish poultry and pig farms. Preventive Veterinary Medicine 31:95-112.

Barbosa, T.M., and S.B. Levy. 2000. The impact of antibiotic use on resistance development
  and persistence. Drug Resistance Updates 3:303-311.

Barker, J.C. 1996. Lagoon design and management for livestock waste treatment and storage
  EBAE 103-83. North Carolina cooperative extension service.

Barza, M., S. Gorbach, and S.J. DeVincent. 2002. The need to improve antimicrobial use in
  agriculture: Ecological and human health consequences - Introduction. Clinical
  Infectious Diseases 34:S71-S72.

Belloc, C., D.N. Lam, J.L. Pellerin, F. Beaudeau, and A. Laval. 2005. Effect of quinolone treatment on selection and persistence of quinolone-resistant Escherichia coli in swine faecal flora. Journal of Applied Microbiology 99:954-959.

Bicudo, J.R., and S.M. Goyal. 2003. Pathogens and manure management systems: A review. Environmental Technology 24:115-130.

Burbee, C.R., R. Green, and M. Matsumoto. 1985. Antibiotics in Animal Feeds - Risks and Costs. American Journal of Agricultural Economics 67:966-970.

Carlson, M.S., and T.J. Fangman. 2000. Swine Antibiotics and feed additives: food safety consideration. Agricultural Pub. G2353.

Cedergren, R., M.W. Gray, Y. Abel, and D. Sankoff. 1989. The Evolutionary Relationships among Known Life Forms. Journal of Molecular Evolution 28:98-112.

Chee-Sanford, J.C., R.I. Aminov, I.J. Krapac, N. Garrigues-Jeanjean, and R.I. Mackie. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Applied and Environmental Microbiology 67:1494-1502.

Chun, J., A. Huq, and R.R. Colwell. 1999. Analysis of 16S-23S rRNA intergenic spacer regions of Vibrio cholerae and Vibrio mimicus. Applied and Environmental Microbiology 65:2202-2208.

Cochran, K. 2000. Dollars and Sense-an economic analysis of alternative hog waste management technologies Environmental Defense.

Condon, C., J. Philips, Z.Y. Fu, C. Squires, and C.L. Squires. 1992. Comparison of the Expression of the 7 Ribosomal-Rna Operons in Escherichia-Coli. Embo Journal 11:4175-4185.

Cromwell, G.L. 2002. Why and how antibiotics are used in swine production. Animal

   Biotechnology 13:7-27.

Davies, J. 1994. Inactivation of Antibiotics and the Dissemination of Resistance Genes.

   Science 264:375-382.

Delsol, A.A., L. Randall, S. Cooles, M.J. Woodward, J. Sunderland, and J.M. Roe. 2005.

   Effect of the growth promoter avilamycin on emergence and persistence of

   antimicrobial resistance in enteric bacteria in the pig. Journal of Applied

   Microbiology 98:564-571.

Dickerson, J.W.J., C. Hagedorn, and A. Hassall. 2007. Detection and remediation of

   human-origin pollution at two public beaches in Virginia using multiple source

   tracking methods. Water Research (Accepted).

Gaskins, H.R., C.T. Collier, and D.B. Anderson. 2002. Antibiotics as growth promotants:

   Mode of action. Animal Biotechnology 13:29-42.

Gautier-Bouchardon, A.V., A.K. Reinhardt, M. Kobisch, and I. Kempf. 2002. In vitro

   development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and

   oxytetracycline in Mycoplasma gallisepticum, Mycoplasma iowae and Mycoplasma

   synoviae. Veterinary Microbiology 88:47-58.

Gebreyes, W.A., and C. Altier. 2002. Molecular characterization of multidrug-resistant

   Salmonella enterica subsp enterica serovar Typhimurium isolates from swine. Journal

   of Clinical Microbiology 40:2813-2822.

Gebreyes, W.A., P.R. Davies, W.E.M. Morrow, J.A. Funk, and C. Altier. 2000.

   Antimicrobial resistance of Salmonella isolates from swine. Journal of Clinical

   Microbiology 38:4633-4636.

Graham, T., E.J. GolsteynThomas, V.P.J. Gannon, and J.E. Thomas. 1996. Genus- and species specific detection of Listeria monocytogenes using polymerase chain reaction assays targeting the 16S/23S intergenic spacer region of the rRNA operon. Canadian Journal of Microbiology 42:1155-1162.

Graves, A.K., C. Hagedorn, R.L. Brooks, and E. Martin. 2007. Microbial source tracking in a rural watershed dominated by cattle. Water Research (In Press).

Graves, A.K., C. Hagedorn, A. Teetor, M. Mahal, A.M. Booth, and R.B. Reneau. 2002. Antibiotic resistance profiles to determine sources of fecal contamination in a rural Virginia watershed. Journal of Environmental Quality 31:1300-1308.

Guan, T.Y., and R.A. Holley. 2003. Pathogen survival in swine manure environments and transmission of human enteric illness: A review. Journal of Environmental Quality 32:383-392.

Gurtler, V., and V.A. Stanisich. 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology-Uk 142:3-16.

Hagedorn, C., S.L. Robinson, J.R. Filtz, S.M. Grubbs, T.A. Angier, and R.B. Reneau. 1999. Determining sources of fecal pollution in a rural virginia watershed with antibiotic resistance patterns in fecal streptococci. Applied and Environmental Microbiology 65:5522-5531.

Hart, W.S., M.W. Heuzenroeder, and M.D. Barton. 2004. Antimicrobial resistance in Campylobacter spp., Escherichia coli and enterococci associated with pigs in australia. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 51:216-221.

Harwood, V.J., J. Whitlock, and V. Withington. 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: Use in predicting the source of fecal contamination in subtropical waters. Applied and Environmental Microbiology 66:3698-3704.

Hill, V.R., and M.D. Sobsey. 1998. Microbial indicator reductions in alternative treatment systems for swine wastewater. Water Science and Technology 38:119-122.

Hill, V.R., and M.D. Sobsey. 2003. Performance of swine waste lagoons for removing Salmonella and enteric microbial indicators. Transactions of the Asae 46:781-788.

Hummel, R., H. Tschape, and W. Witte. 1986. Spread of Plasmid-Mediated Nourseothricin Resistance Due to Antibiotic Use in Animal Husbandry. Journal of Basic Microbiology 26:461-466.

Hunter, J.E.B., M. Bennett, C.A. Hart, J.C. Shelley, and J.R. Walton. 1994. Apramycin-Resistant Escherichia-Coli Isolated from Pigs and a Stockman. Epidemiology and Infection 112:473-480.

Hurst, C.J., and R.L. Crawford. 2002. Manual of environmental microbiology. 2nd ed. ASM Press, Washington, D.C.

Huycke, M.M., D.F. Sahm, and M.S. Gilmore. 1998. Multiple-drug resistant Enterococci: The nature of the problem and an agenda for the future. Emerging Infectious Diseases 4:239-249.

Jensen, M.A., J.A. Webster, and N. Straus. 1993. Rapid Identification of Bacteria on the Basis of Polymerase Chain Reaction-Amplified Ribosomal DNA Spacer Polymorphisms. Applied and Environmental Microbiology 59:945-952.

Khachatourians, G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal 159:1129-1136.

Krieger, D.J., J.H. Bond, and C.L. Barth. 1975. Survival of Salmonella, total coliforms, and fecal coliforms in swine waste lagoon effluents. Proc. of the 3rd International symposium on livestock wastes, St. Joseph, MI.

Kummerer, K. 2003. Significance of antibiotics in the environment (vol 52, pg 5, 2003). Journal of Antimicrobial Chemotherapy 52:317-317.

Langlois, B.E., and K.A. Dawson. 1986. Antibiotic resistance in pigs following a 13 year ban. J. Animal Science:18-32.

Levy, S.B. 1998. The challenge of antibiotic resistance. Scientific American 278:46-53.

Levy, S.B., and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine 10:S122-S129.

Linton, K.B., P.A. Lee, A.J. Rowland, V.N. Baker, Gillespi.Wa, and M.H. Richmond. 1972. Antibiotic Resistance and Transmissible R-Factors in Intestinal Coliform Flora of Healthy Adults and Children in an Urban and a Rural Community. Journal of Hygiene-Cambridge 70:99-&.

Mathew, A.G., W.G. Upchurch, and S.E. Chattin. 1998. Incidence of antibiotic resistance in fecal Escherichia coli isolated from commercial swine farms. Journal of Animal Science 76:429-434.

McAllister, T.A., L.J. Yanke, G.D. Inglis, and M.E. Olson. 2001. Is antibiotic us in dairy cattle causing antibiotic resistance? . Advances in dairy technology:229-247.

Rademaker, J.L.W., and F.J. de Bruijn. 1997. Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer assisted pattern analysis. DNA Markers:151-172.

Schroeder, C.M., C.W. Zhao, C. DebRoy, J. Torcolini, S.H. Zhao, D.G. White, D.D. Wagner, P.F. McDermott, R.D. Walker, and J.H. Meng. 2002. Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food. Applied and Environmental Microbiology 68:576-581.

Sechi, L.A., and L. Daneomoore. 1993. Characterization of Intergenic Spacers in 2 Rrn Operons of Enterococcus-Hirae Atcc-9790. Journal of Bacteriology 175:3213-3219.

Simpson, J.M., J.W. Santo Domingo, and D.J. Reasoner. 2002. Microbial source tracking: State of the science. Environmental Science & Technology 36:5279-5288.

Sobsey, M.D., V.R. Hill, and D. Cole. 1999. Public Health Impact of Intensive Livestock Operations: Human Communicable Disease Concerns. First Annual Public Health Symposium: Public Health Impact of Intensive Livestock Operations, Raleigh, NC.

Stubbs, S.L.J., J.S. Brazier, G.L. O'Neill, and B.I. Duerden. 1999. PCR targeted to the 16S-23S rRNA gene intergenic spacer region of Clostridium difficile and construction of a library consisting of 116 different PCR ribotypes. Journal of Clinical Microbiology 37:461-463.

Sunde, M., K. Fossum, A. Solberg, and H. Sorum. 1998. Antibiotic resistance in Escherichia coli of the normal intestinal flora of swine. Microbial Drug Resistance-Mechanisms Epidemiology and Disease 4:289-299.

Teuber, M. 1999. Spread of antibiotic resistance with food-borne pathogens. Cellular and Molecular Life Sciences 56:755-763.

Vanotti, M.B., P.D. Millner, P.G. Hunt, and A.Q. Ellison. 2005. Removal of pathogen and

indicator microorganisms from liquid swine manure in mufti-step biological and

chemical treatment. Bioresource Technology 96:209-214.

Wegener, H.C., F.M. Aarestrup, L.B. Jensen, A.M. Hammerum, and F. Bager. 1999. Use of

antimicrobial growth promoters in food animals and Enterococcus faecium resistance

to therapeutic antimicrobial drugs in Europe. Emerging Infectious Diseases

5:329-335.

Table 1. Major antibiotic families and their mechanisms of action

Mechanisms of action	Antibiotic families
Inhibition of cell wall synthesis	Penicillins, cephalosporins, carbapenems, daptomycin, monobactams, glycopeptides
Inhibition of cell membrane permeability	Ionophores, polymyxins
Inhibition of protein synthesis	Tetracyclines, aminoglycosides, oxazolidonones, streptogramins, ketolides, macrolides, lincosamides
Inhibition of DNA synthesis	Fluoroquinolones
Inhibition of RNA synthesis	Rifampin
Competitive inhibition of folic acid synthesis	Sulfonamides, methotrexate
Other	Metronidazole

(Khachatourians, 1998; Levy and Marshall, 2004)

Table 2. Stock solutions of antibiotics

Antibiotics	Stock solution concentration (mg/mL)	Solvent
Amoxicillin	2.5	1:1 Water: Methanol
Cephalothin	10	Water
Chlorotetracycline	10	1N NaOH
Erythromycin	10	1:1 Water: Ethanol
Neomycin	10	Water
Oxytetracycline	10	1:1 Water: Methanol
Streptomycin	10	Water
Tetracycline	10	Methanol
Rifampicin	2.5	Methanol
Vancomycin	10	1:1 Water: Ethanol

(Sigma-Aldrich Inc, St. Louis, MO)

(Graves et al., 2002; Hagedorn et al., 1999; Harwood et al., 2000)

Table 3. Concentrations of antibiotic tests for *E. coli* and *Enterococcus*

Antibiotics	Abbreviation	Concentration for *E. coli* (mg/L)	Concentration for *Enterococcus* (mg/L)
Cephalothin	CEP	15, 25, 35	10, 15, 30, 50
Erythromycin	ERY	60, 70, 90, 100	10, 15, 30, 50
Neomycin	NEO	2.5, 5.0, 10	40, 60, 80
Oxytetracycline	OTC	2.5, 5.0, 7.5, 10, 15	20, 40, 60, 80, 100
Streptomycin	STR	2.5, 5.0, 7.5, 10, 15	40, 60, 80, 100
Tetracycline	TET	2.5, 5.0, 7.5, 10, 15	10, 15, 30, 50, 100
Rifampicin	RIF	15, 18.75, 22.5	
Chlorotetracycline	CTC		60, 80, 100
Vancomycin	VAN		2.5
Amoxicillin	AMX		0.625
Control		0	0

(Hagedorn et al., 1999; Harwood et al., 2000)

Table 4. Numbers of *E. coli* and *Enterococcus* isolated from samples

Sample Source		Cool Season		Warm Season	
		E. coli	*Enterococcus*	*E. coli*	*Enterococcus*
Nursery Site	Feces 1	192	192	192	192
	Feces 2	192	192	192	192
	Feces 3	192	192	0	192
	Lagoon 1	192	192	0	192
	Lagoon 2	192	192	0	192
	Lagoon 3	192	192	0	192
	Soils	0	0	0	0
Finishing Site	Feces 1	96	192	192	96
	Feces 2	96	192	192	192
	Feces 3	384	192	192	192
	Feces 4	192	192	192	192
	Lagoon 1	192	192	384	192
	Lagoon 2	192	192	0	192
	Lagoon 3	192	192	0	192
	Soils	0	0	0	0

Note: unit - number of isolates
Nursery Site Feces 1, 2, 3 – three composite fecal samples collected from nursery swine house 1, 2, 3, respectively
Nursery Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of nursery swine lagoon
Nursery Site Soils – soil samples collected from spray field of nursery swine site
Finishing Site Feces 1, 2, 3, 4 – four composite fecal samples collected from finishing swine house 1, 2, 3, 4, respectively
Finishing Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of finishing swine lagoon
Finishing Site Soils – soil samples collected from spray field of finishing swine site

Table 5. Numbers of *E. coli* and *Enterococcus* isolates analyzed by antibiotic resistance analysis (ARA)

Sample Source		Cool Season		Warm Season	
		E. coli	*Enterococcus*	*E. coli*	*Enterococcus*
Nursery Site	Feces 1	192	192	192	101
	Feces 2	192	180	191	181
	Feces 3	191	187	0	120
	Lagoon 1	191	110	0	38
	Lagoon 2	192	24	0	27
	Lagoon 3	190	23	0	10
	Soils	0	0	0	0
Finishing Site	Feces 1	96	77	192	73
	Feces 2	95	51	188	13
	Feces 3	384	135	190	51
	Feces 4	192	20	189	47
	Lagoon 1	192	124	383	66
	Lagoon 2	192	48	0	15
	Lagoon 3	192	49	0	27
	Soils	0	0	0	0

Note: unit - number of isolates
Nursery Site Feces 1, 2, 3 – three composite fecal samples collected from nursery swine house 1, 2, 3, respectively
Nursery Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of nursery swine lagoon
Nursery Site Soils – soil samples collected from spray field of nursery swine site
Finishing Site Feces 1, 2, 3, 4 – four composite fecal samples collected from finishing swine house 1, 2, 3, 4, respectively
Finishing Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of finishing swine lagoon
Finishing Site Soils – soil samples collected from spray field of finishing swine site

Table 6. Numbers of *E. coli* and *Enterococcus* isolates analyzed by PCR

Sample Source		Cool Season		Warm Season	
		E. coli	*Enterococcus*	*E. coli*	*Enterococcus*
Nursery Site	Feces 1	25	22	25	25
	Feces 2	25	25	25	25
	Feces 3	25	25	0	25
	Lagoon 1	25	25	0	25
	Lagoon 2	25	24	0	25
	Lagoon 3	25	23	0	10
	Soils	0	0	0	0
Finishing Site	Feces 1	25	25	25	15
	Feces 2	25	25	25	10
	Feces 3	25	25	25	25
	Feces 4	25	20	25	25
	Lagoon 1	25	25	25	25
	Lagoon 2	25	25	0	15
	Lagoon 3	25	25	0	25
	Soils	0	0	0	0

Note: unit - number of isolates
Nursery Site Feces 1, 2, 3 – three composite fecal samples collected from nursery swine house 1, 2, 3, respectively
Nursery Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of nursery swine lagoon
Nursery Site Soils – soil samples collected from spray field of nursery swine site
Finishing Site Feces 1, 2, 3, 4 – four composite fecal samples collected from finishing swine house 1, 2, 3, 4, respectively
Finishing Site Lagoon 1, 2, 3 – three lagoon liquid samples collected from three different sites of finishing swine lagoon
Finishing Site Soils – soil samples collected from spray field of finishing swine site

Table 7. Significance analysis (P-value) of antibiotic tests and swine stages of development

	P-value			
	Cool season		Warm season	
	E. coli	*Enterococcus*	*E. coli*	*Enterococcus*
Trt	<.0001	<.0001	<.0001	<.0001
Age x Trt	0.0562	<.0001	<.0001	0.2057
Age	0.1929	0.0042	0.018	0.3003

Note: Trt – antibiotic tests

      Age x Trt – the interaction between swine stages of development and antibiotic tests

      Age – swine stage of development (nursery versus finishing)

Table 8. Classification table displaying the numbers and percentages of cool season *E. coli* isolates from four source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing feces	Finishing lagoon	Nursery feces	Nursery lagoon	Total number
Finishing feces	73 **73.00%**	15 20.00	4 5.33	4 5.33	96
Finishing lagoon	5 5.00	39 **52.00%**	6 8.00	8 10.67	58
Nursery feces	6 6.00	9 12.00	50 **66.67%**	3 4.00	68
Nursery lagoon	16 16.00	12 16.00	15 20.00	60 **80.00%**	103
Total number	100	75	75	75	325

Table 9. Classification table displaying the numbers and percentages of cool season *E. coli* isolates from two source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	131 **74.86%**	27 18.00	158
Nursery	44 25.14	123 **82.00%**	167
Total number	175	150	325

Table 10. Classification table displaying the numbers and percentages of cool season *Enterococcus* isolates from four source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing feces	Finishing lagoon	Nursery feces	Nursery lagoon	Total number
Finishing feces	68 **72.34%**	13 17.33	0 0.00	23 31.51	104
Finishing lagoon	14 14.89	42 **56.00%**	2 2.67	21 28.77	79
Nursery feces	4 4.26	16 21.33	73 **97.33%**	0 0.00	93
Nursery lagoon	8 8.51	4 5.33	0 0.00	29 **39.73%**	41
Total number	94	75	75	73	317

Table 11. Classification table displaying the numbers and percentages of cool season *Enterococcus* isolates from two source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	133 **78.70%**	47 31.76	180
Nursery	36 21.30	101 **68.24%**	137
Total number	169	148	317

Table 12. Classification table displaying the numbers and percentages of warm season *E. coli* isolates from three source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing feces	Finishing lagoon	Nursery feces	Total number
Finishing feces	89 **89.00%**	9 36.00	4 8.00	102
Finishing lagoon	4 4.00	13 **52.00%**	0 0.00	17
Nursery feces	7 7.00	3 12.00	46 **92.00%**	56
Total number	100	25	50	175

Table 13. Classification table displaying the numbers and percentages of warm season *E. coli* isolates from two source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	116 **92.80%**	4 8.00	120
Nursery	9 7.20	46 **92.00%**	55
Total number	125	50	175

Table 14. Classification table displaying the numbers and percentages of warm season *Enterococcus* isolates from four source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing feces	Finishing lagoon	Nursery feces	Nursery lagoon	Total number
Finishing feces	45 **60.00%**	7 10.77	7 9.33	11 18.33	70
Finishing lagoon	16 21.33	48 **73.85%**	11 14.67	22 36.67	97
Nursery feces	7 9.33	8 12.31	53 **70.67%**	3 5.00	71
Nursery lagoon	7 9.33	2 3.08	4 5.33	24 **40.00%**	37
Total number	75	65	75	60	275

Table 15. Classification table displaying the numbers and percentages of warm season *Enterococcus* isolates from two source categories by discriminant analysis of ARA data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	114 **81.43%**	48 35.56	162
Nursery	26 18.57	87 **64.44%**	113
Total number	140	135	275

Table 16. Classification table displaying the numbers and percentages of cool season *E. coli* isolates from four source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing Lagoon	Finishing Feces	Nursery Lagoon	Nursery Feces	Total number
Finishing Lagoon	44 **58.67%**	3 3.00%	8 10.67%	11 14.67%	66
Finishing Feces	6 8.00%	89 **89.00%**	14 18.67%	11 14.67%	120
Nursery Lagoon	12 16.00%	6 6.00%	52 **69.33%**	8 10.67%	78
Nursery Feces	13 17.33%	2 2.00%	1 1.33%	45 **60.00%**	61
Total number	75	100	75	75	325

Table 17. Classification table displaying the numbers and percentages of cool season *E. coli* isolates from two source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	142 **81.61%**	40 26.67%	182
Nursery	32 18.39%	110 **73.33%**	142
Total number	174	150	324

Table 18. Classification table displaying the numbers and percentages of cool season *Enterococcus* isolates from four source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing Lagoon	Finishing Feces	Nursery Lagoon	Nursery Feces	Total number
Finishing Lagoon	29 **38.67%**	16 16.84%	2 2.78%	3 4.17%	50
Finishing Feces	21 28.00%	60 **63.16%**	12 16.67%	0 0.00	93
Nursery Lagoon	5 6.67%	8 8.42%	50 **69.44%**	5 6.94%	68
Nursery Feces	20 26.67%	11 11.58%	8 11.11%	64 **88.89%**	103
Total number	75	95	72	72	314

Table 19. Classification table displaying the numbers and percentages of cool season *Enterococcus* isolates from two source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	135 **79.41%**	27 18.75%	162
Nursery	35 20.59%	117 **81.25%**	152
Total number	170	144	314

Table 20. Classification table displaying the numbers and percentages of warm season
E. coli **isolates from three source categories by discriminant analysis of PCR data**

Numbers and percentages of isolates classified as:	Finishing Lagoon	Finishing Feces	Nursery Feces	Total number
Finishing Lagoon	11 **44.00%**	0 0.00	2 4.00%	13
Finishing Feces	6 24.00%	88 **88.00%**	8 16.00%	102
Nursery Feces	8 32.00%	12 12.00%	40 **80.00%**	60
Total number	25	100	50	175

Table 21. Classification table displaying the numbers and percentages of warm season
E. coli **isolates from two source categories by discriminant analysis of PCR data**

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	111 **88.80%**	12 24.00%	123
Nursery	14 11.20%	38 **76.00%**	52
Total number	125	50	175

Table 22. Classification table displaying the numbers and percentages of warm season *Enterococcus* isolates from four source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing Lagoon	Finishing Feces	Nursery Lagoon	Nursery Feces	Total number
Finishing Lagoon	46 **51.11%**	18 24.00%	10 14.29%	11 14.67%	85
Finishing Feces	3 3.33%	22 **29.33%**	6 8.57%	0 0.00	31
Nursery Lagoon	8 8.89%	18 24.00%	38 **54.29%**	4 5.33%	68
Nursery Feces	33 36.67%	17 22.67%	16 22.86%	60 **80.00%**	126
Total number	90	75	70	75	310

Table 23. Classification table displaying the numbers and percentages of warm season *Enterococcus* isolates from two source categories by discriminant analysis of PCR data

Numbers and percentages of isolates classified as:	Finishing	Nursery	Total number
Finishing	94 **56.97%**	41 28.28%	135
Nursery	71 43.03%	104 **71.72%**	175
Total number	165	145	310

Socioeconomic factors

Appropriateness of use

Travel of people of foodstuffs

Infection control measures

Antibiotic Use ➙ **Antibiotic Resistance**

Antibiotic residues

Dose/duration of treatment

Gene transfer

Cross selection
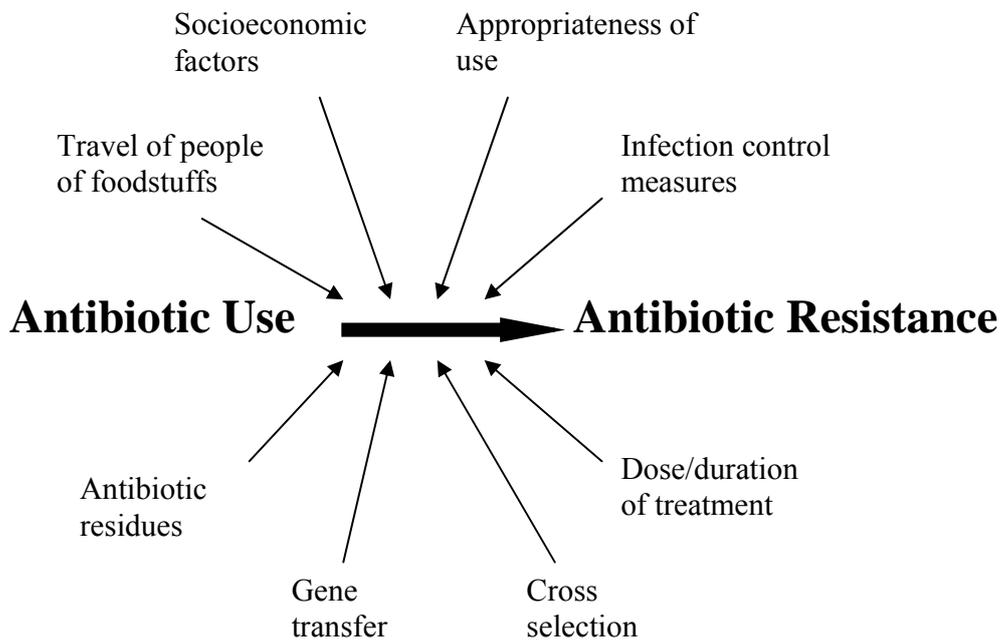
Figure 1. Relationship between antibiotic use and occurrence of antibiotic resistance (Barbosa and Levy, 2000)
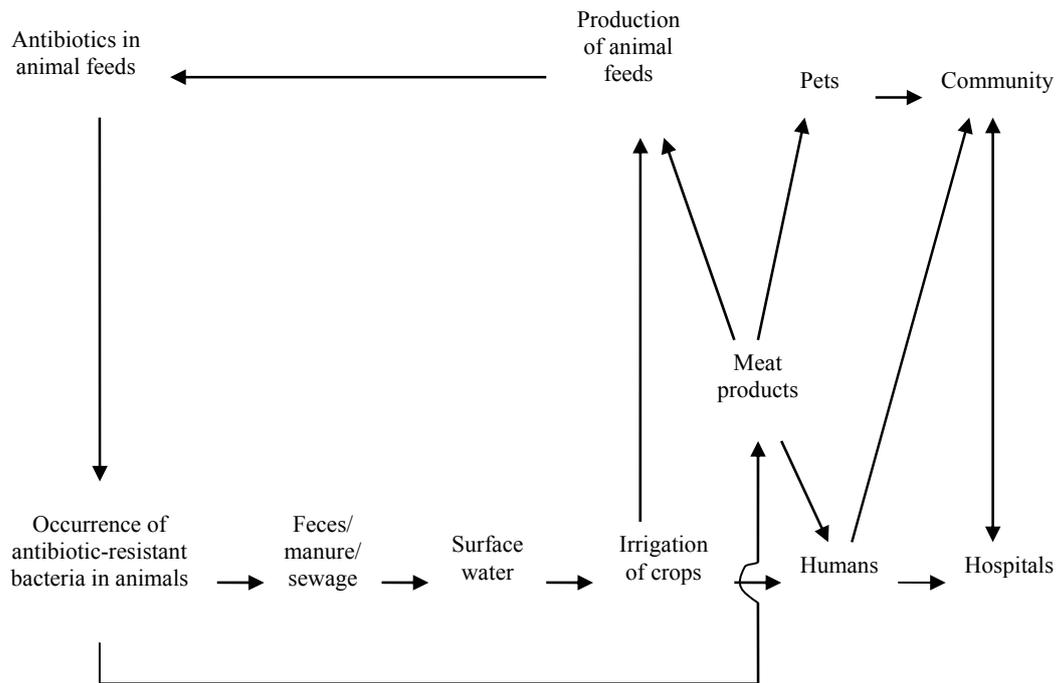
Figure 2. Emergence and transmission of antibiotic-resistant bacteria in agricultural system (Khachatourians, 1998)
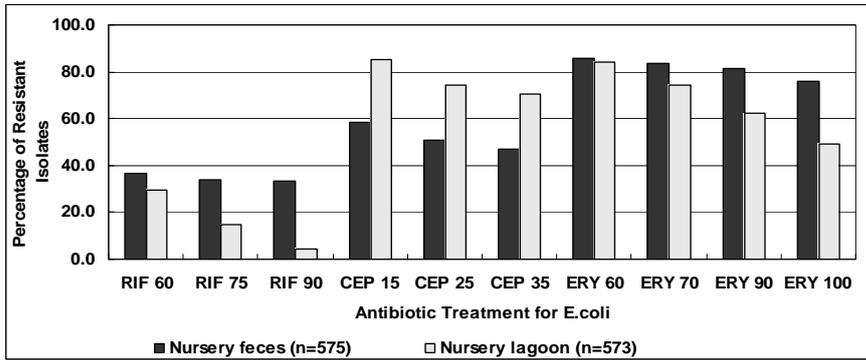
(a)
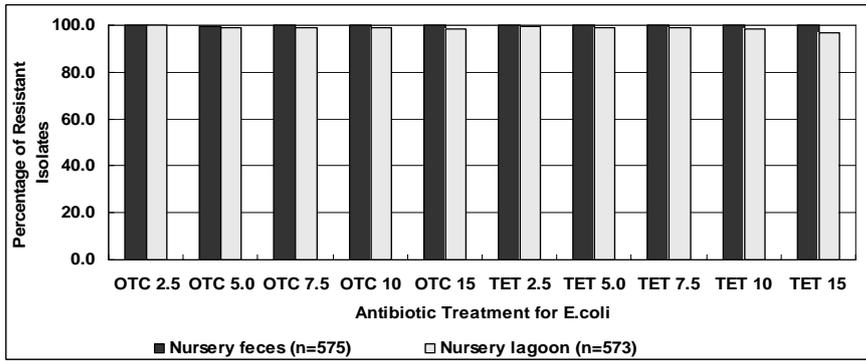
(b)
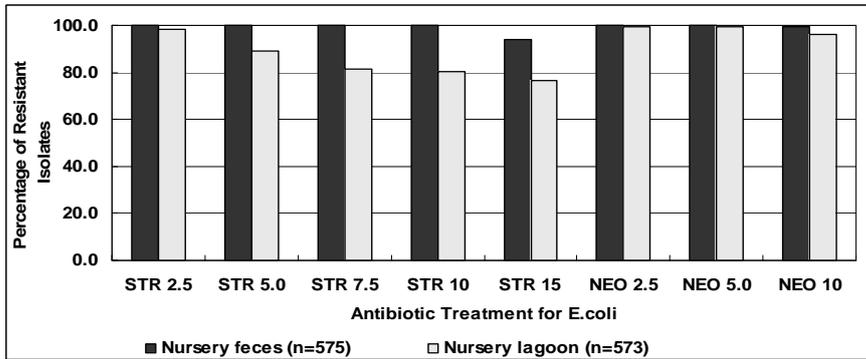
(c)

Figure 3. Percentages of resistant _E. coli_ isolated from three <u>nursery fecal</u> samples and three <u>nursery lagoon liquid</u> samples in <u>cool season</u>.   Concentrations of antibiotics are indicated in Table 3.   Number of isolates from each sample is indicated in Table 5. (a) rifamycin, cephalothin, and erythromycin; (b) oxytetracycline and tetracycline; (c) streptomycin and neomycin.
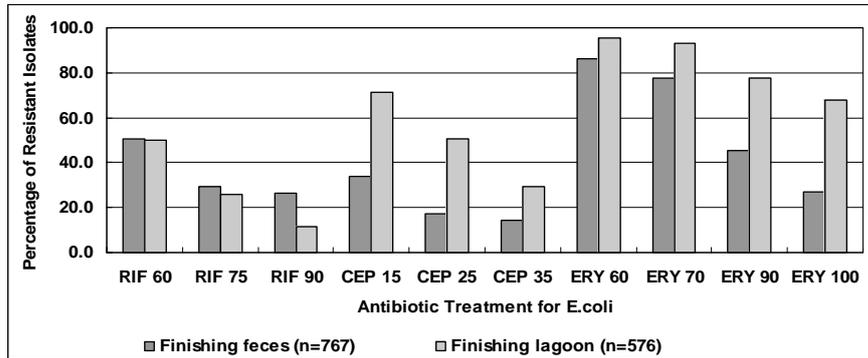
(a)
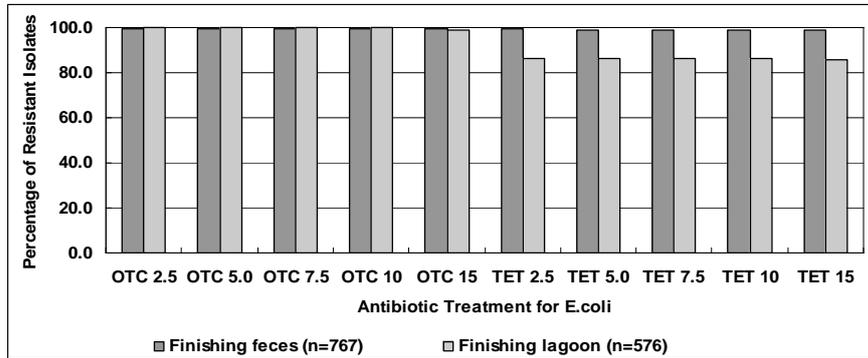
(b)
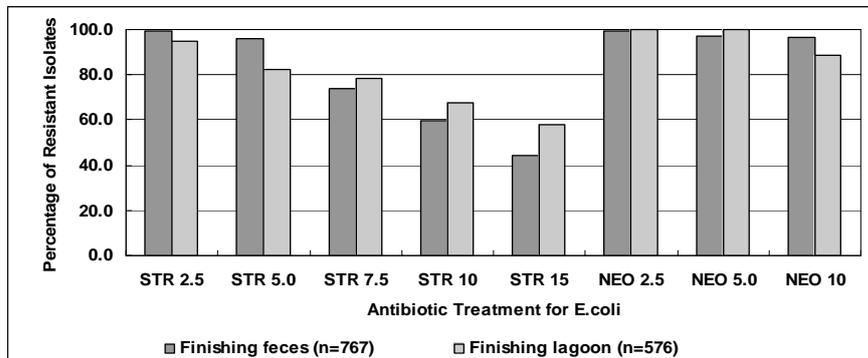
(c)

Figure 4. Percentages of resistant _E. coli_ isolated from four <u>finishing fecal</u> samples and three <u>finishing lagoon liquid</u> samples in <u>cool season</u>.   Concentrations of antibiotics are indicated in Table 3.   Number of isolates from each sample is indicated in Table 5. (a) rifamycin, cephalothin, and erythromycin; (b) oxytetracycline and tetracycline; (c) streptomycin and neomycin.
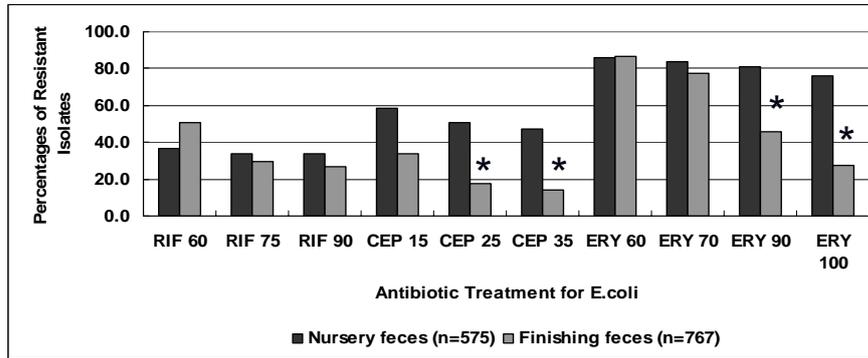
(a)
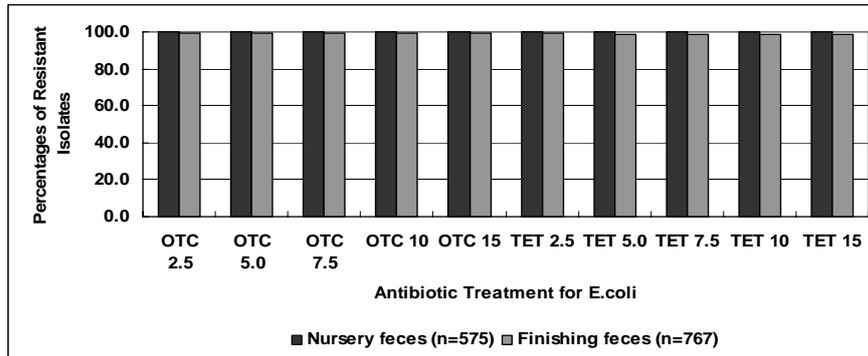
(b)
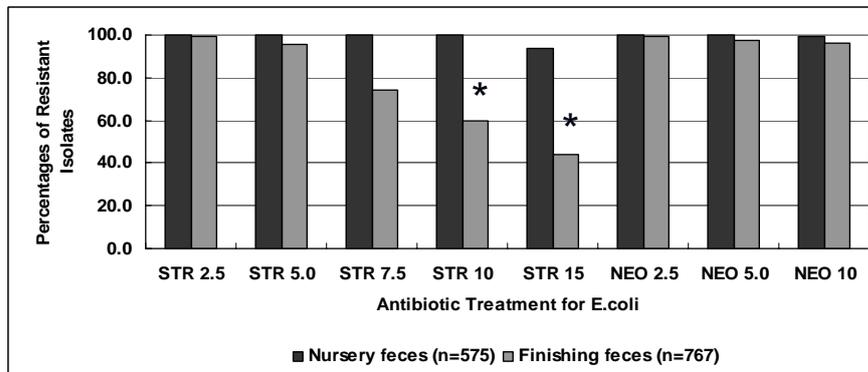
(c)

Figure 5. Percentages of resistant _E. coli_ isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>cool season</u>. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) rifamycin, cephalothin, and erythromycin; (b) oxytetracycline and tetracycline; (c) streptomycin and neomycin.
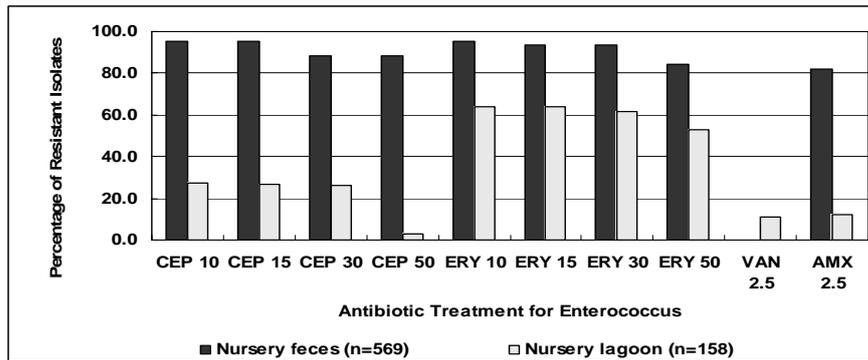
(a)
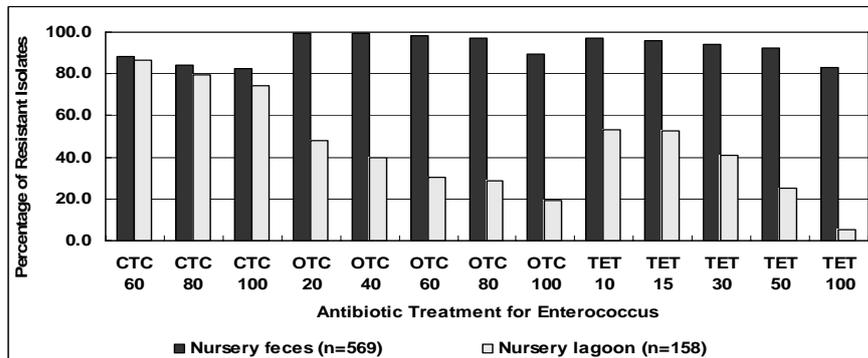
(b)
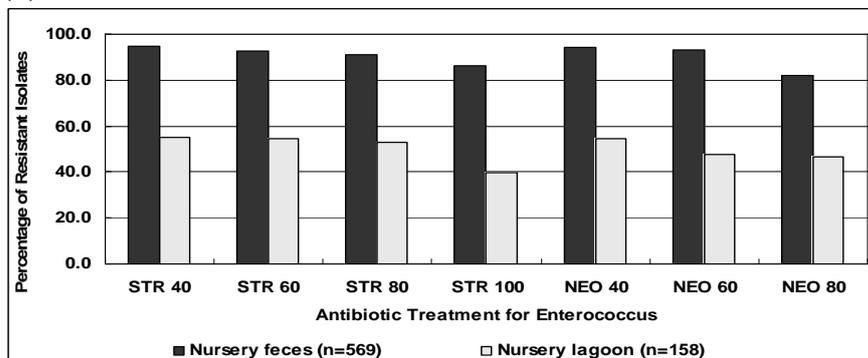
(c)

Figure 6. Percentages of resistant _Enterococcus_ isolated from three <u>nursery fecal</u> samples and three <u>nursery lagoon liquid</u> samples in <u>cool season</u>. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
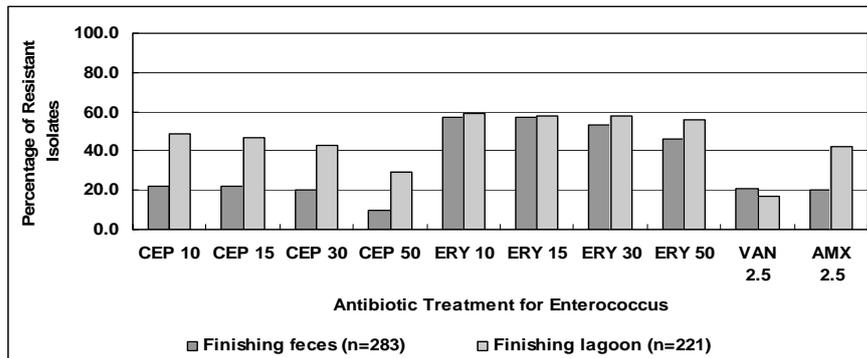
(a)
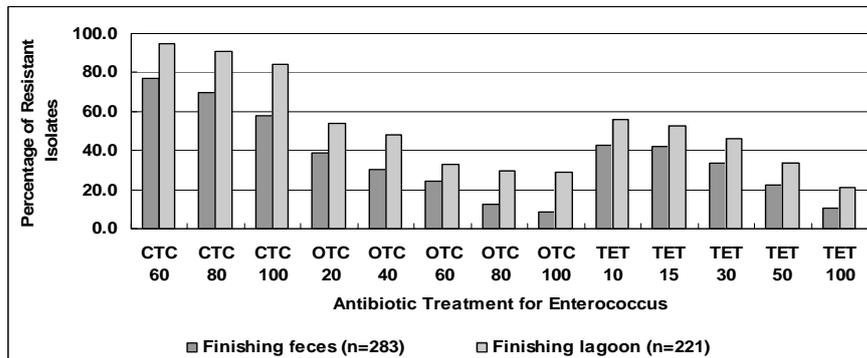
(b)
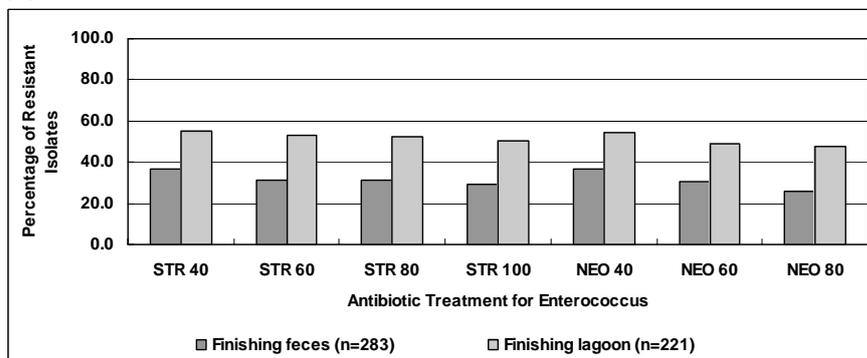
(c)

Figure 7. Percentages of resistant _Enterococcus_ isolated from four <u>finishing fecal</u> samples and three <u>finishing lagoon liquid</u> samples in <u>cool season</u>.   Concentrations of antibiotics are indicated in Table 3.   Number of isolates from each sample is indicated in Table 5.   (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
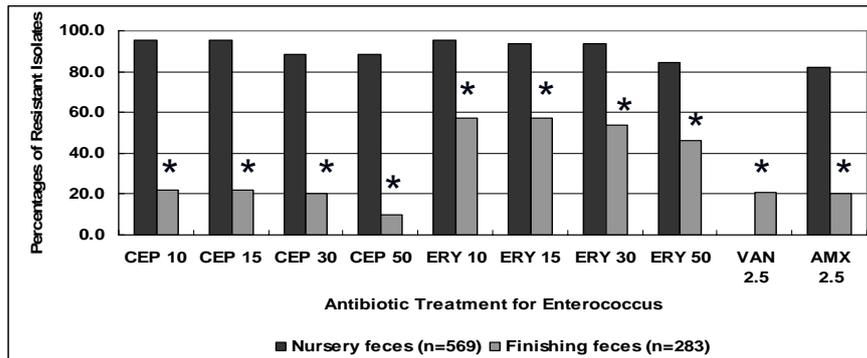
(a)
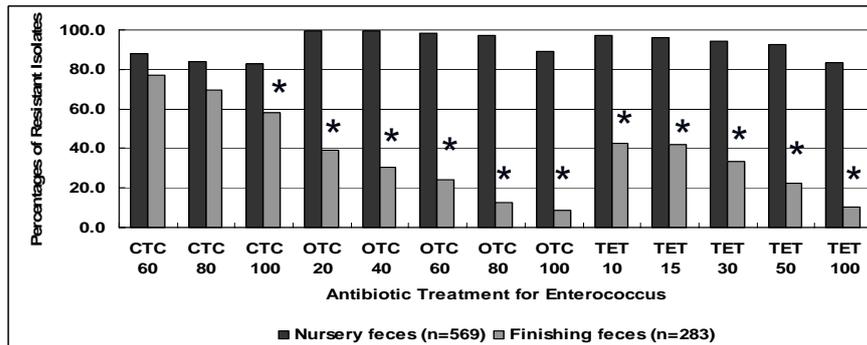
(b)
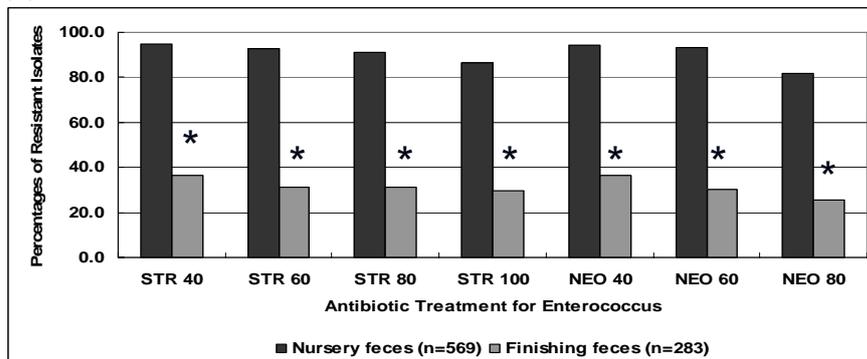
(c)

Figure 8. Percentages of resistant _Enterococcus_ isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>cool season</u>.   Concentrations of antibiotics are indicated in Table 3.   Number of isolates from each sample is indicated in Table 5. (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
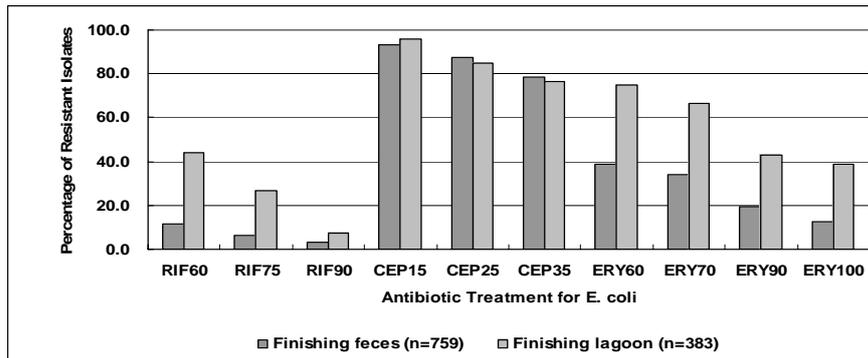
(a)
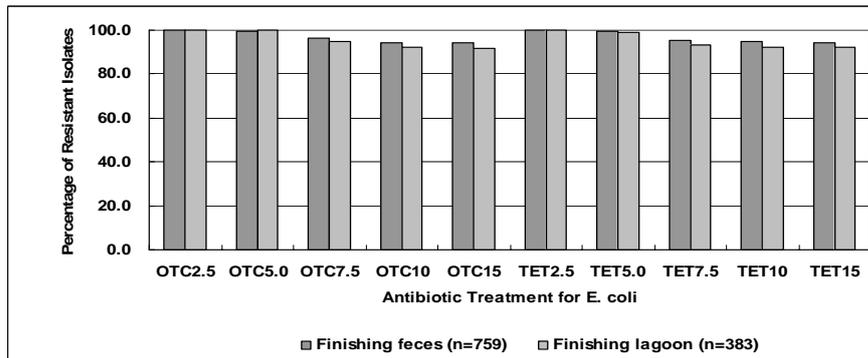
(b)
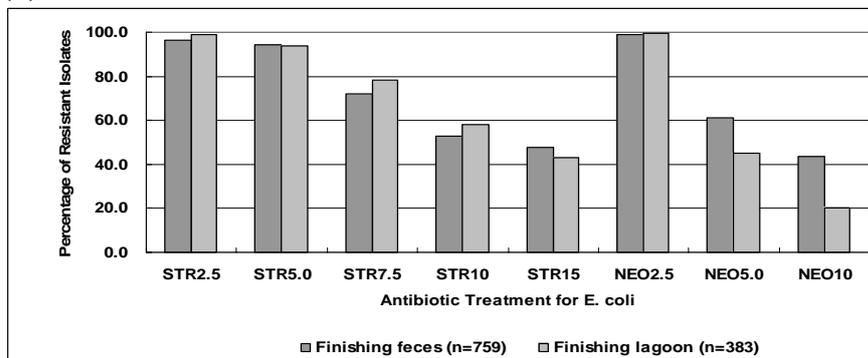
(c)

Figure 9. Percentages of resistant _E. coli_ isolated from four finishing fecal samples and one finishing lagoon liquid samples in warm season. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) rifamycin, cephalothin, and erythromycin; (b) oxytetracycline and tetracycline; (c) streptomycin and neomycin.
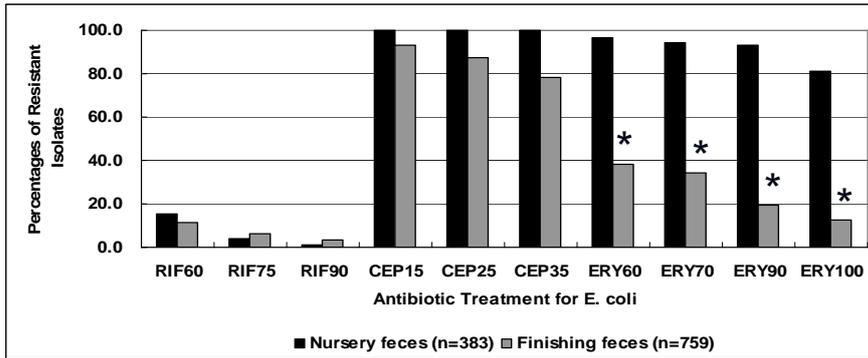
(a)
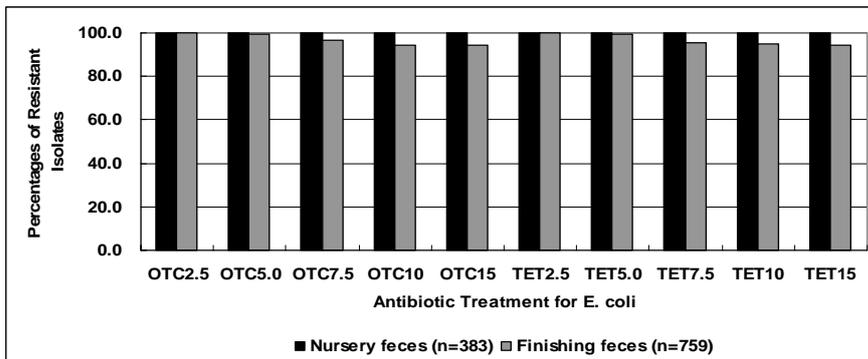
(b)
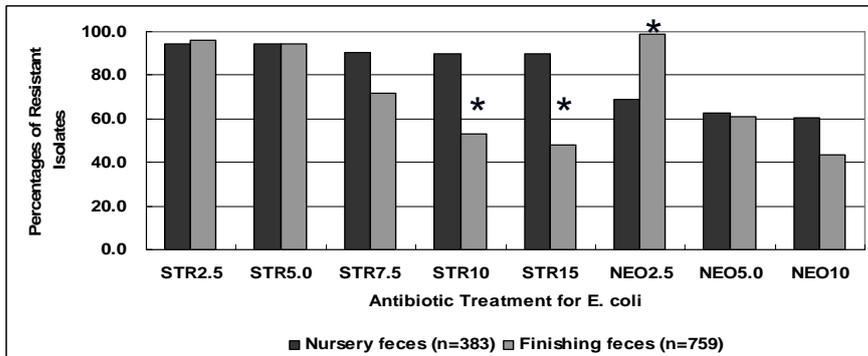
(c)

Figure 10. Percentages of resistant _E. coli_ isolated from two <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>warm season</u>. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) rifamycin, cephalothin, and erythromycin; (b) oxytetracycline and tetracycline; (c) streptomycin and neomycin.
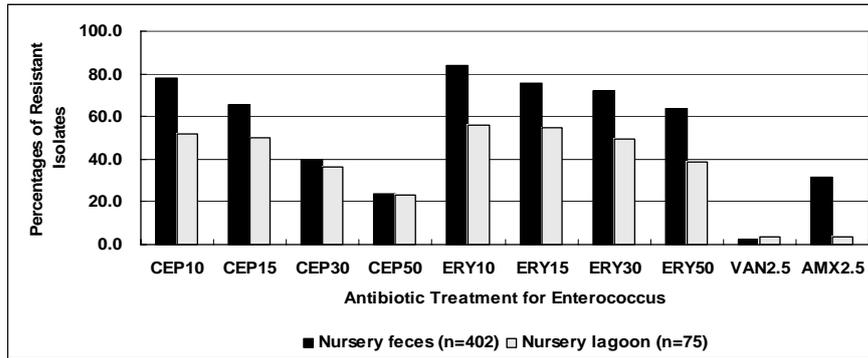
(a)
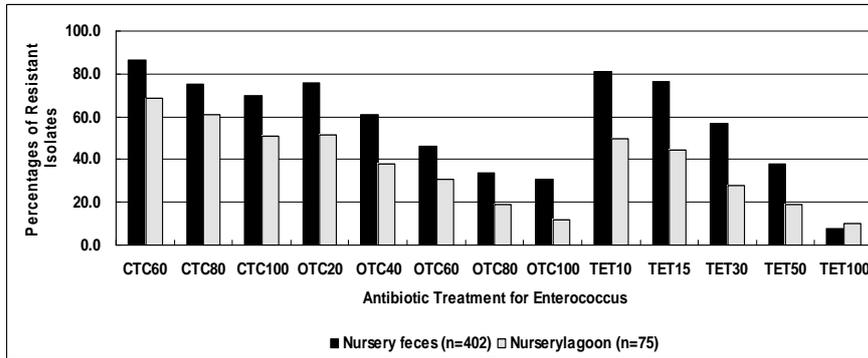
(b)
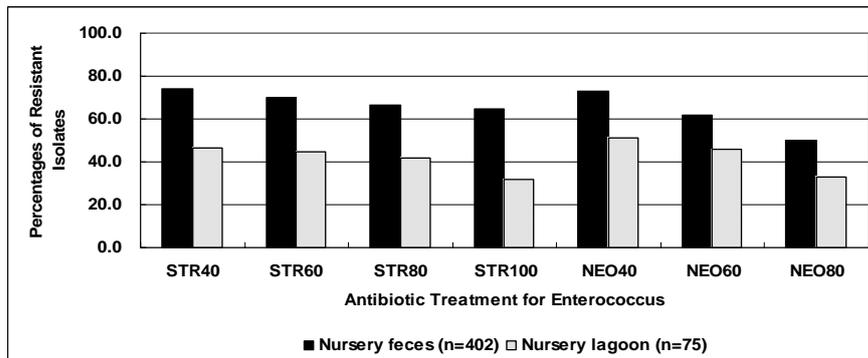
(c)

Figure 11. Percentages of resistant _Enterococcus_ isolated from three <u>nursery fecal</u> samples and three <u>nursery lagoon liquid</u> samples in <u>warm season</u>.   Concentrations of antibiotics are indicated in Table 3.   Number of isolates from each sample is indicated in Table 5.   (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
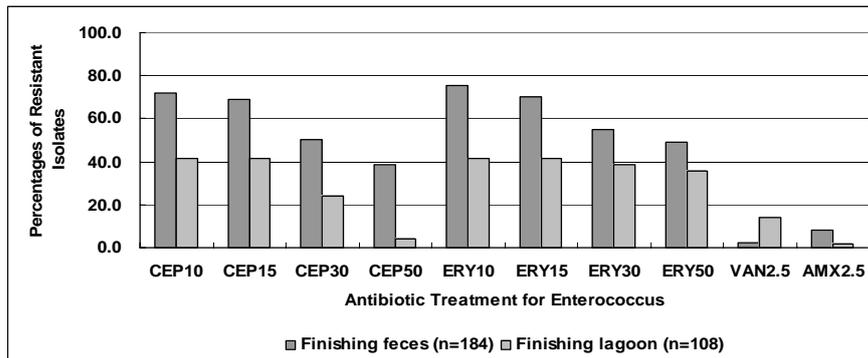
(a)
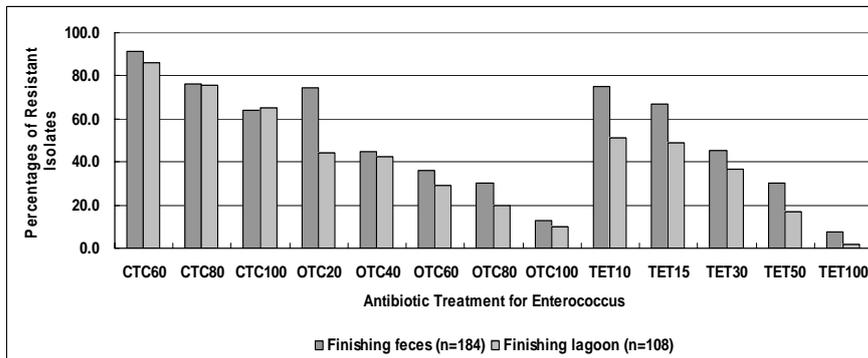
(b)
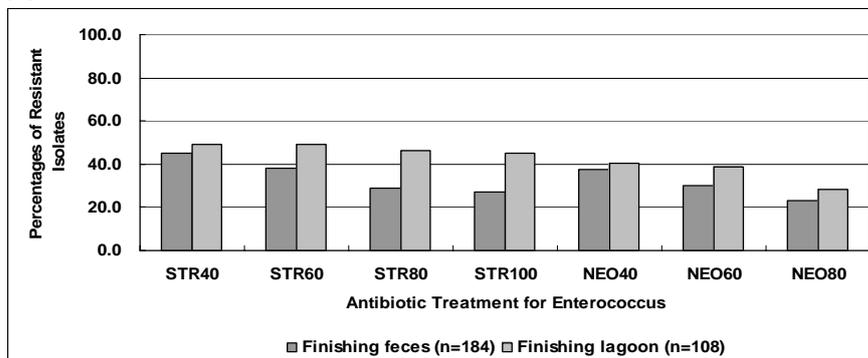
(c)

Figure 12. Percentages of resistant _Enterococcus_ isolated from four finishing fecal samples and three finishing lagoon liquid samples in warm season. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
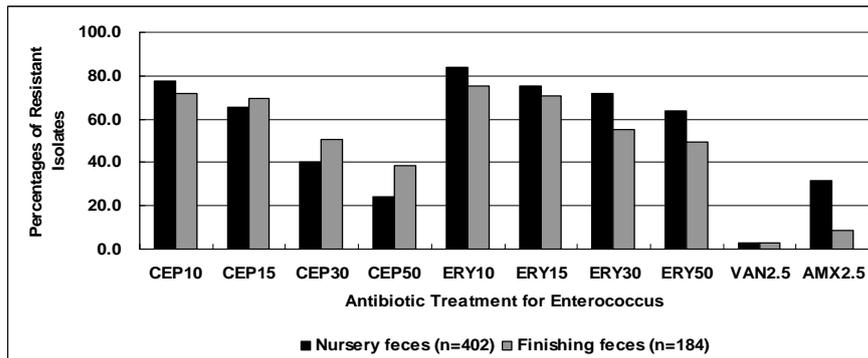
(a)
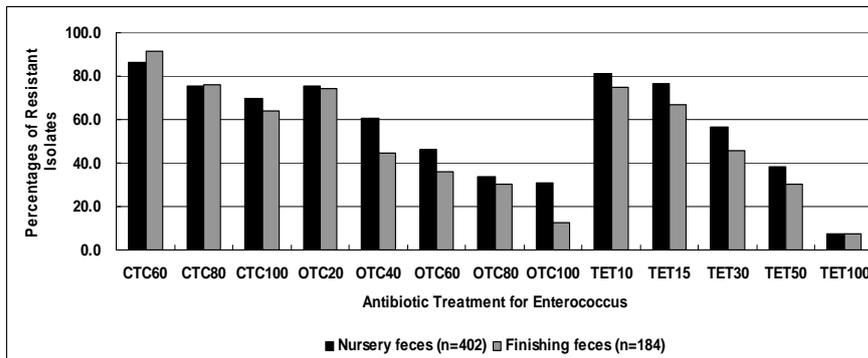
(b)
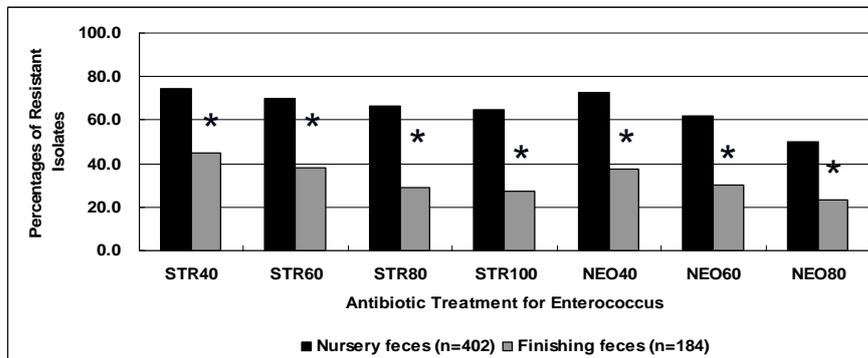
(c)

Figure 13. Percentages of resistant _Enterococcus_ isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>warm season</u>. Concentrations of antibiotics are indicated in Table 3. Number of isolates from each sample is indicated in Table 5. (a) cephalothin, erythromycin, vancomycin, and amoxicillin; (b) chlorotetracycline, oxytetracycline and tetracycline; (c) streptomycin and neomycin.
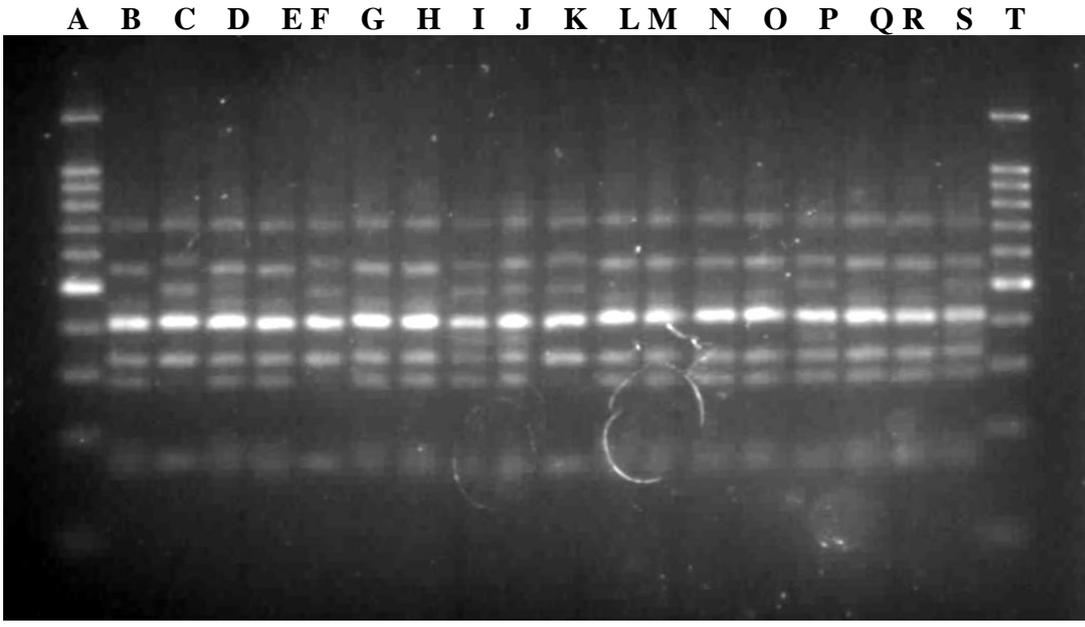
Figure 14. PCR DNA fingerprint patterns of *E. coli* strains.   Lanes A and T contained a standard, a 100 bp ladder
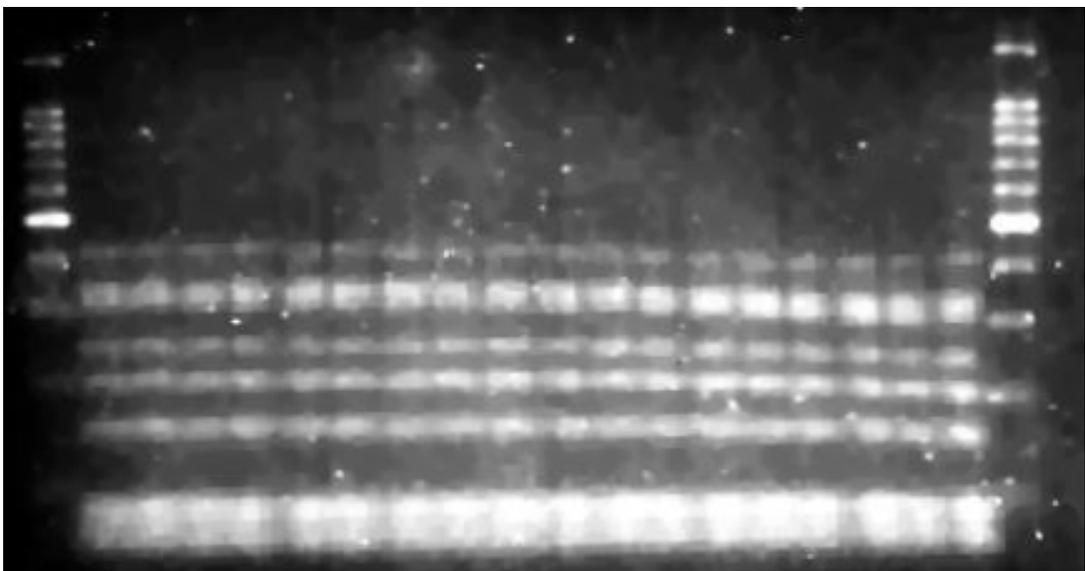
Figure 15. PCR DNA fingerprint patterns of *Enterococcus* strains.   Lanes A and T contained a standard, a 100 bp ladder
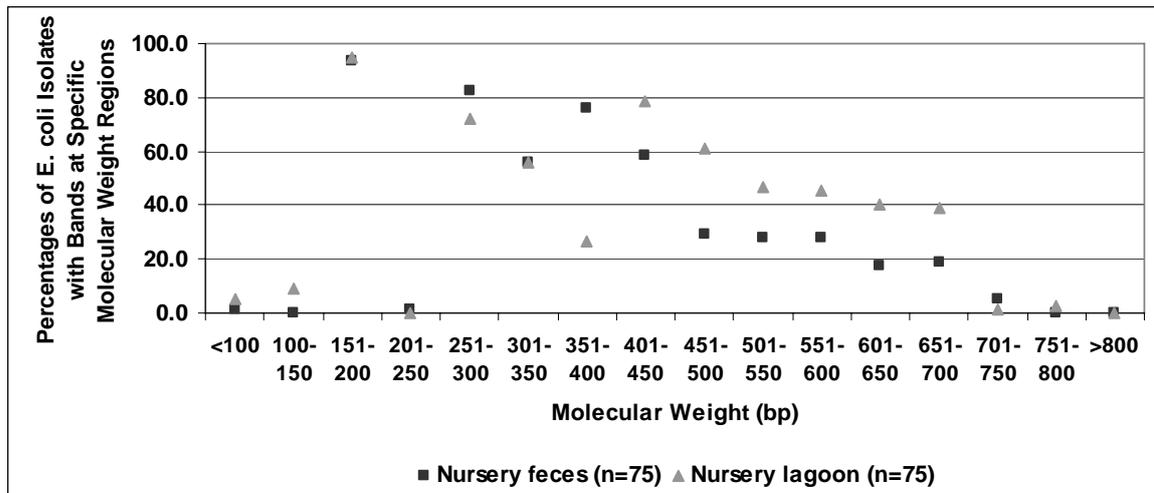
Figure 16. Percentages of _E. coli_ isolates having bands at specific molecular weight regions.   Isolates were isolated from three <u>nursery fecal</u> samples and three <u>nursery lagoon liquid</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
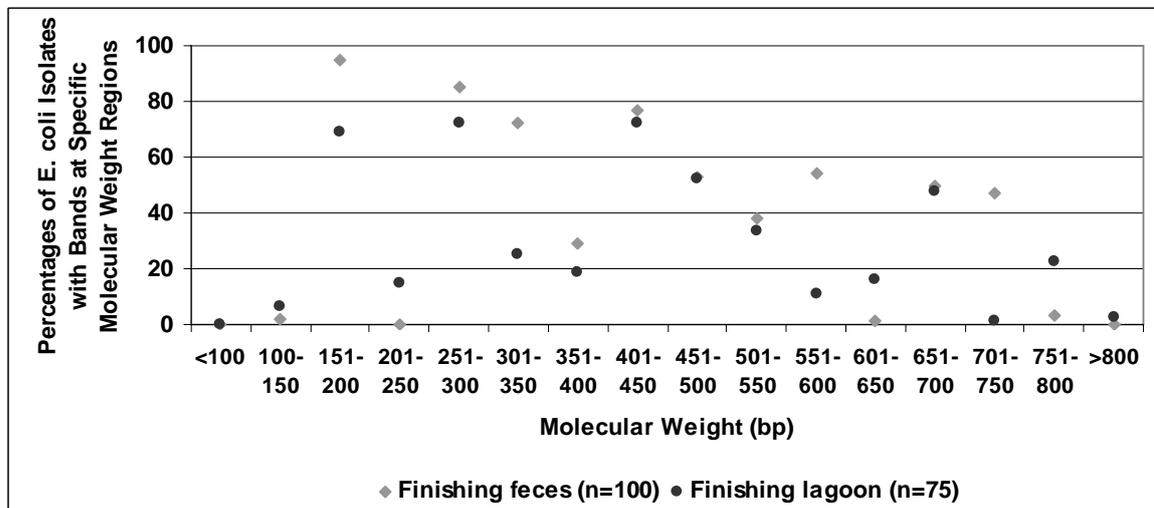
Figure 17. Percentages of _E. coli_ isolates having bands at specific molecular weight regions.   Isolates were isolated from four <u>finishing fecal</u> samples and three <u>finishing lagoon liquid</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
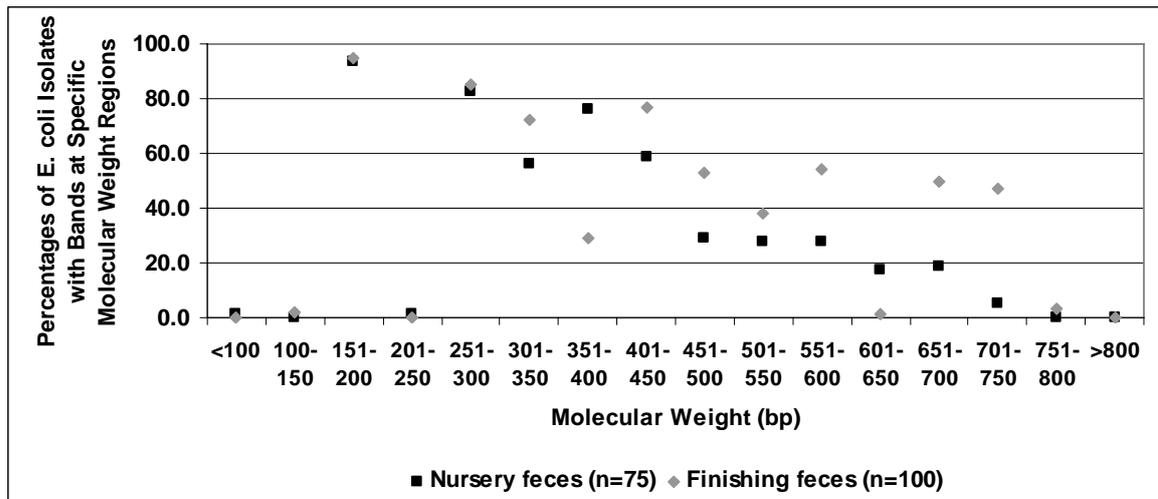
Figure 18. Percentages of _E. coli_ isolates having bands at specific molecular weight regions. Isolates were isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
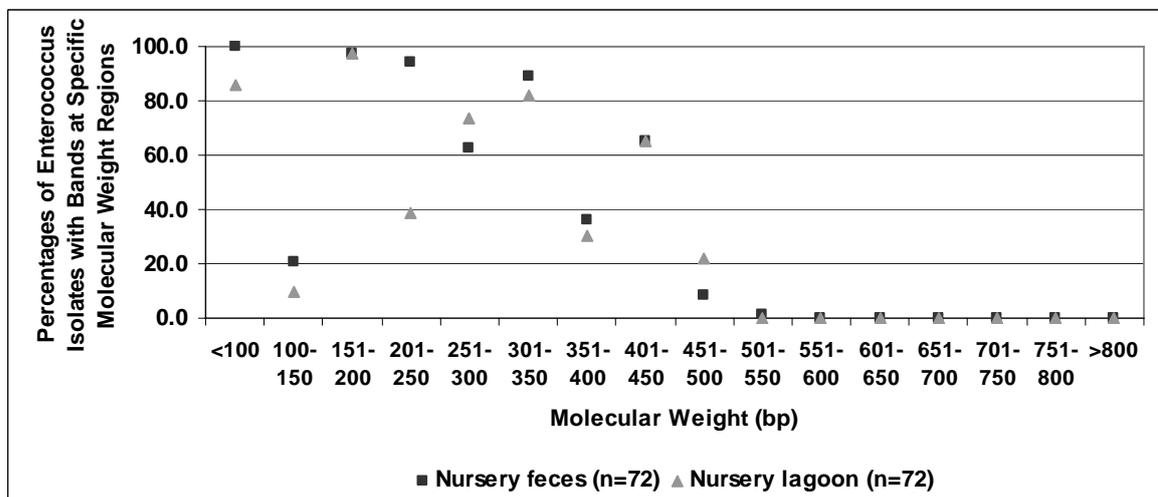
Figure 19. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions. Isolates were isolated from three <u>nursery fecal</u> samples and three <u>nursery lagoon liquid</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
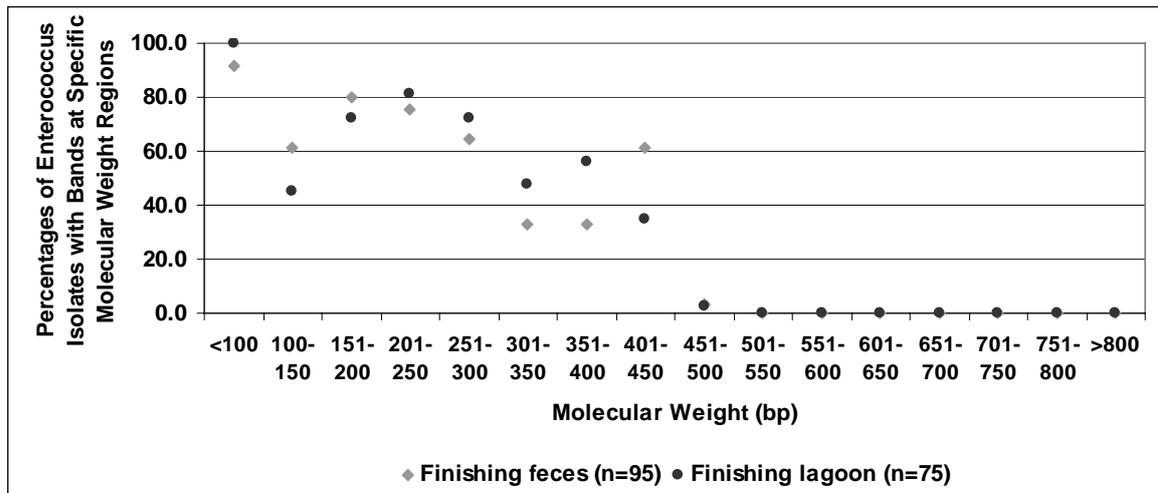
Figure 20. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions. Isolates were isolated from four <u>finishing fecal</u> samples and three <u>finishing lagoon liquid</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
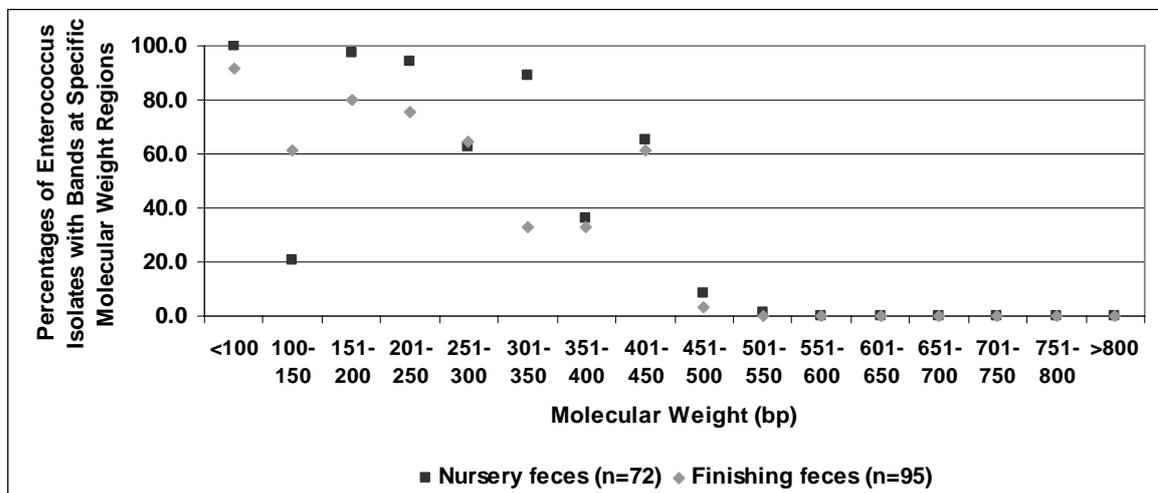
Figure 21. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions. Isolates were isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>cool season</u> (number of isolates from each sample was indicated in Table 6)
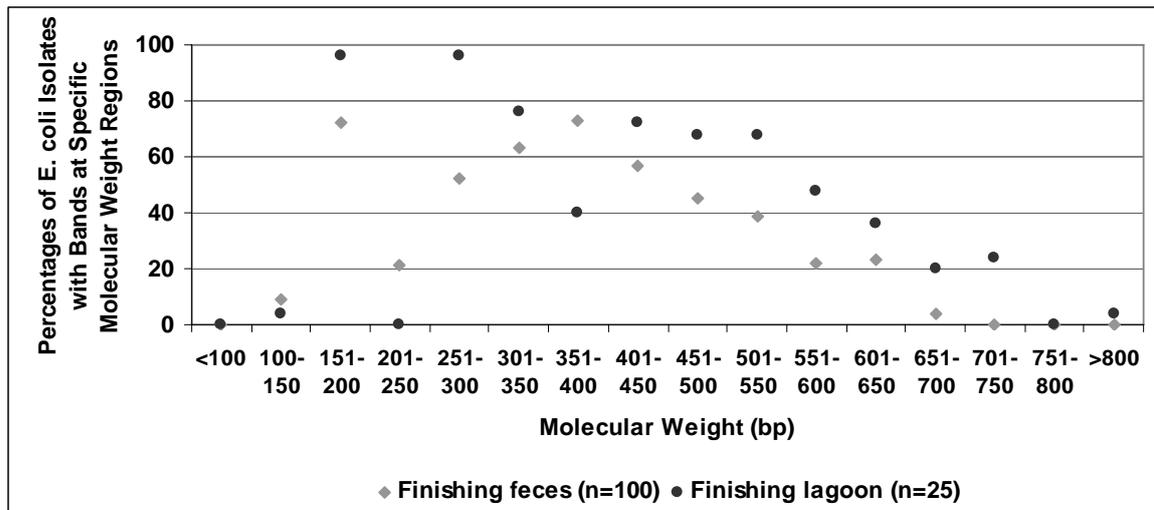
Figure 22. Percentages of _E. coli_ isolates having bands at specific molecular weight regions. Isolates were isolated from four finishing fecal samples and one finishing lagoon liquid samples in warm season (number of isolates from each sample was indicated in Table 6)
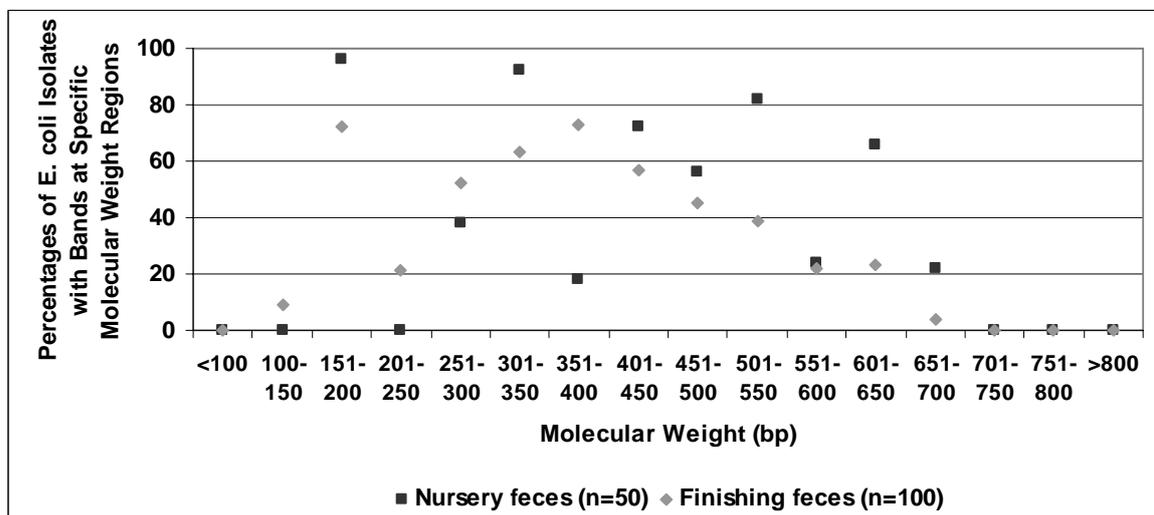
Figure 23. Percentages of _E. coli_ isolates having bands at specific molecular weight regions. Isolates were isolated from two nursery fecal samples and four finishing fecal samples in warm season (number of isolates from each sample was indicated in Table 6)
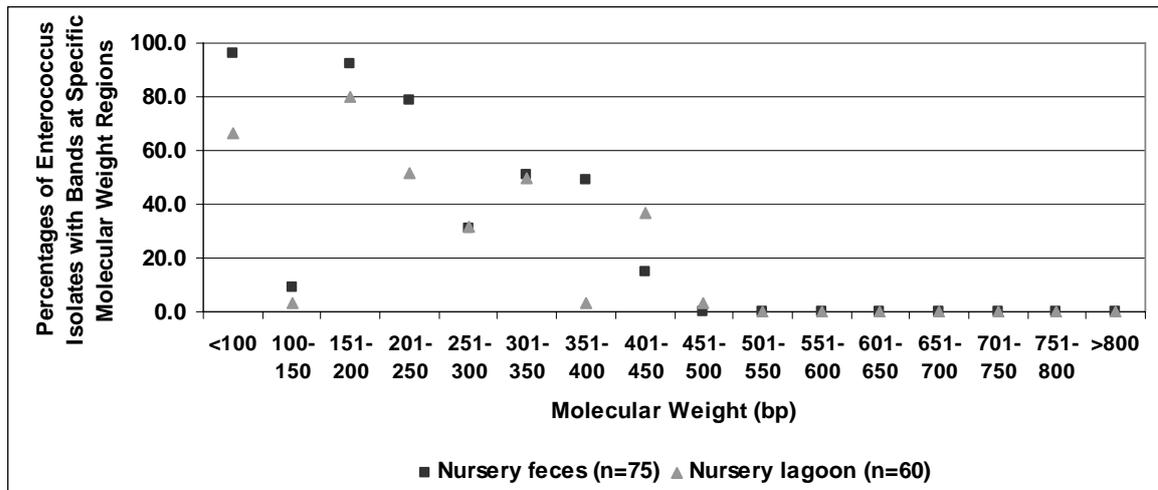
Figure 24. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions.   Isolates were isolated from three nursery fecal samples and three nursery lagoon liquid samples in warm season (number of isolates from each sample was indicated in Table 6)
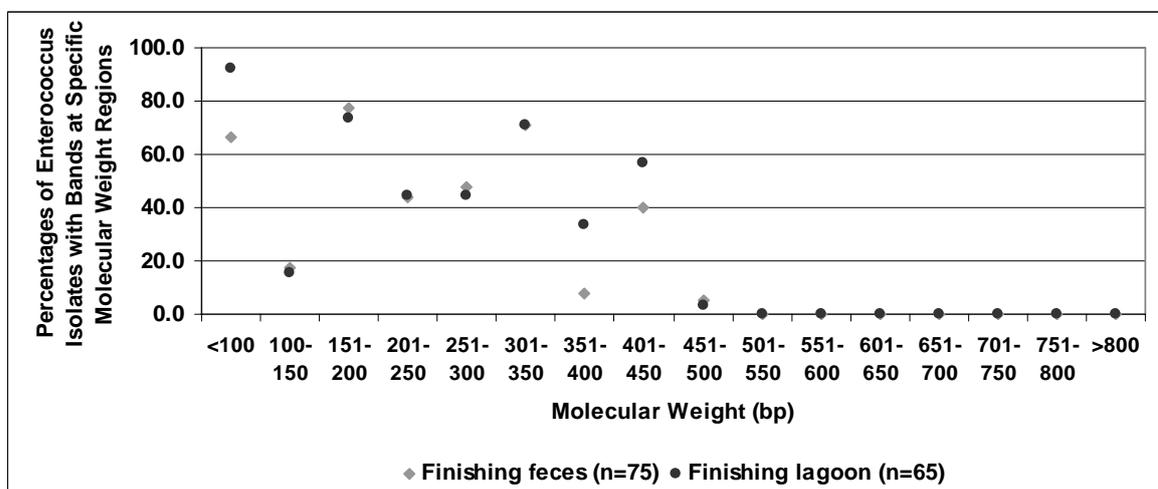
Figure 25. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions.   Isolates were isolated from four finishing fecal samples and three finishing lagoon liquid samples in warm season (number of isolates from each sample was indicated in Table 6)
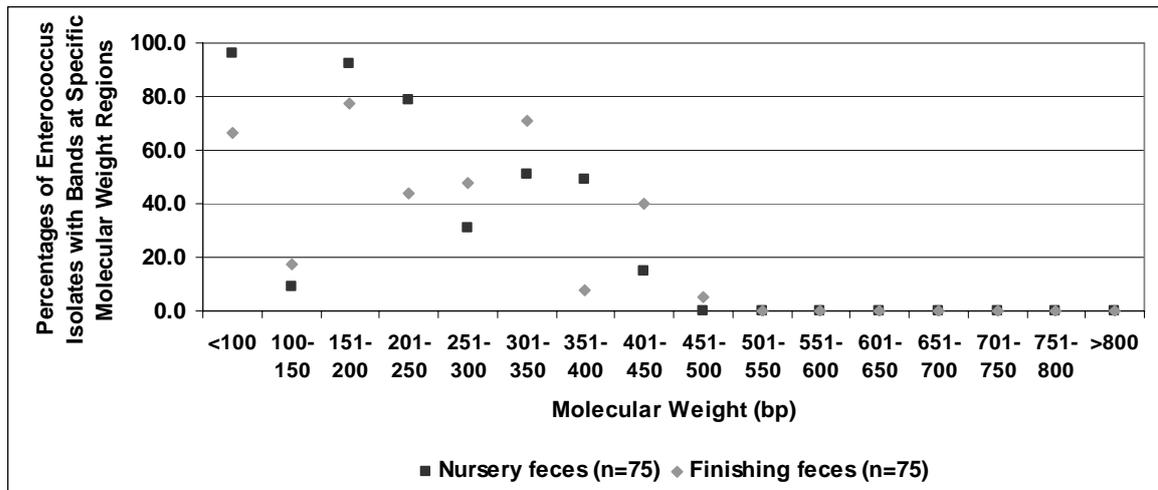
Figure 26. Percentages of _Enterococcus_ isolates having bands at specific molecular weight regions. Isolates were isolated from three <u>nursery fecal</u> samples and four <u>finishing fecal</u> samples in <u>warm season</u> (number of isolates from each sample was indicated in Table 6)

APPENDICES

Appendix A

SAS code

```
**pgm3.sas;
options pageno=1 ls=90;
libname in '.';

**Manure Data -- by bacteria ;

ods rtf   file='pgm3.rtf' style=journal;
ods graphics on;
*ods trace on /listing;
ods trace off;


data a; set in.all;
length loc2 $7;
loc2=compress(loc||sample);
proc sort data=a out=b; by    season bacteria;
proc glm data=b; by    season bacteria;
*ods exclude diff;
where site    ne 'Lagoon';
class     age loc2 site    trt ;
model Pc= age loc2(age)    trt age*trt;
test h=age e=loc2(age);

lsmeans    age|trt / slice= trt    pdiff;
means trt / lsd;
run; quit;
ods rtf close;
ods graphics off;
```

Appendix B

Least squares means percentages of resistant isolates for cool season *E. coli*

The GLM Procedure
Least Squares Means
Season=cool Bacteria=*E. coli*

Table 1. Least squares means and the index numbers of percentages of resistant isolates at different antibiotic concentrations for *E. coli* in cool season

Age	Trt	Pc LSMEAN	LSMEAN Number
Finishing	CEP15	34.134457	1
Finishing	CEP25	17.519189	2
Finishing	CEP35	14.136513	3
Finishing	ERY100	27.290296	4
Finishing	ERY60	86.438459	5
Finishing	ERY70	77.559622	6
Finishing	ERY90	45.668860	7
Finishing	NEO10	96.448739	8
Finishing	NEO2.5	99.210526	9
Finishing	NEO5	97.238213	10
Finishing	OTC10	99.479167	11
Finishing	OTC15	99.479167	12
Finishing	OTC2.5	99.479167	13
Finishing	OTC5	99.479167	14
Finishing	OTC7.5	99.479167	15
Finishing	RIF60	50.374863	16
Finishing	RIF75	29.533306	17
Finishing	RIF90	26.533032	18
Finishing	STR10	59.699836	19
Finishing	STR15	44.261239	20
Finishing	STR2.5	99.476425	21
Finishing	STR5	95.749041	22
Finishing	STR7.5	73.922697	23
Finishing	TET10	98.697917	24
Finishing	TET15	98.697917	25
Finishing	TET2.5	99.479167	26
Finishing	TET5	98.697917	27
Finishing	TET7.5	98.697917	28
Nursery	CEP15	58.314572	29

Age	Trt	Pc LSMEAN	LSMEAN Number
Nursery	CEP25	51.029167	30
Nursery	CEP35	47.197906	31
Nursery	ERY100	76.058333	32
Nursery	ERY60	85.600000	33
Nursery	ERY70	83.866667	34
Nursery	ERY90	81.243056	35
Nursery	NEO10	99.486111	36
Nursery	NEO2.5	100.000000	37
Nursery	NEO5	100.000000	38
Nursery	OTC10	100.000000	39
Nursery	OTC15	100.000000	40
Nursery	OTC2.5	100.000000	41
Nursery	OTC5	99.650960	42
Nursery	OTC7.5	100.000000	43
Nursery	RIF60	36.462500	44
Nursery	RIF75	33.840278	45
Nursery	RIF90	33.666667	46
Nursery	STR10	100.000000	47
Nursery	STR15	93.759722	48
Nursery	STR2.5	100.000000	49
Nursery	STR5	100.000000	50
Nursery	STR7.5	100.000000	51
Nursery	TET10	100.000000	52
Nursery	TET15	99.825480	53
Nursery	TET2.5	100.000000	54
Nursery	TET5	100.000000	55
Nursery	TET7.5	100.000000	56

Table 2. P-values of comparisons of different antibiotic concentrations for *E. coli* in cool season

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.1985	0.1222	0.5954	<.0001	0.0010	0.3713	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	0.1985		0.7929	0.4487	<.0001	<.0001	0.0303	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	0.1222	0.7929		0.3082	<.0001	<.0001	0.0155	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	0.5954	0.4487	0.3082		<.0001	0.0001	0.1552	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		0.4911	0.0019	0.4377	0.3224	0.4025	0.3123	0.3123	0.3123	0.3123
6	0.0010	<.0001	<.0001	0.0001	0.4911		0.0144	0.1442	0.0945	0.1283	0.0906	0.0906	0.0906	0.0906
7	0.3713	0.0303	0.0155	0.1552	0.0019	0.0144		0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	0.4377	0.1442	0.0001		0.8303	0.9511	0.8140	0.8140	0.8140	0.8140
9	<.0001	<.0001	<.0001	<.0001	0.3224	0.0945	<.0001	0.8303		0.8783	0.9834	0.9834	0.9834	0.9834
10	<.0001	<.0001	<.0001	<.0001	0.4025	0.1283	<.0001	0.9511	0.8783		0.8619	0.8619	0.8619	0.8619
11	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619		1.0000	1.0000	1.0000
12	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619	1.0000		1.0000	1.0000
13	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619	1.0000	1.0000		1.0000
14	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619	1.0000	1.0000	1.0000	
15	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619	1.0000	1.0000	1.0000	1.0000
16	0.2088	0.0117	0.0056	0.0749	0.0058	0.0363	0.7150	0.0005	0.0002	0.0004	0.0002	0.0002	0.0002	0.0002
17	0.7210	0.3518	0.2333	0.8618	<.0001	0.0003	0.2117	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	0.5554	0.4845	0.3367	0.9531	<.0001	0.0001	0.1390	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.0488	0.0013	0.0005	0.0129	0.0395	0.1671	0.2771	0.0049	0.0026	0.0041	0.0024	0.0024	0.0024	0.0024
20	0.4323	0.0394	0.0206	0.1891	0.0013	0.0107	0.9130	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	0.3124	0.0906	<.0001	0.8142	0.9835	0.8621	0.9998	0.9998	0.9998	0.9998
22	<.0001	<.0001	<.0001	<.0001	0.4703	0.1595	0.0002	0.9567	0.7882	0.9080	0.7722	0.7722	0.7722	0.7722
23	0.0024	<.0001	<.0001	0.0004	0.3321	0.7777	0.0297	0.0821	0.0513	0.0720	0.0489	0.0489	0.0489	0.0489
24	<.0001	<.0001	<.0001	<.0001	0.3421	0.1025	<.0001	0.8614	0.9683	0.9098	0.9516	0.9516	0.9516	0.9516
25	<.0001	<.0001	<.0001	<.0001	0.3421	0.1025	<.0001	0.8614	0.9683	0.9098	0.9516	0.9516	0.9516	0.9516
26	<.0001	<.0001	<.0001	<.0001	0.3123	0.0906	<.0001	0.8140	0.9834	0.8619	1.0000	1.0000	1.0000	1.0000
27	<.0001	<.0001	<.0001	<.0001	0.3421	0.1025	<.0001	0.8614	0.9683	0.9098	0.9516	0.9516	0.9516	0.9516
28	<.0001	<.0001	<.0001	<.0001	0.3421	0.1025	<.0001	0.8614	0.9683	0.9098	0.9516	0.9516	0.9516	0.9516
29	0.0840	0.0039	0.0018	0.0271	0.0449	0.1681	0.3642	0.0069	0.0038	0.0058	0.0036	0.0036	0.0036	0.0036
30	0.2260	0.0172	0.0089	0.0897	0.0119	0.0582	0.7002	0.0014	0.0007	0.0011	0.0007	0.0007	0.0007	0.0007
31	0.3486	0.0344	0.0187	0.1541	0.0054	0.0305	0.9125	0.0005	0.0003	0.0004	0.0002	0.0002	0.0002	0.0002
32	0.0030	<.0001	<.0001	0.0006	0.4561	0.9141	0.0304	0.1444	0.0978	0.1296	0.0941	0.0941	0.0941	0.0941
33	0.0003	<.0001	<.0001	<.0001	0.9520	0.5636	0.0047	0.4361	0.3289	0.4035	0.3194	0.3194	0.3194	0.3194
34	0.0005	<.0001	<.0001	<.0001	0.8534	0.6505	0.0068	0.3666	0.2712	0.3374	0.2630	0.2630	0.2630	0.2630
35	0.0009	<.0001	<.0001	0.0002	0.7089	0.7913	0.0115	0.2756	0.1980	0.2515	0.1914	0.1914	0.1914	0.1914
36	<.0001	<.0001	<.0001	<.0001	0.3492	0.1167	0.0002	0.8272	0.9842	0.8717	0.9996	0.9996	0.9996	0.9996
37	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
38	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
39	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
40	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
41	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
42	<.0001	<.0001	<.0001	<.0001	0.3432	0.1140	0.0002	0.8180	0.9747	0.8623	0.9901	0.9901	0.9901	0.9901
43	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
44	0.8671	0.1749	0.1103	0.5101	0.0004	0.0036	0.5086	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	0.9831	0.2420	0.1583	0.6380	0.0002	0.0020	0.3959	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	0.9732	0.2470	0.1620	0.6469	0.0002	0.0019	0.3890	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
48	<.0001	<.0001	<.0001	<.0001	0.5990	0.2455	0.0007	0.8468	0.6953	0.8026	0.6811	0.6811	0.6811	0.6811
49	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
50	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
51	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
52	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
53	<.0001	<.0001	<.0001	<.0001	0.3368	0.1112	0.0002	0.8083	0.9647	0.8525	0.9801	0.9801	0.9801	0.9801
54	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
55	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701
56	<.0001	<.0001	<.0001	<.0001	0.3306	0.1085	0.0001	0.7986	0.9548	0.8427	0.9701	0.9701	0.9701	0.9701

(continued)

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	<.0001	0.2088	0.7210	0.5554	0.0488	0.4323	<.0001	<.0001	0.0024	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001	0.0117	0.3518	0.4845	0.0013	0.0394	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.0056	0.2333	0.3367	0.0005	0.0206	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	0.0749	0.8618	0.9531	0.0129	0.1891	<.0001	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.3123	0.0058	<.0001	<.0001	0.0395	0.0013	0.3124	0.4703	0.3321	0.3421	0.3421	0.3123	0.3421	0.3421
6	0.0906	0.0363	0.0003	0.0001	0.1671	0.0107	0.0906	0.1595	0.7777	0.1025	0.1025	0.0906	0.1025	0.1025
7	<.0001	0.7150	0.2117	0.1390	0.2771	0.9130	<.0001	0.0002	0.0297	<.0001	<.0001	<.0001	<.0001	<.0001
8	0.8140	0.0005	<.0001	<.0001	0.0049	<.0001	0.8142	0.9567	0.0821	0.8614	0.8614	0.8140	0.8614	0.8614
9	0.9834	0.0002	<.0001	<.0001	0.0026	<.0001	0.9835	0.7882	0.0513	0.9683	0.9683	0.9834	0.9683	0.9683
10	0.8619	0.0004	<.0001	<.0001	0.0041	<.0001	0.8621	0.9080	0.0720	0.9098	0.9098	0.8619	0.9098	0.9098
11	1.0000	0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516	1.0000	0.9516	0.9516
12	1.0000	0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516	1.0000	0.9516	0.9516
13	1.0000	0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516	1.0000	0.9516	0.9516

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
14	1.0000	0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516	1.0000	0.9516	0.9516
15		0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516	1.0000	0.9516	0.9516
16	0.0002		0.1074	0.0659	0.4696	0.6352	0.0002	0.0006	0.0693	0.0003	0.0003	0.0002	0.0003	0.0003
17	<.0001	0.1074		0.8159	0.0204	0.2541	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	0.0659	0.8159		0.0110	0.1703	<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.0024	0.4696	0.0204	0.0110		0.2320	0.0024	0.0058	0.2707	0.0029	0.0029	0.0024	0.0029	0.0029
20	<.0001	0.6352	0.2541	0.1703	0.2320		<.0001	0.0001	0.0226	<.0001	<.0001	<.0001	<.0001	<.0001
21	0.9998	0.0002	<.0001	<.0001	0.0024	<.0001		0.7724	0.0489	0.9518	0.9518	0.9998	0.9518	0.9518
22	0.7722	0.0006	<.0001	<.0001	0.0058	0.0001	0.7724		0.0919	0.8190	0.8190	0.7722	0.8190	0.8190
23	0.0489	0.0693	0.0007	0.0003	0.2707	0.0226	0.0489	0.0919		0.0561	0.0561	0.0489	0.0561	0.0561
24	0.9516	0.0003	<.0001	<.0001	0.0029	<.0001	0.9518	0.8190	0.0561		1.0000	0.9516	1.0000	1.0000
25	0.9516	0.0003	<.0001	<.0001	0.0029	<.0001	0.9518	0.8190	0.0561	1.0000		0.9516	1.0000	1.0000
26	1.0000	0.0002	<.0001	<.0001	0.0024	<.0001	0.9998	0.7722	0.0489	0.9516	0.9516		0.9516	0.9516
27	0.9516	0.0003	<.0001	<.0001	0.0029	<.0001	0.9518	0.8190	0.0561	1.0000	1.0000	0.9516		1.0000
28	0.9516	0.0003	<.0001	<.0001	0.0029	<.0001	0.9518	0.8190	0.0561	1.0000	1.0000	0.9516	1.0000	
29	0.0036	0.5685	0.0401	0.0237	0.9207	0.3134	0.0036	0.0079	0.2631	0.0043	0.0043	0.0036	0.0043	0.0043
30	0.0007	0.9625	0.1240	0.0800	0.5335	0.6268	0.0007	0.0016	0.1016	0.0008	0.0008	0.0007	0.0008	0.0008
31	0.0002	0.8194	0.2056	0.1391	0.3697	0.8329	0.0002	0.0006	0.0564	0.0003	0.0003	0.0002	0.0003	0.0003
32	0.0941	0.0666	0.0010	0.0005	0.2410	0.0236	0.0941	0.1586	0.8780	0.1054	0.1054	0.0941	0.1054	0.1054
33	0.3194	0.0123	<.0001	<.0001	0.0644	0.0035	0.3195	0.4662	0.4020	0.3473	0.3473	0.3194	0.3473	0.3473
34	0.2630	0.0172	0.0001	<.0001	0.0841	0.0050	0.2631	0.3938	0.4753	0.2875	0.2875	0.2630	0.2875	0.2875
35	0.1914	0.0279	0.0003	0.0001	0.1232	0.0087	0.1915	0.2982	0.5990	0.2110	0.2110	0.1914	0.2110	0.2110
36	0.9996	0.0006	<.0001	<.0001	0.0048	0.0001	0.9994	0.7883	0.0679	0.9548	0.9548	0.9996	0.9548	0.9548
37	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
38	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
39	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
40	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
41	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
42	0.9901	0.0005	<.0001	<.0001	0.0047	0.0001	0.9900	0.7792	0.0662	0.9454	0.9454	0.9901	0.9454	0.9454
43	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
44	<.0001	0.3183	0.6187	0.4759	0.0966	0.5754	<.0001	<.0001	0.0079	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	0.2360	0.7570	0.5997	0.0648	0.4544	<.0001	<.0001	0.0045	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	0.2311	0.7665	0.6084	0.0630	0.4469	<.0001	<.0001	0.0044	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
48	0.6811	0.0022	<.0001	<.0001	0.0155	0.0005	0.6813	0.8863	0.1555	0.7227	0.7227	0.6811	0.7227	0.7227
49	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
50	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
51	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
52	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
53	0.9801	0.0005	<.0001	<.0001	0.0045	0.0001	0.9800	0.7696	0.0644	0.9354	0.9354	0.9801	0.9354	0.9354
54	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
55	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254
56	0.9701	0.0005	<.0001	<.0001	0.0043	<.0001	0.9700	0.7600	0.0626	0.9254	0.9254	0.9701	0.9254	0.9254

(continued)

Dependent Variable: Pc

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	0.0840	0.2260	0.3486	0.0030	0.0003	0.0005	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	0.0039	0.0172	0.0344	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	0.0018	0.0089	0.0187	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	0.0271	0.0897	0.1541	0.0006	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.0449	0.0119	0.0054	0.4561	0.9520	0.8534	0.7089	0.3492	0.3306	0.3306	0.3306	0.3306	0.3306	0.3432
6	0.1681	0.0582	0.0305	0.9141	0.5636	0.6505	0.7913	0.1167	0.1085	0.1085	0.1085	0.1085	0.1085	0.1140
7	0.3642	0.7002	0.9125	0.0304	0.0047	0.0068	0.0115	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002
8	0.0069	0.0014	0.0005	0.1444	0.4361	0.3666	0.2756	0.8272	0.7986	0.7986	0.7986	0.7986	0.7986	0.8180
9	0.0038	0.0007	0.0003	0.0978	0.3289	0.2712	0.1980	0.9842	0.9548	0.9548	0.9548	0.9548	0.9548	0.9747
10	0.0058	0.0011	0.0004	0.1296	0.4035	0.3374	0.2515	0.8717	0.8427	0.8427	0.8427	0.8427	0.8427	0.8623
11	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
12	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
13	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
14	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
15	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
16	0.5685	0.9625	0.8194	0.0666	0.0123	0.0172	0.0279	0.0006	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
17	0.0401	0.1240	0.2056	0.0010	<.0001	0.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	0.0237	0.0800	0.1391	0.0005	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.9207	0.5335	0.3697	0.2410	0.0644	0.0841	0.1232	0.0048	0.0043	0.0043	0.0043	0.0043	0.0043	0.0047
20	0.3134	0.6268	0.8329	0.0236	0.0035	0.0050	0.0087	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001
21	0.0036	0.0007	0.0002	0.0941	0.3195	0.2631	0.1915	0.9994	0.9700	0.9700	0.9700	0.9700	0.9700	0.9900
22	0.0079	0.0016	0.0006	0.1586	0.4662	0.3938	0.2982	0.7883	0.7600	0.7600	0.7600	0.7600	0.7600	0.7792
23	0.2631	0.1016	0.0564	0.8780	0.4020	0.4753	0.5990	0.0679	0.0626	0.0626	0.0626	0.0626	0.0626	0.0662
24	0.0043	0.0008	0.0003	0.1054	0.3473	0.2875	0.2110	0.9548	0.9254	0.9254	0.9254	0.9254	0.9254	0.9454
25	0.0043	0.0008	0.0003	0.1054	0.3473	0.2875	0.2110	0.9548	0.9254	0.9254	0.9254	0.9254	0.9254	0.9454
26	0.0036	0.0007	0.0002	0.0941	0.3194	0.2630	0.1914	0.9996	0.9701	0.9701	0.9701	0.9701	0.9701	0.9901
27	0.0043	0.0008	0.0003	0.1054	0.3473	0.2875	0.2110	0.9548	0.9254	0.9254	0.9254	0.9254	0.9254	0.9454

Dependent Variable: Pc

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
28	0.0043	0.0008	0.0003	0.1054	0.3473	0.2875	0.2110	0.9548	0.9254	0.9254	0.9254	0.9254	0.9254	0.9454
29		0.6245	0.4553	0.2342	0.0683	0.0876	0.1249	0.0063	0.0057	0.0057	0.0057	0.0057	0.0057	0.0061
30	0.6245		0.7968	0.0942	0.0214	0.0287	0.0438	0.0014	0.0012	0.0012	0.0012	0.0012	0.0012	0.0013
31	0.4553	0.7968		0.0540	0.0108	0.0148	0.0234	0.0006	0.0005	0.0005	0.0005	0.0005	0.0005	0.0006
32	0.2342	0.0942	0.0540		0.5216	0.5998	0.7275	0.1169	0.1092	0.1092	0.1092	0.1092	0.1092	0.1144
33	0.0683	0.0214	0.0108	0.5216		0.9072	0.7696	0.3513	0.3339	0.3339	0.3339	0.3339	0.3339	0.3457
34	0.0876	0.0287	0.0148	0.5998	0.9072		0.8600	0.2947	0.2792	0.2792	0.2792	0.2792	0.2792	0.2897
35	0.1249	0.0438	0.0234	0.7275	0.7696	0.8600		0.2213	0.2087	0.2087	0.2087	0.2087	0.2087	0.2172
36	0.0063	0.0014	0.0006	0.1169	0.3513	0.2947	0.2213		0.9724	0.9724	0.9724	0.9724	0.9724	0.9912
37	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724		1.0000	1.0000	1.0000	1.0000	0.9813
38	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000		1.0000	1.0000	1.0000	0.9813
39	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000		1.0000	1.0000	0.9813
40	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000		1.0000	0.9813
41	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000		0.9813
42	0.0061	0.0013	0.0006	0.1144	0.3457	0.2897	0.2172	0.9912	0.9813	0.9813	0.9813	0.9813	0.9813	
43	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
44	0.1434	0.3283	0.4709	0.0086	0.0012	0.0018	0.0031	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	0.1016	0.2491	0.3699	0.0052	0.0007	0.0010	0.0018	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	0.0992	0.2443	0.3638	0.0050	0.0006	0.0009	0.0017	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
48	0.0184	0.0047	0.0021	0.2353	0.5835	0.5064	0.4007	0.7004	0.6750	0.6750	0.6750	0.6750	0.6750	0.6922
49	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
50	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
51	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
52	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
53	0.0059	0.0013	0.0005	0.1118	0.3397	0.2844	0.2129	0.9818	0.9906	0.9906	0.9906	0.9906	0.9906	0.9906
54	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
55	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813
56	0.0057	0.0012	0.0005	0.1092	0.3339	0.2792	0.2087	0.9724	1.0000	1.0000	1.0000	1.0000	1.0000	0.9813

(continued)

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	<.0001	0.8671	0.9831	0.9732	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001	0.1749	0.2420	0.2470	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.1103	0.1583	0.1620	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
4	<.0001	0.5101	0.6380	0.6469	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.3306	0.0004	0.0002	0.0002	0.3306	0.5990	0.3306	0.3306	0.3306	0.3306	0.3368	0.3306	0.3306	0.3306
6	0.1085	0.0036	0.0020	0.0019	0.1085	0.2455	0.1085	0.1085	0.1085	0.1085	0.1112	0.1085	0.1085	0.1085
7	0.0001	0.5086	0.3959	0.3890	0.0001	0.0007	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001
8	0.7986	<.0001	<.0001	<.0001	0.7986	0.8468	0.7986	0.7986	0.7986	0.7986	0.8083	0.7986	0.7986	0.7986
9	0.9548	<.0001	<.0001	<.0001	0.9548	0.6953	0.9548	0.9548	0.9548	0.9548	0.9647	0.9548	0.9548	0.9548
10	0.8427	<.0001	<.0001	<.0001	0.8427	0.8026	0.8427	0.8427	0.8427	0.8427	0.8525	0.8427	0.8427	0.8427
11	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
12	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
13	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
14	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
15	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
16	0.0005	0.3183	0.2360	0.2311	0.0005	0.0022	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
17	<.0001	0.6187	0.7570	0.7665	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	0.4759	0.5997	0.6084	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.0043	0.0966	0.0648	0.0630	0.0043	0.0155	0.0043	0.0043	0.0043	0.0043	0.0045	0.0043	0.0043	0.0043
20	<.0001	0.5754	0.4544	0.4469	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001
21	0.9700	<.0001	<.0001	<.0001	0.9700	0.6813	0.9700	0.9700	0.9700	0.9700	0.9800	0.9700	0.9700	0.9700
22	0.7600	<.0001	<.0001	<.0001	0.7600	0.8863	0.7600	0.7600	0.7600	0.7600	0.7696	0.7600	0.7600	0.7600
23	0.0626	0.0079	0.0045	0.0044	0.0626	0.1555	0.0626	0.0626	0.0626	0.0626	0.0644	0.0626	0.0626	0.0626
24	0.9254	<.0001	<.0001	<.0001	0.9254	0.7227	0.9254	0.9254	0.9254	0.9254	0.9354	0.9254	0.9254	0.9254
25	0.9254	<.0001	<.0001	<.0001	0.9254	0.7227	0.9254	0.9254	0.9254	0.9254	0.9354	0.9254	0.9254	0.9254
26	0.9701	<.0001	<.0001	<.0001	0.9701	0.6811	0.9701	0.9701	0.9701	0.9701	0.9801	0.9701	0.9701	0.9701
27	0.9254	<.0001	<.0001	<.0001	0.9254	0.7227	0.9254	0.9254	0.9254	0.9254	0.9354	0.9254	0.9254	0.9254
28	0.9254	<.0001	<.0001	<.0001	0.9254	0.7227	0.9254	0.9254	0.9254	0.9254	0.9354	0.9254	0.9254	0.9254
29	0.0057	0.1434	0.1016	0.0992	0.0057	0.0184	0.0057	0.0057	0.0057	0.0057	0.0059	0.0057	0.0057	0.0057
30	0.0012	0.3283	0.2491	0.2443	0.0012	0.0047	0.0012	0.0012	0.0012	0.0012	0.0013	0.0012	0.0012	0.0012
31	0.0005	0.4709	0.3699	0.3638	0.0005	0.0021	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
32	0.1092	0.0086	0.0052	0.0050	0.1092	0.2353	0.1092	0.1092	0.1092	0.1092	0.1118	0.1092	0.1092	0.1092
33	0.3339	0.0012	0.0007	0.0006	0.3339	0.5835	0.3339	0.3339	0.3339	0.3339	0.3397	0.3339	0.3339	0.3339
34	0.2792	0.0018	0.0010	0.0009	0.2792	0.5064	0.2792	0.2792	0.2792	0.2792	0.2844	0.2792	0.2792	0.2792
35	0.2087	0.0031	0.0018	0.0017	0.2087	0.4007	0.2087	0.2087	0.2087	0.2087	0.2129	0.2087	0.2087	0.2087
36	0.9724	<.0001	<.0001	<.0001	0.9724	0.7004	0.9724	0.9724	0.9724	0.9724	0.9818	0.9724	0.9724	0.9724
37	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
38	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
39	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
40	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
41	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
42	0.9813	<.0001	<.0001	<.0001	0.9813	0.6922	0.9813	0.9813	0.9813	0.9813	0.9906	0.9813	0.9813	0.9813
43		<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
44	<.0001		0.8601	0.8509	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	0.8601		0.9907	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	0.8509	0.9907		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	1.0000	<.0001	<.0001	<.0001		0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
48	0.6750	0.0002	<.0001	<.0001	0.6750		0.6750	0.6750	0.6750	0.6750	0.6835	0.6750	0.6750	0.6750
49	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750		1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
50	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000		1.0000	1.0000	0.9906	1.0000	1.0000	1.0000
51	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000		1.0000	0.9906	1.0000	1.0000	1.0000
52	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000		0.9906	1.0000	1.0000	1.0000
53	0.9906	<.0001	<.0001	<.0001	0.9906	0.6835	0.9906	0.9906	0.9906	0.9906		0.9906	0.9906	0.9906
54	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906		1.0000	1.0000
55	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000		1.0000
56	1.0000	<.0001	<.0001	<.0001	1.0000	0.6750	1.0000	1.0000	1.0000	1.0000	0.9906	1.0000	1.0000	

Appendix C

Least squares means of percentages of resistant isolates for cool season *Enterococcus*

The GLM Procedure
Least Squares Means
Season=cool Bacteria=*Enterococcus*

Table 1. Least squares means and the index numbers of percentages of resistant isolates at different antibiotic concentrations for *Enterococcus* in cool season

Age	Trt	Pc LSMEAN	LSMEAN Number
Finishing	AMX2.5	20.0220341	1
Finishing	CEP10	22.1695244	2
Finishing	CEP15	21.9843392	3
Finishing	CEP30	20.3545964	4
Finishing	CEP50	9.9537037	5
Finishing	CTC100	57.9319101	6
Finishing	CTC60	77.2425586	7
Finishing	CTC80	69.7840449	8
Finishing	ERY10	57.3682908	9
Finishing	ERY15	57.3682908	10
Finishing	ERY30	53.4902951	11
Finishing	ERY50	46.1346660	12
Finishing	NEO40	36.7005135	13
Finishing	NEO60	30.3092196	14
Finishing	NEO80	25.5382961	15
Finishing	OTC100	8.8425926	16
Finishing	OTC20	38.9804912	17
Finishing	OTC40	30.4834762	18
Finishing	OTC60	24.3043177	19
Finishing	OTC80	12.3983179	20
Finishing	STR100	29.4319950	21
Finishing	STR40	36.6018377	22
Finishing	STR60	31.2493280	23
Finishing	STR80	31.3864132	24
Finishing	TET10	42.7410661	25
Finishing	TET100	10.5092593	26
Finishing	TET15	42.0917155	27
Finishing	TET30	33.5072716	28
Finishing	TET50	22.6751761	29

Age	Trt	Pc LSMEAN	LSMEAN Number
Finishing	*VAN2.5*	20.6804742	30
Nursery	*AMX2.5*	81.8758307	31
Nursery	*CEP10*	95.5436720	32
Nursery	*CEP15*	95.5436720	33
Nursery	*CEP30*	88.4135472	34
Nursery	*CEP50*	88.4135472	35
Nursery	*CTC100*	82.5397942	36
Nursery	*CTC60*	88.1600447	37
Nursery	*CTC80*	84.1376028	38
Nursery	*ERY10*	95.3654189	39
Nursery	*ERY15*	93.5828877	40
Nursery	*ERY30*	93.4092766	41
Nursery	*ERY50*	84.5430407	42
Nursery	*NEO40*	94.2058452	43
Nursery	*NEO60*	93.3266782	44
Nursery	*NEO80*	81.8934117	45
Nursery	*OTC100*	89.3217000	46
Nursery	*OTC20*	99.2869875	47
Nursery	*OTC40*	99.2869875	48
Nursery	*OTC60*	98.4097945	49
Nursery	*OTC80*	97.1620227	50
Nursery	*STR100*	86.3283864	51
Nursery	*STR40*	94.8334741	52
Nursery	*STR60*	92.5208255	53
Nursery	*STR80*	90.9351155	54
Nursery	*TET10*	97.0062858	55
Nursery	*TET100*	83.2966664	56
Nursery	*TET15*	95.7782156	57
Nursery	*TET30*	94.2248530	58
Nursery	*TET50*	92.6325269	59
Nursery	*VAN2.5*	0.1736111	60

Table 2. P-values of comparisons of different antibiotic concentrations for *Enterococcus* in cool season

*Least Squares Means for effect Age*Trt*
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		0.7837	0.8019	0.9661	0.1993	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011	0.0344	0.1898	0.4811
2	0.7837		0.9811	0.8165	0.1199	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0026	0.0648	0.2990	0.6668
3	0.8019	0.9811		0.8350	0.1256	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0024	0.0615	0.2882	0.6497
4	0.9661	0.8165	0.8350		0.1850	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012	0.0381	0.2044	0.5079
5	0.1993	0.1199	0.1256	0.1850		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0008	0.0101	0.0478
6	<.0001	<.0001	<.0001	<.0001	<.0001		0.0146	0.1312	0.9426	0.9426	0.5704	0.1330	0.0073	0.0005	<.0001
7	<.0001	<.0001	<.0001	<.0001	<.0001	0.0146		0.3411	0.0120	0.0120	0.0028	0.0001	<.0001	<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001	0.1312	0.3411		0.1140	0.1140	0.0387	0.0029	<.0001	<.0001	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001	0.9426	0.0120	0.1140		1.0000	0.6202	0.1524	0.0090	0.0007	<.0001
10	<.0001	<.0001	<.0001	<.0001	<.0001	0.9426	0.0120	0.1140	1.0000		0.6202	0.1524	0.0090	0.0007	<.0001
11	<.0001	<.0001	<.0001	<.0001	<.0001	0.5704	0.0028	0.0387	0.6202	0.6202		0.3478	0.0332	0.0035	0.0005
12	0.0011	0.0026	0.0024	0.0012	<.0001	0.1330	0.0001	0.0029	0.1524	0.1524	0.3478		0.2290	0.0445	0.0093
13	0.0344	0.0648	0.0615	0.0381	0.0008	0.0073	<.0001	<.0001	0.0090	0.0090	0.0332	0.2290		0.4144	0.1550
14	0.1898	0.2990	0.2882	0.2044	0.0101	0.0005	<.0001	<.0001	0.0007	0.0007	0.0035	0.0445	0.4144		0.5422
15	0.4811	0.6668	0.6497	0.5079	0.0478	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.0093	0.1550	0.5422	
16	0.1544	0.0900	0.0945	0.1426	0.8870	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.0067	0.0342
17	0.0164	0.0330	0.0311	0.0184	0.0003	0.0165	<.0001	0.0001	0.0199	0.0199	0.0652	0.3611	0.7707	0.2686	0.0873
18	0.1824	0.2888	0.2782	0.1967	0.0095	0.0006	<.0001	<.0001	0.0008	0.0008	0.0037	0.0469	0.4272	0.9822	0.5275
19	0.5843	0.7849	0.7668	0.6138	0.0681	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0059	0.1146	0.4431	0.8747
20	0.3305	0.2128	0.2216	0.3100	0.7547	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0022	0.0232	0.0946
21	0.2301	0.3539	0.3418	0.2470	0.0137	0.0004	<.0001	<.0001	0.0005	0.0005	0.0025	0.0341	0.3535	0.9107	0.6188
22	0.0354	0.0666	0.0632	0.0392	0.0008	0.0071	<.0001	<.0001	0.0087	0.0087	0.0322	0.2242	0.9899	0.4217	0.1587
23	0.1527	0.2468	0.2374	0.1651	0.0072	0.0008	<.0001	<.0001	0.0010	0.0010	0.0050	0.0586	0.4862	0.9043	0.4657
24	0.1477	0.2398	0.2305	0.1599	0.0068	0.0009	<.0001	<.0001	0.0011	0.0011	0.0053	0.0609	0.4973	0.8905	0.4551
25	0.0042	0.0093	0.0087	0.0048	<.0001	0.0537	<.0001	0.0007	0.0631	0.0631	0.1708	0.6645	0.4405	0.1136	0.0292
26	0.2251	0.1376	0.1439	0.2094	0.9434	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0010	0.0123	0.0562
27	0.0054	0.0118	0.0110	0.0061	<.0001	0.0443	<.0001	0.0005	0.0523	0.0523	0.1465	0.6054	0.4910	0.1335	0.0357
28	0.0863	0.1487	0.1422	0.0943	0.0030	0.0021	<.0001	<.0001	0.0027	0.0027	0.0115	0.1080	0.6832	0.6827	0.3092
29	0.7345	0.9485	0.9296	0.7668	0.1055	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0031	0.0746	0.3299	0.7144
30	0.9329	0.8490	0.8676	0.9668	0.1717	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0014	0.0420	0.2195	0.5349
31	<.0001	<.0001	<.0001	<.0001	<.0001	0.0052	0.5836	0.1538	0.0042	0.0042	0.0010	<.0001	<.0001	<.0001	<.0001
32	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0317	0.0027	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
33	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0317	0.0027	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
34	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.1874	0.0288	0.0003	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.1874	0.0288	0.0003	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	0.0041	0.5310	0.1326	0.0033	0.0033	0.0007	<.0001	<.0001	<.0001	<.0001
37	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.1976	0.0310	0.0004	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	<.0001	<.0001	0.0023	0.4150	0.0909	0.0018	0.0018	0.0004	<.0001	<.0001	<.0001	<.0001
39	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0333	0.0029	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
40	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0546	0.0054	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
41	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0572	0.0058	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	0.0020	0.3882	0.0823	0.0016	0.0016	0.0003	<.0001	<.0001	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0462	0.0044	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0585	0.0060	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	<.0001	<.0001	0.0051	0.5822	0.1532	0.0042	0.0042	0.0010	<.0001	<.0001	<.0001	<.0001
46	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.1543	0.0219	0.0002	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0099	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0099	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
49	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0132	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
50	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0195	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
51	<.0001	<.0001	<.0001	<.0001	<.0001	0.0010	0.2832	0.0517	0.0008	0.0008	0.0002	<.0001	<.0001	<.0001	<.0001
52	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0388	0.0035	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0721	0.0079	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
54	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.1067	0.0133	0.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
55	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0205	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
56	<.0001	<.0001	<.0001	<.0001	<.0001	0.0031	0.4740	0.1113	0.0025	0.0025	0.0005	<.0001	<.0001	<.0001	<.0001
57	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0296	0.0025	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
58	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0459	0.0043	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
59	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0701	0.0076	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
60	0.0200	0.0101	0.0107	0.0180	0.2481	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.0031

(continued)

Dependent Variable: Pc

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.1544	0.0164	0.1824	0.5843	0.3305	0.2301	0.0354	0.1527	0.1477	0.0042	0.2251	0.0054	0.0863	0.7345	0.9329
2	0.0900	0.0330	0.2888	0.7849	0.2128	0.3539	0.0666	0.2468	0.2398	0.0093	0.1376	0.0118	0.1487	0.9485	0.8490
3	0.0945	0.0311	0.2782	0.7668	0.2216	0.3418	0.0632	0.2374	0.2305	0.0087	0.1439	0.0110	0.1422	0.9296	0.8676
4	0.1426	0.0184	0.1967	0.6138	0.3100	0.2470	0.0392	0.1651	0.1599	0.0048	0.2094	0.0061	0.0943	0.7668	0.9668
5	0.8870	0.0003	0.0095	0.0681	0.7547	0.0137	0.0008	0.0072	0.0068	<.0001	0.9434	<.0001	0.0030	0.1055	0.1717
6	<.0001	0.0165	0.0006	<.0001	<.0001	0.0004	0.0071	0.0008	0.0009	0.0537	<.0001	0.0443	0.0021	<.0001	<.0001
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	<.0001	0.0005	<.0001	<.0001	<.0001
9	<.0001	0.0199	0.0008	<.0001	<.0001	0.0005	0.0087	0.0010	0.0011	0.0631	<.0001	0.0523	0.0027	<.0001	<.0001
10	<.0001	0.0199	0.0008	<.0001	<.0001	0.0005	0.0087	0.0010	0.0011	0.0631	<.0001	0.0523	0.0027	<.0001	<.0001
11	<.0001	0.0652	0.0037	0.0003	<.0001	0.0025	0.0322	0.0050	0.0053	0.1708	<.0001	0.1465	0.0115	0.0001	<.0001
12	<.0001	0.3611	0.0469	0.0059	<.0001	0.0341	0.2242	0.0586	0.0609	0.6645	<.0001	0.6054	0.1080	0.0031	0.0014
13	0.0005	0.7707	0.4272	0.1146	0.0022	0.3535	0.9899	0.4862	0.4973	0.4405	0.0010	0.4910	0.6832	0.0746	0.0420
14	0.0067	0.2686	0.9822	0.4431	0.0232	0.9107	0.4217	0.9043	0.8905	0.1136	0.0123	0.1335	0.6827	0.3299	0.2195
15	0.0342	0.0873	0.5275	0.8747	0.0946	0.6188	0.1587	0.4657	0.4551	0.0292	0.0562	0.0357	0.3092	0.7144	0.5349
16		0.0002	0.0063	0.0496	0.6495	0.0093	0.0005	0.0047	0.0045	<.0001	0.8313	<.0001	0.0019	0.0786	0.1317

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
17	0.0002		0.2783	0.0622	0.0009	0.2234	0.7611	0.3238	0.3324	0.6308	0.0004	0.6909	0.4845	0.0385	0.0205
18	0.0063	0.2783		0.4301	0.0220	0.8931	0.4346	0.9220	0.9081	0.1187	0.0116	0.1393	0.6992	0.3190	0.2114
19	0.0496	0.0622	0.4301		0.1295	0.5124	0.1175	0.3753	0.3659	0.0196	0.0794	0.0242	0.2405	0.8350	0.6433
20	0.6495	0.0009	0.0220	0.1295		0.0308	0.0023	0.0170	0.0163	0.0002	0.8092	0.0002	0.0077	0.1902	0.2906
21	0.0093	0.2234	0.8931	0.5124	0.0308		0.3601	0.8163	0.8027	0.0905	0.0166	0.1071	0.6025	0.3883	0.2643
22	0.0005	0.7611	0.4346	0.1175	0.0023	0.3601		0.4942	0.5053	0.4330	0.0011	0.4832	0.6925	0.0766	0.0433
23	0.0047	0.3238	0.9220	0.3753	0.0170	0.8163	0.4942		0.9860	0.1433	0.0088	0.1671	0.7729	0.2740	0.1780
24	0.0045	0.3324	0.9081	0.3659	0.0163	0.8027	0.5053	0.9860		0.1481	0.0084	0.1725	0.7863	0.2665	0.1725
25	<.0001	0.6308	0.1187	0.0196	0.0002	0.0905	0.4330	0.1433	0.1481		<.0001	0.9338	0.2389	0.0112	0.0054
26	0.8313	0.0004	0.0116	0.0794	0.8092	0.0166	0.0011	0.0088	0.0084	<.0001		<.0001	0.0038	0.1214	0.1948
27	<.0001	0.6909	0.1393	0.0242	0.0002	0.1071	0.4832	0.1671	0.1725	0.9338	<.0001		0.2734	0.0140	0.0069
28	0.0019	0.4845	0.6992	0.2405	0.0077	0.6025	0.6925	0.7729	0.7863	0.2389	0.0038	0.2734		0.1675	0.1026
29	0.0786	0.0385	0.3190	0.8350	0.1902	0.3883	0.0766	0.2740	0.2665	0.0112	0.1214	0.0140	0.1675		0.7987
30	0.1317	0.0205	0.2114	0.6433	0.2906	0.2643	0.0433	0.1780	0.1725	0.0054	0.1948	0.0069	0.1026	0.7987	
31	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
32	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
33	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
34	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
37	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
39	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
40	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
41	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
49	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
50	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
51	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
52	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
54	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
55	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
56	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
57	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
58	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
59	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
60	0.3058	<.0001	0.0004	0.0048	0.1494	0.0007	<.0001	0.0003	0.0003	<.0001	0.2224	<.0001	0.0001	0.0085	0.0163

(continued)

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	0.0052	<.0001	<.0001	0.0004	0.0004	0.0041	0.0005	0.0023	<.0001	<.0001	<.0001	0.0020	<.0001	<.0001	0.0051
7	0.5836	0.0317	0.0317	0.1874	0.1874	0.5310	0.1976	0.4150	0.0333	0.0546	0.0572	0.3882	0.0462	0.0585	0.5822
8	0.1538	0.0027	0.0027	0.0288	0.0288	0.1326	0.0310	0.0909	0.0029	0.0054	0.0058	0.0823	0.0044	0.0060	0.1532
9	0.0042	<.0001	<.0001	0.0003	0.0003	0.0033	0.0004	0.0018	<.0001	<.0001	<.0001	0.0016	<.0001	<.0001	0.0042
10	0.0042	<.0001	<.0001	0.0003	0.0003	0.0033	0.0004	0.0018	<.0001	<.0001	<.0001	0.0016	<.0001	<.0001	0.0042
11	0.0010	<.0001	<.0001	<.0001	<.0001	0.0007	<.0001	0.0004	<.0001	<.0001	<.0001	0.0003	<.0001	<.0001	0.0010
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
26	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
27	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
28	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
29	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
30	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
31		0.1317	0.1317	0.4696	0.4696	0.9414	0.4870	0.8023	0.1368	0.1962	0.2029	0.7678	0.1736	0.2061	0.9984
32	0.1317		1.0000	0.4304	0.4304	0.1514	0.4142	0.2079	0.9843	0.8282	0.8132	0.2244	0.8823	0.8061	0.1322
33	0.1317	1.0000		0.4304	0.4304	0.1514	0.4142	0.2079	0.9843	0.8282	0.8132	0.2244	0.8823	0.8061	0.1322
34	0.4696	0.4304	0.4304		1.0000	0.5158	0.9776	0.6361	0.4420	0.5673	0.5804	0.6684	0.5216	0.5867	0.4708

i/j	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
35	0.4696	0.4304	0.4304	1.0000		0.5158	0.9776	0.6361	0.4420	0.5673	0.5804	0.6684	0.5216	0.5867	0.4708
36	0.9414	0.1514	0.1514	0.5158	0.5158		0.5341	0.8596	0.1571	0.2227	0.2300	0.8245	0.1978	0.2335	0.9430
37	0.4870	0.4142	0.4142	0.9776	0.9776	0.5341		0.6562	0.4255	0.5485	0.5614	0.6889	0.5036	0.5675	0.4882
38	0.8023	0.2079	0.2079	0.6361	0.6361	0.8596	0.6562		0.2151	0.2966	0.3055	0.9642	0.2660	0.3099	0.8038
39	0.1368	0.9843	0.9843	0.4420	0.4420	0.1571	0.4255	0.2151		0.8436	0.8286	0.2320	0.8979	0.8214	0.1373
40	0.1962	0.8282	0.8282	0.5673	0.5673	0.2227	0.5485	0.2966	0.8436		0.9847	0.3177	0.9450	0.9774	0.1969
41	0.2029	0.8132	0.8132	0.5804	0.5804	0.2300	0.5614	0.3055	0.8286	0.9847		0.3271	0.9297	0.9927	0.2036
42	0.7678	0.2244	0.2244	0.6684	0.6684	0.8245	0.6889	0.9642	0.2320	0.3177	0.3271		0.2857	0.3316	0.7693
43	0.1736	0.8823	0.8823	0.5216	0.5216	0.1978	0.5036	0.2660	0.8979	0.9450	0.9297	0.2857		0.9225	0.1742
44	0.2061	0.8061	0.8061	0.5867	0.5867	0.2335	0.5675	0.3099	0.8214	0.9774	0.9927	0.3316	0.9225		0.2068
45	0.9984	0.1322	0.1322	0.4708	0.4708	0.9430	0.4882	0.8038	0.1373	0.1969	0.2036	0.7693	0.1742	0.2068	
46	0.4103	0.4913	0.4913	0.9199	0.9199	0.4532	0.8977	0.5662	0.5038	0.6372	0.6510	0.5969	0.5889	0.6576	0.4114
47	0.0554	0.6786	0.6786	0.2298	0.2298	0.0653	0.2192	0.0951	0.6643	0.5280	0.5155	0.1042	0.5740	0.5096	0.0557
48	0.0554	0.6786	0.6786	0.2298	0.2298	0.0653	0.2192	0.0951	0.6643	0.5280	0.5155	0.1042	0.5740	0.5096	0.0557
49	0.0688	0.7510	0.7510	0.2694	0.2694	0.0805	0.2575	0.1156	0.7361	0.5932	0.5800	0.1263	0.6417	0.5738	0.0690
50	0.0922	0.8578	0.8578	0.3335	0.3335	0.1070	0.3198	0.1508	0.8423	0.6920	0.6779	0.1638	0.7435	0.6712	0.0925
51	0.6222	0.3085	0.3085	0.8174	0.8174	0.6750	0.8393	0.8084	0.3179	0.4224	0.4336	0.8433	0.3838	0.4389	0.6236
52	0.1529	0.9373	0.9373	0.4776	0.4776	0.1749	0.4604	0.2375	0.9530	0.8899	0.8747	0.2556	0.9446	0.8675	0.1534
53	0.2397	0.7379	0.7379	0.6494	0.6494	0.2702	0.6294	0.3541	0.7529	0.9064	0.9216	0.3777	0.8520	0.9289	0.2405
54	0.3167	0.6101	0.6101	0.7801	0.7801	0.3534	0.7587	0.4522	0.6239	0.7694	0.7842	0.4795	0.7173	0.7912	0.3176
55	0.0955	0.8714	0.8714	0.3422	0.3422	0.1108	0.3282	0.1557	0.8559	0.7047	0.6905	0.1690	0.7566	0.6838	0.0959
56	0.8750	0.1765	0.1765	0.5713	0.5713	0.9332	0.5905	0.9258	0.1828	0.2558	0.2639	0.8903	0.2283	0.2678	0.8765
57	0.1253	0.9793	0.9793	0.4154	0.4154	0.1442	0.3996	0.1988	0.9635	0.8080	0.7931	0.2148	0.8618	0.7861	0.1258
58	0.1729	0.8839	0.8839	0.5203	0.5203	0.1971	0.5023	0.2651	0.8995	0.9433	0.9281	0.2847	0.9983	0.9208	0.1736
59	0.2348	0.7473	0.7473	0.6406	0.6406	0.2649	0.6206	0.3477	0.7623	0.9162	0.9315	0.3711	0.8617	0.9387	0.2356
60	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

(continued)

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0200
2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0101
3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0107
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0180
5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2481
6	0.0003	<.0001	<.0001	<.0001	<.0001	0.0010	<.0001	<.0001	0.0001	<.0001	0.0031	<.0001	<.0001	<.0001	<.0001
7	0.1543	0.0099	0.0099	0.0132	0.0195	0.2832	0.0388	0.0721	0.1067	0.0205	0.4740	0.0296	0.0459	0.0701	<.0001
8	0.0219	0.0006	0.0006	0.0009	0.0015	0.0517	0.0035	0.0079	0.0133	0.0015	0.1113	0.0025	0.0043	0.0076	<.0001
9	0.0002	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	0.0001	<.0001	0.0025	<.0001	<.0001	<.0001	<.0001
10	0.0002	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	0.0001	<.0001	0.0025	<.0001	<.0001	<.0001	<.0001

Dependent Variable: Pc

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
11	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0031
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3058
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0048
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1494
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
26	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2224
27	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
28	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001
29	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0085
30	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0163
31	0.4103	0.0554	0.0554	0.0688	0.0922	0.6222	0.1529	0.2397	0.3167	0.0955	0.8750	0.1253	0.1729	0.2348	<.0001
32	0.4913	0.6786	0.6786	0.7510	0.8578	0.3085	0.9373	0.7379	0.6101	0.8714	0.1765	0.9793	0.8839	0.7473	<.0001
33	0.4913	0.6786	0.6786	0.7510	0.8578	0.3085	0.9373	0.7379	0.6101	0.8714	0.1765	0.9793	0.8839	0.7473	<.0001
34	0.9199	0.2298	0.2298	0.2694	0.3335	0.8174	0.4776	0.6494	0.7801	0.3422	0.5713	0.4154	0.5203	0.6406	<.0001
35	0.9199	0.2298	0.2298	0.2694	0.3335	0.8174	0.4776	0.6494	0.7801	0.3422	0.5713	0.4154	0.5203	0.6406	<.0001
36	0.4532	0.0653	0.0653	0.0805	0.1070	0.6750	0.1749	0.2702	0.3534	0.1108	0.9332	0.1442	0.1971	0.2649	<.0001
37	0.8977	0.2192	0.2192	0.2575	0.3198	0.8393	0.4604	0.6294	0.7587	0.3282	0.5905	0.3996	0.5023	0.6206	<.0001
38	0.5662	0.0951	0.0951	0.1156	0.1508	0.8084	0.2375	0.3541	0.4522	0.1557	0.9258	0.1988	0.2651	0.3477	<.0001
39	0.5038	0.6643	0.6643	0.7361	0.8423	0.3179	0.9530	0.7529	0.6239	0.8559	0.1828	0.9635	0.8995	0.7623	<.0001
40	0.6372	0.5280	0.5280	0.5932	0.6920	0.4224	0.8899	0.9064	0.7694	0.7047	0.2558	0.8080	0.9433	0.9162	<.0001
41	0.6510	0.5155	0.5155	0.5800	0.6779	0.4336	0.8747	0.9216	0.7842	0.6905	0.2639	0.7931	0.9281	0.9315	<.0001
42	0.5969	0.1042	0.1042	0.1263	0.1638	0.8433	0.2556	0.3777	0.4795	0.1690	0.8903	0.2148	0.2847	0.3711	<.0001
43	0.5889	0.5740	0.5740	0.6417	0.7435	0.3838	0.9446	0.8520	0.7173	0.7566	0.2283	0.8618	0.9983	0.8617	<.0001
44	0.6576	0.5096	0.5096	0.5738	0.6712	0.4389	0.8675	0.9289	0.7912	0.6838	0.2678	0.7861	0.9208	0.9387	<.0001
45	0.4114	0.0557	0.0557	0.0690	0.0925	0.6236	0.1534	0.2405	0.3176	0.0959	0.8765	0.1258	0.1736	0.2356	<.0001
46		0.2709	0.2709	0.3152	0.3860	0.7404	0.5420	0.7233	0.8582	0.3955	0.5051	0.4751	0.5874	0.7140	<.0001
47	0.2709		1.0000	0.9226	0.8140	0.1528	0.6221	0.4542	0.3559	0.8007	0.0783	0.6977	0.5754	0.4617	<.0001
48	0.2709	1.0000		0.9226	0.8140	0.1528	0.6221	0.4542	0.3559	0.8007	0.0783	0.6977	0.5754	0.4617	<.0001
49	0.3152	0.9226	0.9226		0.8901	0.1824	0.6922	0.5147	0.4085	0.8765	0.0959	0.7708	0.6433	0.5227	<.0001
50	0.3860	0.8140	0.8140	0.8901		0.2315	0.7966	0.6075	0.4909	0.9862	0.1263	0.8782	0.7451	0.6162	<.0001
51	0.7404	0.1528	0.1528	0.1824	0.2315		0.3471	0.4933	0.6102	0.2383	0.7372	0.2964	0.3826	0.4856	<.0001
52	0.5420	0.6221	0.6221	0.6922	0.7966	0.3471		0.7979	0.6661	0.8099	0.2028	0.9167	0.9463	0.8075	<.0001

Dependent Variable: Pc

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
53	0.7233	0.4542	0.4542	0.5147	0.6075	0.4933	0.7979		0.8606	0.6196	0.3080	0.7184	0.8504	0.9901	<.0001
54	0.8582	0.3559	0.3559	0.4085	0.4909	0.6102	0.6661	0.8606		0.5018	0.3983	0.5920	0.7158	0.8509	<.0001
55	0.3955	0.8007	0.8007	0.8765	0.9862	0.2383	0.8099	0.6196	0.5018		0.1306	0.8919	0.7582	0.6284	<.0001
56	0.5051	0.0783	0.0783	0.0959	0.1263	0.7372	0.2028	0.3080	0.3983	0.1306		0.1684	0.2275	0.3022	<.0001
57	0.4751	0.6977	0.6977	0.7708	0.8782	0.2964	0.9167	0.7184	0.5920	0.8919	0.1684		0.8635	0.7277	<.0001
58	0.5874	0.5754	0.5754	0.6433	0.7451	0.3826	0.9463	0.8504	0.7158	0.7582	0.2275	0.8635		0.8601	<.0001
59	0.7140	0.4617	0.4617	0.5227	0.6162	0.4856	0.8075	0.9901	0.8509	0.6284	0.3022	0.7277	0.8601		<.0001
60	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Appendix D

Least squares means of percentages of resistant isolates for warm season *E. coli*

The GLM Procedure
Least Squares Means
Season=warm Bacteria=*E. coli*

Table 1. Least squares means and the index numbers of percentages of resistant isolates at different antibiotic concentrations for *E. coli* in warm season

	Trt	*Pc LSMEAN*	*LSMEAN Number*
Finishing	*CEP15*	93.260035	1
Finishing	*CEP25*	87.308892	2
Finishing	*CEP35*	78.293639	3
Finishing	*ERY100*	12.725746	4
Finishing	*ERY60*	38.496542	5
Finishing	*ERY70*	34.150818	6
Finishing	*ERY90*	19.313325	7
Finishing	*NEO10*	43.563449	8
Finishing	*NEO2.5*	98.815093	9
Finishing	*NEO5*	60.974628	10
Finishing	*OTC10*	94.188659	11
Finishing	*OTC15*	94.188659	12
Finishing	*OTC2.5*	100.000000	13
Finishing	*OTC5*	99.475055	14
Finishing	*OTC7.5*	96.439557	15
Finishing	*RIF60*	11.413599	16
Finishing	*RIF75*	6.307799	17
Finishing	*RIF90*	3.289561	18
Finishing	*STR10*	53.106927	19
Finishing	*STR15*	47.836647	20
Finishing	*STR2.5*	96.172375	21
Finishing	*STR5*	94.070484	22
Finishing	*STR7.5*	71.768664	23
Finishing	*TET10*	94.728994	24
Finishing	*TET15*	94.199894	25
Finishing	*TET2.5*	100.000000	26
Finishing	*TET5*	99.604567	27
Finishing	*TET7.5*	95.515052	28
Nursery	*CEP15*	100.000000	29

	Trt	Pc LSMEAN	LSMEAN Number
Nursery	CEP25	100.000000	30
Nursery	CEP35	100.000000	31
Nursery	ERY100	80.977312	32
Nursery	ERY60	96.345986	33
Nursery	ERY70	94.002236	34
Nursery	ERY90	93.216896	35
Nursery	NEO10	60.400305	36
Nursery	NEO2.5	69.010417	37
Nursery	NEO5	62.753599	38
Nursery	OTC10	100.000000	39
Nursery	OTC15	100.000000	40
Nursery	OTC2.5	100.000000	41
Nursery	OTC5	100.000000	42
Nursery	OTC7.5	100.000000	43
Nursery	RIF60	15.374127	44
Nursery	RIF75	3.906250	45
Nursery	RIF90	1.302083	46
Nursery	STR10	90.095986	47
Nursery	STR15	89.834206	48
Nursery	STR2.5	94.531250	49
Nursery	STR5	94.270833	50
Nursery	STR7.5	90.619546	51
Nursery	TET10	100.000000	52
Nursery	TET15	100.000000	53
Nursery	TET2.5	100.000000	54
Nursery	TET5	100.000000	55
Nursery	TET7.5	100.000000	56

Table 2. P-values of comparisons of different antibiotic concentrations for *E. coli* in warm season

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.5495	0.1340	<.0001	<.0001	<.0001	<.0001	<.0001	0.5763	0.0015	0.9255	0.9255	0.4980	0.5320
2	0.5495		0.3651	<.0001	<.0001	<.0001	<.0001	<.0001	0.2483	0.0091	0.4891	0.4891	0.2032	0.2223
3	0.1340	0.3651		<.0001	0.0001	<.0001	<.0001	0.0007	0.0408	0.0834	0.1117	0.1117	0.0307	0.0349
4	<.0001	<.0001	<.0001		0.0106	0.0329	0.5077	0.0024	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	0.0001	0.0106		0.6620	0.0556	0.6103	<.0001	0.0253	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	<.0001	0.0329	0.6620		0.1373	0.3444	<.0001	0.0079	<.0001	<.0001	<.0001	<.0001
7	<.0001	<.0001	<.0001	0.5077	0.0556	0.1373		0.0160	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	<.0001	0.0007	0.0024	0.6103	0.3444	0.0160		<.0001	0.0818	<.0001	<.0001	<.0001	<.0001
9	0.5763	0.2483	0.0408	<.0001	<.0001	<.0001	<.0001	<.0001		0.0002	0.6416	0.6416	0.9051	0.9470
10	0.0015	0.0091	0.0834	<.0001	0.0253	0.0079	<.0001	0.0818	0.0002		0.0011	0.0011	0.0001	0.0002
11	0.9255	0.4891	0.1117	<.0001	<.0001	<.0001	<.0001	<.0001	0.6416	0.0011		1.0000	0.5589	0.5949
12	0.9255	0.4891	0.1117	<.0001	<.0001	<.0001	<.0001	<.0001	0.6416	0.0011	1.0000		0.5589	0.5949
13	0.4980	0.2032	0.0307	<.0001	<.0001	<.0001	<.0001	<.0001	0.9051	0.0001	0.5589	0.5589		0.9579
14	0.5320	0.2223	0.0349	<.0001	<.0001	<.0001	<.0001	<.0001	0.9470	0.0002	0.5949	0.5949	0.9579	
15	0.7490	0.3590	0.0699	<.0001	<.0001	<.0001	<.0001	<.0001	0.8110	0.0005	0.8208	0.8208	0.7201	0.7600
16	<.0001	<.0001	<.0001	0.8949	0.0073	0.0237	0.4272	0.0016	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	0.5187	0.0016	0.0059	0.1923	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	<.0001	0.3432	0.0006	0.0024	0.1089	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	0.0008	0.0125	<.0001	0.1434	0.0585	0.0009	0.3378	<.0001	0.4291	<.0001	<.0001	<.0001	<.0001
20	<.0001	0.0001	0.0027	0.0006	0.3481	0.1702	0.0048	0.6672	<.0001	0.1878	<.0001	<.0001	<.0001	<.0001
21	0.7695	0.3732	0.0741	<.0001	<.0001	<.0001	<.0001	<.0001	0.7903	0.0006	0.8418	0.8418	0.7001	0.7396
22	0.9350	0.4966	0.1144	<.0001	<.0001	<.0001	<.0001	<.0001	0.6331	0.0012	0.9905	0.9905	0.5510	0.5867
23	0.0323	0.1199	0.5118	<.0001	0.0011	0.0002	<.0001	0.0053	0.0074	0.2786	0.0257	0.0257	0.0053	0.0061
24	0.8825	0.4557	0.1002	<.0001	<.0001	<.0001	<.0001	<.0001	0.6810	0.0009	0.9566	0.9566	0.5960	0.6330
25	0.9246	0.4884	0.1115	<.0001	<.0001	<.0001	<.0001	<.0001	0.6424	0.0011	0.9991	0.9991	0.5597	0.5957
26	0.4980	0.2032	0.0307	<.0001	<.0001	<.0001	<.0001	<.0001	0.9051	0.0001	0.5589	0.5589	1.0000	0.9579
27	0.5235	0.2175	0.0338	<.0001	<.0001	<.0001	<.0001	<.0001	0.9367	0.0002	0.5859	0.5859	0.9683	0.9896
28	0.8205	0.4096	0.0852	<.0001	<.0001	<.0001	<.0001	<.0001	0.7398	0.0007	0.8938	0.8938	0.6518	0.6903
29	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
30	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
31	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
32	0.3139	0.6030	0.8255	<.0001	0.0007	0.0002	<.0001	0.0026	0.1446	0.1023	0.2789	0.2789	0.1200	0.1305
33	0.7998	0.4582	0.1399	<.0001	<.0001	<.0001	<.0001	<.0001	0.8392	0.0043	0.8593	0.8593	0.7640	0.7971
34	0.9514	0.5825	0.1984	<.0001	<.0001	<.0001	<.0001	<.0001	0.6926	0.0076	0.9878	0.9878	0.6223	0.6530
35	0.9972	0.6275	0.2216	<.0001	<.0001	<.0001	<.0001	<.0001	0.6456	0.0091	0.9363	0.9363	0.5775	0.6073
36	0.0079	0.0287	0.1434	0.0002	0.0740	0.0328	0.0010	0.1683	0.0020	0.9624	0.0064	0.0064	0.0015	0.0017
37	0.0483	0.1346	0.4461	<.0001	0.0134	0.0049	<.0001	0.0384	0.0157	0.5094	0.0405	0.0405	0.0121	0.0136

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
38	0.0135	0.0456	0.2033	<.0001	0.0482	0.0203	0.0005	0.1169	0.0037	0.8838	0.0109	0.0109	0.0027	0.0031
39	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
40	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
41	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
42	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
43	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
44	<.0001	<.0001	<.0001	0.8277	0.0595	0.1249	0.7462	0.0221	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	0.4691	0.0052	0.0142	0.2071	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	<.0001	<.0001	0.3488	0.0028	0.0079	0.1408	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.7949	0.8188	0.3331	<.0001	<.0001	<.0001	<.0001	0.0002	0.4742	0.0182	0.7367	0.7367	0.4164	0.4415
48	0.7783	0.8356	0.3439	<.0001	<.0001	<.0001	<.0001	0.0002	0.4610	0.0192	0.7205	0.7205	0.4042	0.4288
49	0.9168	0.5531	0.1839	<.0001	<.0001	<.0001	<.0001	<.0001	0.7249	0.0067	0.9775	0.9775	0.6533	0.6846
50	0.9338	0.5675	0.1909	<.0001	<.0001	<.0001	<.0001	<.0001	0.7089	0.0071	0.9946	0.9946	0.6379	0.6690
51	0.8282	0.7856	0.3122	<.0001	<.0001	<.0001	<.0001	0.0002	0.5011	0.0162	0.7693	0.7693	0.4414	0.4673
52	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
53	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
54	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
55	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656
56	0.5799	0.2982	0.0766	<.0001	<.0001	<.0001	<.0001	<.0001	0.9224	0.0017	0.6331	0.6331	1.0000	0.9656

(continued)

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.7490	<.0001	<.0001	<.0001	<.0001	<.0001	0.7695	0.9350	0.0323	0.8825	0.9246	0.4980	0.5235	0.8205
2	0.3590	<.0001	<.0001	<.0001	0.0008	0.0001	0.3732	0.4966	0.1199	0.4557	0.4884	0.2032	0.2175	0.4096
3	0.0699	<.0001	<.0001	<.0001	0.0125	0.0027	0.0741	0.1144	0.5118	0.1002	0.1115	0.0307	0.0338	0.0852
4	<.0001	0.8949	0.5187	0.3432	<.0001	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	0.0073	0.0016	0.0006	0.1434	0.3481	<.0001	<.0001	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001
6	<.0001	0.0237	0.0059	0.0024	0.0585	0.1702	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
7	<.0001	0.4272	0.1923	0.1089	0.0009	0.0048	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	0.0016	0.0003	<.0001	0.3378	0.6672	<.0001	<.0001	0.0053	<.0001	<.0001	<.0001	<.0001	<.0001
9	0.8110	<.0001	<.0001	<.0001	<.0001	<.0001	0.7903	0.6331	0.0074	0.6810	0.6424	0.9051	0.9367	0.7398
10	0.0005	<.0001	<.0001	<.0001	0.4291	0.1878	0.0006	0.0012	0.2786	0.0009	0.0011	0.0001	0.0002	0.0007
11	0.8208	<.0001	<.0001	<.0001	<.0001	<.0001	0.8418	0.9905	0.0257	0.9566	0.9991	0.5589	0.5859	0.8938
12	0.8208	<.0001	<.0001	<.0001	<.0001	<.0001	0.8418	0.9905	0.0257	0.9566	0.9991	0.5589	0.5859	0.8938
13	0.7201	<.0001	<.0001	<.0001	<.0001	<.0001	0.7001	0.5510	0.0053	0.5960	0.5597	1.0000	0.9683	0.6518

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
14	0.7600	<.0001	<.0001	<.0001	<.0001	<.0001	0.7396	0.5867	0.0061	0.6330	0.5957	0.9579	0.9896	0.6903
15		<.0001	<.0001	<.0001	<.0001	<.0001	0.9785	0.8116	0.0143	0.8633	0.8217	0.7201	0.7501	0.9259
16	<.0001		0.6075	0.4142	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	0.6075		0.7613	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	0.4142	0.7613		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001		0.5960	<.0001	<.0001	0.0624	<.0001	<.0001	<.0001	<.0001	<.0001
20	<.0001	0.0004	<.0001	<.0001	0.5960		<.0001	<.0001	0.0174	<.0001	<.0001	<.0001	<.0001	<.0001
21	0.9785	<.0001	<.0001	<.0001	<.0001	<.0001		0.8325	0.0154	0.8845	0.8426	0.7001	0.7298	0.9472
22	0.8116	<.0001	<.0001	<.0001	<.0001	<.0001	0.8325		0.0265	0.9472	0.9896	0.5510	0.5778	0.8844
23	0.0143	<.0001	<.0001	<.0001	0.0624	0.0174	0.0154	0.0265		0.0224	0.0256	0.0053	0.0059	0.0183
24	0.8633	<.0001	<.0001	<.0001	<.0001	<.0001	0.8845	0.9472	0.0224		0.9575	0.5960	0.6238	0.9369
25	0.8217	<.0001	<.0001	<.0001	<.0001	<.0001	0.8426	0.9896	0.0256	0.9575		0.5597	0.5867	0.8947
26	0.7201	<.0001	<.0001	<.0001	<.0001	<.0001	0.7001	0.5510	0.0053	0.5960	0.5597		0.9683	0.6518
27	0.7501	<.0001	<.0001	<.0001	<.0001	<.0001	0.7298	0.5778	0.0059	0.6238	0.5867	0.9683		0.6807
28	0.9259	<.0001	<.0001	<.0001	<.0001	<.0001	0.9472	0.8844	0.0183	0.9369	0.8947	0.6518	0.6807	
29	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
30	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
31	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
32	0.2055	<.0001	<.0001	<.0001	0.0236	0.0074	0.2134	0.2832	0.4498	0.2598	0.2785	0.1200	0.1279	0.2337
33	0.9939	<.0001	<.0001	<.0001	0.0005	0.0001	0.9886	0.8517	0.0454	0.8943	0.8600	0.7640	0.7889	0.9456
34	0.8413	<.0001	<.0001	<.0001	0.0010	0.0002	0.8585	0.9955	0.0698	0.9524	0.9870	0.6223	0.6454	0.9011
35	0.7912	<.0001	<.0001	<.0001	0.0013	0.0003	0.8081	0.9441	0.0801	0.9011	0.9356	0.5775	0.5998	0.8502
36	0.0037	0.0001	<.0001	<.0001	0.5492	0.3030	0.0039	0.0065	0.3511	0.0056	0.0063	0.0015	0.0016	0.0046
37	0.0259	<.0001	<.0001	<.0001	0.1930	0.0840	0.0273	0.0414	0.8207	0.0364	0.0404	0.0121	0.0132	0.0312
38	0.0065	<.0001	<.0001	<.0001	0.4286	0.2218	0.0069	0.0112	0.4593	0.0097	0.0109	0.0027	0.0030	0.0081
39	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
40	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
41	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
42	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
43	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
44	<.0001	0.7449	0.4568	0.3217	0.0024	0.0087	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	0.5376	0.8436	0.9596	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	0.4067	0.6809	0.8703	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.6024	<.0001	<.0001	<.0001	0.0029	0.0007	0.6177	0.7440	0.1340	0.7035	0.7360	0.4164	0.4352	0.6562
48	0.5875	<.0001	<.0001	<.0001	0.0031	0.0008	0.6027	0.7278	0.1396	0.6876	0.7198	0.4042	0.4227	0.6408
49	0.8754	<.0001	<.0001	<.0001	0.0009	0.0002	0.8927	0.9698	0.0635	0.9870	0.9783	0.6533	0.6768	0.9356
50	0.8586	<.0001	<.0001	<.0001	0.0010	0.0002	0.8758	0.9869	0.0665	0.9700	0.9953	0.6379	0.6613	0.9186
51	0.6326	<.0001	<.0001	<.0001	0.0025	0.0006	0.6483	0.7767	0.1234	0.7356	0.7686	0.4414	0.4608	0.6876

*Least Squares Means for effect Age*Trt*
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
52	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
53	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
54	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
55	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125
56	0.7699	<.0001	<.0001	<.0001	0.0002	<.0001	0.7531	0.6262	0.0219	0.6650	0.6338	1.0000	0.9741	0.7125

(continued)

*Least Squares Means for effect Age*Trt*
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	0.5799	0.5799	0.5799	0.3139	0.7998	0.9514	0.9972	0.0079	0.0483	0.0135	0.5799	0.5799	0.5799	0.5799
2	0.2982	0.2982	0.2982	0.6030	0.4582	0.5825	0.6275	0.0287	0.1346	0.0456	0.2982	0.2982	0.2982	0.2982
3	0.0766	0.0766	0.0766	0.8255	0.1399	0.1984	0.2216	0.1434	0.4461	0.2033	0.0766	0.0766	0.0766	0.0766
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	0.0740	0.0134	0.0482	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	0.0328	0.0049	0.0203	<.0001	<.0001	<.0001	<.0001
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0010	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001
8	<.0001	<.0001	<.0001	0.0026	<.0001	<.0001	<.0001	0.1683	0.0384	0.1169	<.0001	<.0001	<.0001	<.0001
9	0.9224	0.9224	0.9224	0.1446	0.8392	0.6926	0.6456	0.0020	0.0157	0.0037	0.9224	0.9224	0.9224	0.9224
10	0.0017	0.0017	0.0017	0.1023	0.0043	0.0076	0.0091	0.9624	0.5094	0.8838	0.0017	0.0017	0.0017	0.0017
11	0.6331	0.6331	0.6331	0.2789	0.8593	0.9878	0.9363	0.0064	0.0405	0.0109	0.6331	0.6331	0.6331	0.6331
12	0.6331	0.6331	0.6331	0.2789	0.8593	0.9878	0.9363	0.0064	0.0405	0.0109	0.6331	0.6331	0.6331	0.6331
13	1.0000	1.0000	1.0000	0.1200	0.7640	0.6223	0.5775	0.0015	0.0121	0.0027	1.0000	1.0000	1.0000	1.0000
14	0.9656	0.9656	0.9656	0.1305	0.7971	0.6530	0.6073	0.0017	0.0136	0.0031	0.9656	0.9656	0.9656	0.9656
15	0.7699	0.7699	0.7699	0.2055	0.9939	0.8413	0.7912	0.0037	0.0259	0.0065	0.7699	0.7699	0.7699	0.7699
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.0002	0.0002	0.0002	0.0236	0.0005	0.0010	0.0013	0.5492	0.1930	0.4286	0.0002	0.0002	0.0002	0.0002
20	<.0001	<.0001	<.0001	0.0074	0.0001	0.0002	0.0003	0.3030	0.0840	0.2218	<.0001	<.0001	<.0001	<.0001
21	0.7531	0.7531	0.7531	0.2134	0.9886	0.8585	0.8081	0.0039	0.0273	0.0069	0.7531	0.7531	0.7531	0.7531
22	0.6262	0.6262	0.6262	0.2832	0.8517	0.9955	0.9441	0.0065	0.0414	0.0112	0.6262	0.6262	0.6262	0.6262
23	0.0219	0.0219	0.0219	0.4498	0.0454	0.0698	0.0801	0.3511	0.8207	0.4593	0.0219	0.0219	0.0219	0.0219
24	0.6650	0.6650	0.6650	0.2598	0.8943	0.9524	0.9011	0.0056	0.0364	0.0097	0.6650	0.6650	0.6650	0.6650
25	0.6338	0.6338	0.6338	0.2785	0.8600	0.9870	0.9356	0.0063	0.0404	0.0109	0.6338	0.6338	0.6338	0.6338
26	1.0000	1.0000	1.0000	0.1200	0.7640	0.6223	0.5775	0.0015	0.0121	0.0027	1.0000	1.0000	1.0000	1.0000
27	0.9741	0.9741	0.9741	0.1279	0.7889	0.6454	0.5998	0.0016	0.0132	0.0030	0.9741	0.9741	0.9741	0.9741

Dependent Variable: Pc

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
28	0.7125	0.7125	0.7125	0.2337	0.9456	0.9011	0.8502	0.0046	0.0312	0.0081	0.7125	0.7125	0.7125	0.7125
29		1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
30	1.0000		1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
31	1.0000	1.0000		0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
32	0.1776	0.1776	0.1776		0.2754	0.3549	0.3845	0.1450	0.3952	0.1964	0.1776	0.1776	0.1776	0.1776
33	0.7948	0.7948	0.7948	0.2754		0.8675	0.8238	0.0117	0.0538	0.0183	0.7948	0.7948	0.7948	0.7948
34	0.6696	0.6696	0.6696	0.3549	0.8675		0.9554	0.0182	0.0774	0.0279	0.6696	0.6696	0.6696	0.6696
35	0.6294	0.6294	0.6294	0.3845	0.8238	0.9554		0.0211	0.0871	0.0320	0.6294	0.6294	0.6294	0.6294
36	0.0056	0.0056	0.0056	0.1450	0.0117	0.0182	0.0211		0.5404	0.8670	0.0056	0.0056	0.0056	0.0056
37	0.0292	0.0292	0.0292	0.3952	0.0538	0.0774	0.0871	0.5404		0.6562	0.0292	0.0292	0.0292	0.0292
38	0.0091	0.0091	0.0091	0.1964	0.0183	0.0279	0.0320	0.8670	0.6562		0.0091	0.0091	0.0091	0.0091
39	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091		1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000		1.0000	1.0000
41	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000		1.0000
42	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	
43	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0017	0.0002	0.0010	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.4814	0.4814	0.4814	0.5167	0.6566	0.7810	0.8242	0.0364	0.1354	0.0537	0.4814	0.4814	0.4814	0.4814
48	0.4699	0.4699	0.4699	0.5288	0.6432	0.7668	0.8098	0.0381	0.1403	0.0560	0.4699	0.4699	0.4699	0.4699
49	0.6972	0.6972	0.6972	0.3357	0.8972	0.9700	0.9255	0.0165	0.0714	0.0254	0.6972	0.6972	0.6972	0.6972
50	0.6836	0.6836	0.6836	0.3451	0.8826	0.9847	0.9402	0.0174	0.0743	0.0266	0.6836	0.6836	0.6836	0.6836
51	0.5048	0.5048	0.5048	0.4930	0.6837	0.8098	0.8533	0.0333	0.1261	0.0494	0.5048	0.5048	0.5048	0.5048
52	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
53	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
54	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
55	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000
56	1.0000	1.0000	1.0000	0.1776	0.7948	0.6696	0.6294	0.0056	0.0292	0.0091	1.0000	1.0000	1.0000	1.0000

(continued)

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	0.5799	<.0001	<.0001	<.0001	0.7949	0.7783	0.9168	0.9338	0.8282	0.5799	0.5799	0.5799	0.5799	0.5799
2	0.2982	<.0001	<.0001	<.0001	0.8188	0.8356	0.5531	0.5675	0.7856	0.2982	0.2982	0.2982	0.2982	0.2982
3	0.0766	<.0001	<.0001	<.0001	0.3331	0.3439	0.1839	0.1909	0.3122	0.0766	0.0766	0.0766	0.0766	0.0766

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
4	<.0001	0.8277	0.4691	0.3488	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	0.0595	0.0052	0.0028	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	<.0001	0.1249	0.0142	0.0079	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7	<.0001	0.7462	0.2071	0.1408	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	<.0001	0.0221	0.0015	0.0007	0.0002	0.0002	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
9	0.9224	<.0001	<.0001	<.0001	0.4742	0.4610	0.7249	0.7089	0.5011	0.9224	0.9224	0.9224	0.9224	0.9224
10	0.0017	0.0003	<.0001	<.0001	0.0182	0.0192	0.0067	0.0071	0.0162	0.0017	0.0017	0.0017	0.0017	0.0017
11	0.6331	<.0001	<.0001	<.0001	0.7367	0.7205	0.9775	0.9946	0.7693	0.6331	0.6331	0.6331	0.6331	0.6331
12	0.6331	<.0001	<.0001	<.0001	0.7367	0.7205	0.9775	0.9946	0.7693	0.6331	0.6331	0.6331	0.6331	0.6331
13	1.0000	<.0001	<.0001	<.0001	0.4164	0.4042	0.6533	0.6379	0.4414	1.0000	1.0000	1.0000	1.0000	1.0000
14	0.9656	<.0001	<.0001	<.0001	0.4415	0.4288	0.6846	0.6690	0.4673	0.9656	0.9656	0.9656	0.9656	0.9656
15	0.7699	<.0001	<.0001	<.0001	0.6024	0.5875	0.8754	0.8586	0.6326	0.7699	0.7699	0.7699	0.7699	0.7699
16	<.0001	0.7449	0.5376	0.4067	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	0.4568	0.8436	0.6809	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	0.3217	0.9596	0.8703	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.0002	0.0024	<.0001	<.0001	0.0029	0.0031	0.0009	0.0010	0.0025	0.0002	0.0002	0.0002	0.0002	0.0002
20	<.0001	0.0087	0.0005	0.0002	0.0007	0.0008	0.0002	0.0002	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001
21	0.7531	<.0001	<.0001	<.0001	0.6177	0.6027	0.8927	0.8758	0.6483	0.7531	0.7531	0.7531	0.7531	0.7531
22	0.6262	<.0001	<.0001	<.0001	0.7440	0.7278	0.9698	0.9869	0.7767	0.6262	0.6262	0.6262	0.6262	0.6262
23	0.0219	<.0001	<.0001	<.0001	0.1340	0.1396	0.0635	0.0665	0.1234	0.0219	0.0219	0.0219	0.0219	0.0219
24	0.6650	<.0001	<.0001	<.0001	0.7035	0.6876	0.9870	0.9700	0.7356	0.6650	0.6650	0.6650	0.6650	0.6650
25	0.6338	<.0001	<.0001	<.0001	0.7360	0.7198	0.9783	0.9953	0.7686	0.6338	0.6338	0.6338	0.6338	0.6338
26	1.0000	<.0001	<.0001	<.0001	0.4164	0.4042	0.6533	0.6379	0.4414	1.0000	1.0000	1.0000	1.0000	1.0000
27	0.9741	<.0001	<.0001	<.0001	0.4352	0.4227	0.6768	0.6613	0.4608	0.9741	0.9741	0.9741	0.9741	0.9741
28	0.7125	<.0001	<.0001	<.0001	0.6562	0.6408	0.9356	0.9186	0.6876	0.7125	0.7125	0.7125	0.7125	0.7125
29	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
30	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
31	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
32	0.1776	<.0001	<.0001	<.0001	0.5167	0.5288	0.3357	0.3451	0.4930	0.1776	0.1776	0.1776	0.1776	0.1776
33	0.7948	<.0001	<.0001	<.0001	0.6566	0.6432	0.8972	0.8826	0.6837	0.7948	0.7948	0.7948	0.7948	0.7948
34	0.6696	<.0001	<.0001	<.0001	0.7810	0.7668	0.9700	0.9847	0.8098	0.6696	0.6696	0.6696	0.6696	0.6696
35	0.6294	<.0001	<.0001	<.0001	0.8242	0.8098	0.9255	0.9402	0.8533	0.6294	0.6294	0.6294	0.6294	0.6294
36	0.0056	0.0017	0.0001	<.0001	0.0364	0.0381	0.0165	0.0174	0.0333	0.0056	0.0056	0.0056	0.0056	0.0056
37	0.0292	0.0002	<.0001	<.0001	0.1354	0.1403	0.0714	0.0743	0.1261	0.0292	0.0292	0.0292	0.0292	0.0292
38	0.0091	0.0010	<.0001	<.0001	0.0537	0.0560	0.0254	0.0266	0.0494	0.0091	0.0091	0.0091	0.0091	0.0091
39	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
41	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000

Dependent Variable: Pc

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
42	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
43		<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	1.0000
44	<.0001		0.4151	0.3177	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	0.4151		0.8530	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	<.0001	0.3177	0.8530		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.4814	<.0001	<.0001	<.0001		0.9851	0.7523	0.7664	0.9703	0.4814	0.4814	0.4814	0.4814	0.4814
48	0.4699	<.0001	<.0001	<.0001	0.9851		0.7382	0.7522	0.9554	0.4699	0.4699	0.4699	0.4699	0.4699
49	0.6972	<.0001	<.0001	<.0001	0.7523	0.7382		0.9852	0.7807	0.6972	0.6972	0.6972	0.6972	0.6972
50	0.6836	<.0001	<.0001	<.0001	0.7664	0.7522	0.9852		0.7950	0.6836	0.6836	0.6836	0.6836	0.6836
51	0.5048	<.0001	<.0001	<.0001	0.9703	0.9554	0.7807	0.7950		0.5048	0.5048	0.5048	0.5048	0.5048
52	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048		1.0000	1.0000	1.0000	1.0000
53	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000		1.0000	1.0000	1.0000
54	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000		1.0000	1.0000
55	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000		1.0000
56	1.0000	<.0001	<.0001	<.0001	0.4814	0.4699	0.6972	0.6836	0.5048	1.0000	1.0000	1.0000	1.0000	

Appendix E

Least squares means of percentages of resistant isolates for warm season *Enterococcus*

The GLM Procedure
Least Squares Means
Season=warm Bacteria=*Enterococcus*

Table 1. Least squares means and the index numbers of percentages of resistant isolates at different antibiotic concentrations for *Enterococcus* in warm season

Age	Trt	Pc LSMEAN	LSMEAN Number
Finishing	AMX2.5	7.0991993	1
Finishing	CEP10	72.0359639	2
Finishing	CEP15	69.2802251	3
Finishing	CEP30	50.3705567	4
Finishing	CEP50	38.5383930	5
Finishing	CTC100	64.1666040	6
Finishing	CTC60	91.2720524	7
Finishing	CTC80	76.0262323	8
Finishing	ERY10	75.3163201	9
Finishing	ERY15	70.4520667	10
Finishing	ERY30	54.9459106	11
Finishing	ERY50	49.2557104	12
Finishing	NEO40	37.7440760	13
Finishing	NEO60	30.0517683	14
Finishing	NEO80	23.4081568	15
Finishing	OTC100	12.5593636	16
Finishing	OTC20	74.3286414	17
Finishing	OTC40	44.5496720	18
Finishing	OTC60	36.1625856	19
Finishing	OTC80	30.1927836	20
Finishing	STR100	27.0648615	21
Finishing	STR40	44.9990285	22
Finishing	STR60	38.2076867	23
Finishing	STR80	28.9879385	24
Finishing	TET10	74.7406861	25
Finishing	TET100	7.2799332	26
Finishing	TET15	66.5995055	27
Finishing	TET30	45.5827512	28
Finishing	TET50	30.1661983	29

Age	Trt	Pc LSMEAN	LSMEAN Number
Finishing	VAN2.5	2.6178557	30
Nursery	AMX2.5	31.7465462	31
Nursery	CEP10	77.7731281	32
Nursery	CEP15	65.6224283	33
Nursery	CEP30	39.9758097	34
Nursery	CEP50	24.0754214	35
Nursery	CTC100	69.7823332	36
Nursery	CTC60	86.1340706	37
Nursery	CTC80	75.1833415	38
Nursery	ERY10	83.8416328	39
Nursery	ERY15	75.5418346	40
Nursery	ERY30	71.7278413	41
Nursery	ERY50	63.7217907	42
Nursery	NEO40	72.9368835	43
Nursery	NEO60	61.6670161	44
Nursery	NEO80	49.8475041	45
Nursery	OTC100	30.7149955	46
Nursery	OTC20	75.4494648	47
Nursery	OTC40	60.8108145	48
Nursery	OTC60	46.0324320	49
Nursery	OTC80	33.5668180	50
Nursery	STR100	64.6183652	51
Nursery	STR40	74.4028864	52
Nursery	STR60	69.9810824	53
Nursery	STR80	66.7195753	54
Nursery	TET10	81.0899598	55
Nursery	TET100	7.5360575	56
Nursery	TET15	76.5798279	57
Nursery	TET30	56.7050793	58
Nursery	TET50	38.1308918	59
Nursery	VAN2.5	2.6402640	60

Table 2. P-values of comparisons of different antibiotic concentrations for *Enterococcus* in warm season

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		<.0001	<.0001	0.0004	0.0093	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	0.0112	0.0563	0.1737
2	<.0001		0.8176	0.0714	0.0057	0.5105	0.1091	0.7385	0.7837	0.8946	0.1542	0.0582	0.0047	0.0006	<.0001
3	<.0001	0.8176		0.1151	0.0110	0.6688	0.0673	0.5726	0.6137	0.9219	0.2315	0.0954	0.0091	0.0013	0.0002
4	0.0004	0.0714	0.1151		0.3230	0.2494	0.0008	0.0332	0.0383	0.0945	0.7019	0.9257	0.2916	0.0907	0.0253
5	0.0093	0.0057	0.0110	0.3230		0.0334	<.0001	0.0020	0.0025	0.0083	0.1712	0.3705	0.9470	0.4780	0.2067
6	<.0001	0.5105	0.6688	0.2494	0.0334		0.0246	0.3218	0.3516	0.5991	0.4408	0.2134	0.0283	0.0049	0.0008
7	<.0001	0.1091	0.0673	0.0008	<.0001	0.0246		0.2033	0.1832	0.0831	0.0028	0.0006	<.0001	<.0001	<.0001
8	<.0001	0.7385	0.5726	0.0332	0.0020	0.3218	0.2033		0.9526	0.6410	0.0793	0.0264	0.0016	0.0002	<.0001
9	<.0001	0.7837	0.6137	0.0383	0.0025	0.3516	0.1832	0.9526		0.6841	0.0899	0.0305	0.0020	0.0002	<.0001
10	<.0001	0.8946	0.9219	0.0945	0.0083	0.5991	0.0831	0.6410	0.6841		0.1958	0.0777	0.0069	0.0009	0.0001
11	<.0001	0.1542	0.2315	0.7019	0.1712	0.4408	0.0028	0.0793	0.0899	0.1958		0.6341	0.1515	0.0387	0.0091
12	0.0006	0.0582	0.0954	0.9257	0.3705	0.2134	0.0006	0.0264	0.0305	0.0777	0.6341		0.3362	0.1096	0.0319
13	0.0112	0.0047	0.0091	0.2916	0.9470	0.0283	<.0001	0.0016	0.0020	0.0069	0.1515	0.3362		0.5201	0.2315
14	0.0563	0.0006	0.0013	0.0907	0.4780	0.0049	<.0001	0.0002	0.0002	0.0009	0.0387	0.1096	0.5201		0.5785
15	0.1737	<.0001	0.0002	0.0253	0.2067	0.0008	<.0001	<.0001	<.0001	0.0001	0.0091	0.0319	0.2315	0.5785	
16	0.6479	<.0001	<.0001	0.0019	0.0311	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.0025	0.0365	0.1447	0.3647
17	<.0001	0.8479	0.6728	0.0465	0.0032	0.3957	0.1577	0.8870	0.9341	0.7457	0.1064	0.0373	0.0026	0.0003	<.0001
18	0.0021	0.0226	0.0399	0.6263	0.6151	0.1023	0.0001	0.0092	0.0109	0.0315	0.3850	0.6938	0.5693	0.2263	0.0785
19	0.0161	0.0031	0.0062	0.2356	0.8424	0.0203	<.0001	0.0011	0.0013	0.0047	0.1176	0.2743	0.8947	0.6093	0.2868
20	0.0548	0.0006	0.0013	0.0929	0.4853	0.0050	<.0001	0.0002	0.0002	0.0009	0.0398	0.1122	0.5278	0.9906	0.5704
21	0.0964	0.0002	0.0005	0.0527	0.3378	0.0023	<.0001	<.0001	<.0001	0.0004	0.0208	0.0649	0.3722	0.8027	0.7597
22	0.0018	0.0249	0.0436	0.6532	0.5890	0.1103	0.0002	0.0103	0.0121	0.0346	0.4058	0.7218	0.5441	0.2123	0.0724
23	0.0101	0.0052	0.0102	0.3097	0.9779	0.0312	<.0001	0.0019	0.0022	0.0077	0.1627	0.3560	0.9691	0.4953	0.2168
24	0.0686	0.0004	0.0009	0.0752	0.4247	0.0037	<.0001	0.0001	0.0002	0.0007	0.0312	0.0915	0.4642	0.9291	0.6407
25	<.0001	0.8210	0.6479	0.0429	0.0029	0.3769	0.1680	0.9143	0.9616	0.7198	0.0992	0.0343	0.0023	0.0003	<.0001
26	0.9879	<.0001	<.0001	0.0004	0.0097	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0006	0.0117	0.0583	0.1785
27	<.0001	0.6493	0.8225	0.1758	0.0200	0.8387	0.0404	0.4307	0.4662	0.7472	0.3303	0.1482	0.0168	0.0026	0.0004
28	0.0016	0.0282	0.0489	0.6888	0.5558	0.1215	0.0002	0.0118	0.0138	0.0389	0.4338	0.7586	0.5122	0.1950	0.0651
29	0.0551	0.0006	0.0013	0.0925	0.4839	0.0050	<.0001	0.0002	0.0002	0.0009	0.0396	0.1118	0.5263	0.9924	0.5720
30	0.7077	<.0001	<.0001	<.0001	0.0031	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0038	0.0229	0.0835
31	0.0578	0.0021	0.0042	0.1505	0.5990	0.0130	<.0001	0.0008	0.0009	0.0031	0.0739	0.1763	0.6423	0.8955	0.5186
32	<.0001	0.6568	0.5109	0.0352	0.0028	0.2928	0.2966	0.8924	0.8491	0.5708	0.0786	0.0285	0.0023	0.0003	<.0001
33	<.0001	0.6194	0.7769	0.2385	0.0373	0.9102	0.0484	0.4208	0.4531	0.7084	0.4087	0.2061	0.0321	0.0065	0.0013
34	0.0118	0.0140	0.0244	0.4212	0.9113	0.0625	0.0001	0.0058	0.0069	0.0194	0.2473	0.4726	0.8627	0.4425	0.2006
35	0.1898	0.0003	0.0006	0.0431	0.2636	0.0022	<.0001	<.0001	0.0001	0.0004	0.0179	0.0526	0.2906	0.6435	0.9588
36	<.0001	0.8614	0.9690	0.1341	0.0166	0.6636	0.0975	0.6287	0.6682	0.9586	0.2515	0.1134	0.0140	0.0025	0.0004
37	<.0001	0.2757	0.1930	0.0062	0.0003	0.0904	0.6907	0.4341	0.4026	0.2256	0.0167	0.0048	0.0003	<.0001	<.0001
38	<.0001	0.8074	0.6476	0.0561	0.0051	0.3940	0.2138	0.9479	0.9918	0.7140	0.1185	0.0461	0.0042	0.0006	<.0001
39	<.0001	0.3611	0.2603	0.0104	0.0006	0.1290	0.5651	0.5451	0.5093	0.3005	0.0265	0.0081	0.0005	<.0001	<.0001
40	<.0001	0.7860	0.6278	0.0527	0.0047	0.3788	0.2242	0.9701	0.9861	0.6934	0.1122	0.0432	0.0039	0.0006	<.0001

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
41	<.0001	0.9810	0.8496	0.0996	0.0110	0.5583	0.1315	0.7392	0.7810	0.9213	0.1949	0.0833	0.0093	0.0015	0.0003
42	<.0001	0.5198	0.6669	0.3019	0.0526	0.9725	0.0342	0.3412	0.3697	0.6023	0.4969	0.2635	0.0457	0.0099	0.0021
43	<.0001	0.9444	0.7770	0.0820	0.0085	0.4972	0.1569	0.8109	0.8538	0.8474	0.1648	0.0681	0.0071	0.0011	0.0002
44	<.0001	0.4223	0.5556	0.3821	0.0748	0.8465	0.0230	0.2670	0.2913	0.4965	0.6028	0.3371	0.0654	0.0153	0.0035
45	0.0011	0.0872	0.1337	0.9677	0.3816	0.2683	0.0016	0.0440	0.0500	0.1120	0.6929	0.9634	0.3492	0.1267	0.0420
46	0.0689	0.0017	0.0033	0.1294	0.5447	0.0104	<.0001	0.0006	0.0007	0.0024	0.0621	0.1524	0.5863	0.9590	0.5716
47	<.0001	0.7915	0.6328	0.0536	0.0048	0.3827	0.2215	0.9644	0.9918	0.6987	0.1138	0.0439	0.0040	0.0006	<.0001
48	<.0001	0.3851	0.5121	0.4192	0.0860	0.7949	0.0194	0.2396	0.2622	0.4556	0.6497	0.3714	0.0755	0.0183	0.0043
49	0.0030	0.0454	0.0733	0.7369	0.5618	0.1615	0.0006	0.0213	0.0245	0.0601	0.4902	0.8028	0.5211	0.2169	0.0812
50	0.0418	0.0033	0.0063	0.1943	0.7002	0.0189	<.0001	0.0012	0.0015	0.0048	0.0993	0.2254	0.7463	0.7854	0.4318
51	<.0001	0.5658	0.7180	0.2707	0.0448	0.9721	0.0404	0.3775	0.4078	0.6514	0.4541	0.2351	0.0388	0.0082	0.0017
52	<.0001	0.8545	0.6916	0.0642	0.0061	0.4283	0.1926	0.8999	0.9436	0.7596	0.1332	0.0529	0.0051	0.0008	0.0001
53	<.0001	0.8735	0.9567	0.1302	0.0159	0.6525	0.1006	0.6397	0.6795	0.9709	0.2452	0.1099	0.0135	0.0023	0.0004
54	<.0001	0.6805	0.8428	0.2066	0.0303	0.8432	0.0587	0.4713	0.5057	0.7725	0.3624	0.1774	0.0260	0.0051	0.0010
55	<.0001	0.4834	0.3609	0.0184	0.0012	0.1912	0.4307	0.6949	0.6548	0.4104	0.0443	0.0147	0.0010	0.0001	<.0001
56	0.9730	<.0001	<.0001	0.0011	0.0174	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0015	0.0204	0.0827	0.2200
57	<.0001	0.7249	0.5719	0.0438	0.0037	0.3370	0.2561	0.9658	0.9220	0.6351	0.0953	0.0357	0.0030	0.0004	<.0001
58	0.0002	0.2361	0.3308	0.6238	0.1607	0.5635	0.0082	0.1359	0.1508	0.2878	0.8916	0.5641	0.1433	0.0404	0.0108
59	0.0173	0.0094	0.0169	0.3438	0.9748	0.0452	<.0001	0.0038	0.0045	0.0132	0.1940	0.3894	0.9761	0.5317	0.2551
60	0.7298	<.0001	<.0001	0.0003	0.0061	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.0072	0.0351	0.1092

(continued)

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.6479	<.0001	0.0021	0.0161	0.0548	0.0964	0.0018	0.0101	0.0686	<.0001	0.9879	<.0001	0.0016	0.0551	0.7077
2	<.0001	0.8479	0.0226	0.0031	0.0006	0.0002	0.0249	0.0052	0.0004	0.8210	<.0001	0.6493	0.0282	0.0006	<.0001
3	<.0001	0.6728	0.0399	0.0062	0.0013	0.0005	0.0436	0.0102	0.0009	0.6479	<.0001	0.8225	0.0489	0.0013	<.0001
4	0.0019	0.0465	0.6263	0.2356	0.0929	0.0527	0.6532	0.3097	0.0752	0.0429	0.0004	0.1758	0.6888	0.0925	<.0001
5	0.0311	0.0032	0.6151	0.8424	0.4853	0.3378	0.5890	0.9779	0.4247	0.0029	0.0097	0.0200	0.5558	0.4839	0.0031
6	<.0001	0.3957	0.1023	0.0203	0.0050	0.0023	0.1103	0.0312	0.0037	0.3769	<.0001	0.8387	0.1215	0.0050	<.0001
7	<.0001	0.1577	0.0001	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	0.1680	<.0001	0.0404	0.0002	<.0001	<.0001
8	<.0001	0.8870	0.0092	0.0011	0.0002	<.0001	0.0103	0.0019	0.0001	0.9143	<.0001	0.4307	0.0118	0.0002	<.0001
9	<.0001	0.9341	0.0109	0.0013	0.0002	<.0001	0.0121	0.0022	0.0002	0.9616	<.0001	0.4662	0.0138	0.0002	<.0001
10	<.0001	0.7457	0.0315	0.0047	0.0009	0.0004	0.0346	0.0077	0.0007	0.7198	<.0001	0.7472	0.0389	0.0009	<.0001
11	0.0005	0.1064	0.3850	0.1176	0.0398	0.0208	0.4058	0.1627	0.0312	0.0992	0.0001	0.3303	0.4338	0.0396	<.0001
12	0.0025	0.0373	0.6938	0.2743	0.1122	0.0649	0.7218	0.3560	0.0915	0.0343	0.0006	0.1482	0.7586	0.1118	0.0001
13	0.0365	0.0026	0.5693	0.8947	0.5278	0.3722	0.5441	0.9691	0.4642	0.0023	0.0117	0.0168	0.5122	0.5263	0.0038
14	0.1447	0.0003	0.2263	0.6093	0.9906	0.8027	0.2123	0.4953	0.9291	0.0003	0.0583	0.0026	0.1950	0.9924	0.0229
15	0.3647	<.0001	0.0785	0.2868	0.5704	0.7597	0.0724	0.2168	0.6407	<.0001	0.1785	0.0004	0.0651	0.5720	0.0835
16		<.0001	0.0082	0.0498	0.1416	0.2260	0.0073	0.0332	0.1706	<.0001	0.6588	<.0001	0.0064	0.1422	0.4060

Least Squares Means for effect Age*Trt
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Pc

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
17	<.0001		0.0137	0.0017	0.0003	0.0001	0.0151	0.0029	0.0002	0.9725	<.0001	0.5181	0.0172	0.0003	<.0001
18	0.0082	0.0137		0.4832	0.2308	0.1449	0.9700	0.5958	0.1942	0.0125	0.0022	0.0666	0.9311	0.2299	0.0006
19	0.0498	0.0017	0.4832		0.6176	0.4470	0.4601	0.8641	0.5485	0.0015	0.0167	0.0118	0.4310	0.6160	0.0056
20	0.1416	0.0003	0.2308	0.6176		0.7935	0.2166	0.5028	0.9197	0.0003	0.0567	0.0027	0.1991	0.9982	0.0222
21	0.2260	0.0001	0.1449	0.4470	0.7935		0.1349	0.3519	0.8722	0.0001	0.0994	0.0012	0.1228	0.7953	0.0422
22	0.0073	0.0151	0.9700	0.4601	0.2166	0.1349		0.5701	0.1817	0.0138	0.0019	0.0723	0.9610	0.2158	0.0005
23	0.0332	0.0029	0.5958	0.8641	0.5028	0.3519	0.5701		0.4409	0.0026	0.0105	0.0186	0.5374	0.5014	0.0033
24	0.1706	0.0002	0.1942	0.5485	0.9197	0.8722	0.1817	0.4409		0.0002	0.0709	0.0020	0.1664	0.9215	0.0286
25	<.0001	0.9725	0.0125	0.0015	0.0003	0.0001	0.0138	0.0026	0.0002		<.0001	0.4961	0.0157	0.0003	<.0001
26	0.6588	<.0001	0.0022	0.0167	0.0567	0.0994	0.0019	0.0105	0.0709	<.0001		<.0001	0.0016	0.0570	0.6965
27	<.0001	0.5181	0.0666	0.0118	0.0027	0.0012	0.0723	0.0186	0.0020	0.4961	<.0001		0.0802	0.0027	<.0001
28	0.0064	0.0172	0.9311	0.4310	0.1991	0.1228	0.9610	0.5374	0.1664	0.0157	0.0016	0.0802		0.1983	0.0004
29	0.1422	0.0003	0.2299	0.6160	0.9982	0.7953	0.2158	0.5014	0.9215	0.0003	0.0570	0.0027	0.1983		0.0223
30	0.4060	<.0001	0.0006	0.0056	0.0222	0.0422	0.0005	0.0033	0.0286	<.0001	0.6965	<.0001	0.0004	0.0223	
31	0.1387	0.0012	0.3221	0.7323	0.9042	0.7169	0.3055	0.6168	0.8308	0.0011	0.0596	0.0077	0.2847	0.9026	0.0253
32	<.0001	0.7896	0.0109	0.0015	0.0003	0.0001	0.0120	0.0026	0.0002	0.8143	<.0001	0.3873	0.0136	0.0003	<.0001
33	<.0001	0.5004	0.1042	0.0237	0.0067	0.0033	0.1117	0.0351	0.0051	0.4803	<.0001	0.9397	0.1221	0.0067	<.0001
34	0.0351	0.0085	0.7231	0.7677	0.4490	0.3180	0.6972	0.8911	0.3952	0.0078	0.0122	0.0406	0.6641	0.4477	0.0043
35	0.3730	0.0001	0.1143	0.3498	0.6357	0.8169	0.1066	0.2746	0.7036	0.0001	0.1945	0.0012	0.0973	0.6372	0.0980
36	<.0001	0.7247	0.0521	0.0100	0.0025	0.0012	0.0564	0.0155	0.0019	0.7010	<.0001	0.8053	0.0624	0.0025	<.0001
37	<.0001	0.3611	0.0015	0.0002	<.0001	<.0001	0.0017	0.0003	<.0001	0.3781	<.0001	0.1317	0.0020	<.0001	<.0001
38	<.0001	0.9472	0.0187	0.0029	0.0006	0.0003	0.0205	0.0047	0.0005	0.9726	<.0001	0.5064	0.0230	0.0006	<.0001
39	<.0001	0.4616	0.0027	0.0003	<.0001	<.0001	0.0030	0.0005	<.0001	0.4812	<.0001	0.1830	0.0035	<.0001	<.0001
40	<.0001	0.9251	0.0174	0.0027	0.0006	0.0002	0.0191	0.0043	0.0004	0.9505	<.0001	0.4888	0.0215	0.0006	<.0001
41	<.0001	0.8403	0.0367	0.0065	0.0016	0.0007	0.0398	0.0103	0.0012	0.8155	<.0001	0.6912	0.0443	0.0016	<.0001
42	0.0001	0.4118	0.1390	0.0341	0.0102	0.0051	0.1484	0.0496	0.0079	0.3939	<.0001	0.8236	0.1614	0.0102	<.0001
43	<.0001	0.9141	0.0292	0.0050	0.0011	0.0005	0.0318	0.0079	0.0008	0.8889	<.0001	0.6236	0.0355	0.0011	<.0001
44	0.0002	0.3274	0.1861	0.0497	0.0158	0.0081	0.1979	0.0707	0.0123	0.3120	<.0001	0.7024	0.2140	0.0157	<.0001
45	0.0044	0.0594	0.6816	0.2900	0.1294	0.0792	0.7073	0.3679	0.1077	0.0553	0.0012	0.1957	0.7412	0.1289	0.0003
46	0.1610	0.0009	0.2848	0.6731	0.9677	0.7774	0.2695	0.5618	0.8936	0.0008	0.0710	0.0061	0.2505	0.9661	0.0308
47	<.0001	0.9308	0.0178	0.0027	0.0006	0.0003	0.0195	0.0044	0.0004	0.9562	<.0001	0.4933	0.0219	0.0006	<.0001
48	0.0003	0.2959	0.2090	0.0577	0.0188	0.0098	0.2218	0.0815	0.0147	0.2815	<.0001	0.6539	0.2392	0.0187	<.0001
49	0.0104	0.0297	0.9086	0.4450	0.2210	0.1432	0.9362	0.5447	0.1880	0.0274	0.0031	0.1126	0.9722	0.2202	0.0010
50	0.1052	0.0019	0.3954	0.8406	0.7938	0.6146	0.3765	0.7193	0.7229	0.0017	0.0432	0.0114	0.3526	0.7922	0.0176
51	<.0001	0.4523	0.1216	0.0288	0.0084	0.0041	0.1301	0.0422	0.0064	0.4334	<.0001	0.8780	0.1418	0.0084	<.0001
52	<.0001	0.9954	0.0219	0.0035	0.0008	0.0003	0.0240	0.0057	0.0006	0.9791	<.0001	0.5457	0.0268	0.0008	<.0001
53	<.0001	0.7363	0.0503	0.0096	0.0024	0.0011	0.0545	0.0148	0.0018	0.7124	<.0001	0.7934	0.0603	0.0024	<.0001
54	<.0001	0.5558	0.0875	0.0190	0.0052	0.0025	0.0940	0.0285	0.0040	0.5346	<.0001	0.9926	0.1031	0.0052	<.0001
55	<.0001	0.6006	0.0052	0.0006	0.0001	<.0001	0.0058	0.0011	<.0001	0.6230	<.0001	0.2627	0.0066	0.0001	<.0001
56	0.6972	<.0001	0.0047	0.0279	0.0808	0.1318	0.0042	0.0186	0.0981	<.0001	0.9842	<.0001	0.0037	0.0812	0.7033
57	<.0001	0.8616	0.0141	0.0021	0.0004	0.0002	0.0154	0.0034	0.0003	0.8867	<.0001	0.4399	0.0174	0.0004	<.0001
58	0.0008	0.1735	0.3471	0.1131	0.0414	0.0229	0.3652	0.1533	0.0331	0.1638	0.0002	0.4438	0.3895	0.0412	<.0001

Dependent Variable: Pc

i/j	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
59	0.0491	0.0057	0.6192	0.8788	0.5388	0.3919	0.5949	0.9953	0.4791	0.0051	0.0179	0.0287	0.5640	0.5375	0.0066
60	0.4427	<.0001	0.0014	0.0102	0.0342	0.0600	0.0013	0.0065	0.0427	<.0001	0.7193	<.0001	0.0011	0.0344	0.9986

(continued)

Dependent Variable: Pc

i/j	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
1	0.0578	<.0001	<.0001	0.0118	0.1898	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011
2	0.0021	0.6568	0.6194	0.0140	0.0003	0.8614	0.2757	0.8074	0.3611	0.7860	0.9810	0.5198	0.9444	0.4223	0.0872
3	0.0042	0.5109	0.7769	0.0244	0.0006	0.9690	0.1930	0.6476	0.2603	0.6278	0.8496	0.6669	0.7770	0.5556	0.1337
4	0.1505	0.0352	0.2385	0.4212	0.0431	0.1341	0.0062	0.0561	0.0104	0.0527	0.0996	0.3019	0.0820	0.3821	0.9677
5	0.5990	0.0028	0.0373	0.9113	0.2636	0.0166	0.0003	0.0051	0.0006	0.0047	0.0110	0.0526	0.0085	0.0748	0.3816
6	0.0130	0.2928	0.9102	0.0625	0.0022	0.6636	0.0904	0.3940	0.1290	0.3788	0.5583	0.9725	0.4972	0.8465	0.2683
7	<.0001	0.2966	0.0484	0.0001	<.0001	0.0975	0.6907	0.2138	0.5651	0.2242	0.1315	0.0342	0.1569	0.0230	0.0016
8	0.0008	0.8924	0.4208	0.0058	<.0001	0.6287	0.4341	0.9479	0.5451	0.9701	0.7392	0.3412	0.8109	0.2670	0.0440
9	0.0009	0.8491	0.4531	0.0069	0.0001	0.6682	0.4026	0.9918	0.5093	0.9861	0.7810	0.3697	0.8538	0.2913	0.0500
10	0.0031	0.5708	0.7084	0.0194	0.0004	0.9586	0.2256	0.7140	0.3005	0.6934	0.9213	0.6023	0.8474	0.4965	0.1120
11	0.0739	0.0786	0.4087	0.2473	0.0179	0.2515	0.0167	0.1185	0.0265	0.1122	0.1949	0.4969	0.1648	0.6028	0.6929
12	0.1763	0.0285	0.2061	0.4726	0.0526	0.1134	0.0048	0.0461	0.0081	0.0432	0.0833	0.2635	0.0681	0.3371	0.9634
13	0.6423	0.0023	0.0321	0.8627	0.2906	0.0140	0.0003	0.0042	0.0005	0.0039	0.0093	0.0457	0.0071	0.0654	0.3492
14	0.8955	0.0003	0.0065	0.4425	0.6435	0.0025	<.0001	0.0006	<.0001	0.0006	0.0015	0.0099	0.0011	0.0153	0.1267
15	0.5186	<.0001	0.0013	0.2006	0.9588	0.0004	<.0001	<.0001	<.0001	<.0001	0.0003	0.0021	0.0002	0.0035	0.0420
16	0.1387	<.0001	<.0001	0.0351	0.3730	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0002	0.0044
17	0.0012	0.7896	0.5004	0.0085	0.0001	0.7247	0.3611	0.9472	0.4616	0.9251	0.8403	0.4118	0.9141	0.3274	0.0594
18	0.3221	0.0109	0.1042	0.7231	0.1143	0.0521	0.0015	0.0187	0.0027	0.0174	0.0367	0.1390	0.0292	0.1861	0.6816
19	0.7323	0.0015	0.0237	0.7677	0.3498	0.0100	0.0002	0.0029	0.0003	0.0027	0.0065	0.0341	0.0050	0.0497	0.2900
20	0.9042	0.0003	0.0067	0.4490	0.6357	0.0025	<.0001	0.0006	<.0001	0.0006	0.0016	0.0102	0.0011	0.0158	0.1294
21	0.7169	0.0001	0.0033	0.3180	0.8169	0.0012	<.0001	0.0003	<.0001	0.0002	0.0007	0.0051	0.0005	0.0081	0.0792
22	0.3055	0.0120	0.1117	0.6972	0.1066	0.0564	0.0017	0.0205	0.0030	0.0191	0.0398	0.1484	0.0318	0.1979	0.7073
23	0.6168	0.0026	0.0351	0.8911	0.2746	0.0155	0.0003	0.0047	0.0005	0.0043	0.0103	0.0496	0.0079	0.0707	0.3679
24	0.8308	0.0002	0.0051	0.3952	0.7036	0.0019	<.0001	0.0005	<.0001	0.0004	0.0012	0.0079	0.0008	0.0123	0.1077
25	0.0011	0.8143	0.4803	0.0078	0.0001	0.7010	0.3781	0.9726	0.4812	0.9505	0.8155	0.3939	0.8889	0.3120	0.0553
26	0.0596	<.0001	<.0001	0.0122	0.1945	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012
27	0.0077	0.3873	0.9397	0.0406	0.0012	0.8053	0.1317	0.5064	0.1830	0.4888	0.6912	0.8236	0.6236	0.7024	0.1957
28	0.2847	0.0136	0.1221	0.6641	0.0973	0.0624	0.0020	0.0230	0.0035	0.0215	0.0443	0.1614	0.0355	0.2140	0.7412
29	0.9026	0.0003	0.0067	0.4477	0.6372	0.0025	<.0001	0.0006	<.0001	0.0006	0.0016	0.0102	0.0011	0.0157	0.1289
30	0.0253	<.0001	<.0001	0.0043	0.0980	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
31		0.0011	0.0151	0.5512	0.5785	0.0065	0.0001	0.0020	0.0002	0.0018	0.0043	0.0217	0.0033	0.0315	0.1909
32	0.0011		0.3792	0.0068	0.0001	0.5628	0.5448	0.8511	0.6602	0.8716	0.6614	0.3094	0.7260	0.2443	0.0445
33	0.0151	0.3792		0.0647	0.0030	0.7631	0.1387	0.4888	0.1881	0.4726	0.6583	0.8905	0.5963	0.7744	0.2540
34	0.5512	0.0068	0.0647		0.2503	0.0321	0.0010	0.0116	0.0018	0.0108	0.0226	0.0869	0.0180	0.1175	0.4748

i/j	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
35	0.5785	0.0001	0.0030	0.2503		0.0011	<.0001	0.0003	<.0001	0.0003	0.0007	0.0046	0.0005	0.0071	0.0634
36	0.0065	0.5628	0.7631	0.0321	0.0011		0.2372	0.6956	0.3092	0.6765	0.8879	0.6606	0.8192	0.5567	0.1500
37	0.0001	0.5448	0.1387	0.0010	<.0001	0.2372		0.4280	0.8681	0.4432	0.2974	0.1059	0.3397	0.0778	0.0094
38	0.0020	0.8511	0.4888	0.0116	0.0003	0.6956	0.4280		0.5307	0.9793	0.8023	0.4068	0.8707	0.3281	0.0679
39	0.0002	0.6602	0.1881	0.0018	<.0001	0.3092	0.8681	0.5307		0.5478	0.3807	0.1463	0.4299	0.1096	0.0148
40	0.0018	0.8716	0.4726	0.0108	0.0003	0.6765	0.4432	0.9793	0.5478		0.7823	0.3923	0.8503	0.3155	0.0642
41	0.0043	0.6614	0.6583	0.0226	0.0007	0.8879	0.2974	0.8023	0.3807	0.7823		0.5620	0.9302	0.4664	0.1144
42	0.0217	0.3094	0.8905	0.0869	0.0046	0.6606	0.1059	0.4068	0.1463	0.3923	0.5620		0.5046	0.8816	0.3155
43	0.0033	0.7260	0.5963	0.0180	0.0005	0.8192	0.3397	0.8707	0.4299	0.8503	0.9302	0.5046		0.4146	0.0959
44	0.0315	0.2443	0.7744	0.1175	0.0071	0.5567	0.0778	0.3281	0.1096	0.3155	0.4664	0.8816	0.4146		0.3923
45	0.1909	0.0445	0.2540	0.4748	0.0634	0.1500	0.0094	0.0679	0.0148	0.0642	0.1144	0.3155	0.0959	0.3923	
46	0.9404	0.0008	0.0123	0.5025	0.6306	0.0052	<.0001	0.0015	0.0002	0.0014	0.0034	0.0179	0.0026	0.0262	0.1670
47	0.0018	0.8663	0.4768	0.0110	0.0003	0.6814	0.4392	0.9846	0.5433	0.9947	0.7874	0.3960	0.8555	0.3187	0.0651
48	0.0366	0.2202	0.7274	0.1326	0.0085	0.5159	0.0681	0.2985	0.0967	0.2867	0.4294	0.8329	0.3802	0.9505	0.4274
49	0.3014	0.0226	0.1572	0.6608	0.1131	0.0868	0.0042	0.0360	0.0068	0.0339	0.0642	0.2012	0.0527	0.2583	0.7822
50	0.8951	0.0016	0.0214	0.6425	0.4919	0.0095	0.0002	0.0030	0.0004	0.0027	0.0063	0.0302	0.0049	0.0432	0.2392
51	0.0183	0.3412	0.9420	0.0757	0.0038	0.7083	0.1205	0.4444	0.1650	0.4291	0.6066	0.9482	0.5469	0.8307	0.2854
52	0.0024	0.8071	0.5249	0.0136	0.0004	0.7378	0.3959	0.9549	0.4943	0.9342	0.8463	0.4394	0.9154	0.3568	0.0768
53	0.0062	0.5725	0.7522	0.0310	0.0011	0.9885	0.2429	0.7063	0.3160	0.6871	0.8993	0.6502	0.8304	0.5471	0.1460
54	0.0122	0.4236	0.9366	0.0542	0.0024	0.8244	0.1609	0.5399	0.2159	0.5229	0.7167	0.8280	0.6524	0.7143	0.2226
55	0.0005	0.8101	0.2634	0.0033	<.0001	0.4131	0.7148	0.6687	0.8420	0.6877	0.4978	0.2094	0.5549	0.1607	0.0248
56	0.0809	<.0001	<.0001	0.0199	0.2319	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0025
57	0.0014	0.9311	0.4277	0.0088	0.0002	0.6224	0.4891	0.9194	0.5989	0.9400	0.7252	0.3522	0.7918	0.2808	0.0543
58	0.0721	0.1284	0.5184	0.2266	0.0192	0.3441	0.0343	0.1819	0.0508	0.1736	0.2773	0.6113	0.2406	0.7192	0.6194
59	0.6437	0.0046	0.0478	0.8936	0.3093	0.0230	0.0007	0.0080	0.0011	0.0074	0.0159	0.0652	0.0126	0.0897	0.3964
60	0.0363	<.0001	<.0001	0.0075	0.1219	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0008

(continued)

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1	0.0689	<.0001	<.0001	0.0030	0.0418	<.0001	<.0001	<.0001	<.0001	<.0001	0.9730	<.0001	0.0002	0.0173	0.7298
2	0.0017	0.7915	0.3851	0.0454	0.0033	0.5658	0.8545	0.8735	0.6805	0.4834	<.0001	0.7249	0.2361	0.0094	<.0001
3	0.0033	0.6328	0.5121	0.0733	0.0063	0.7180	0.6916	0.9567	0.8428	0.3609	<.0001	0.5719	0.3308	0.0169	<.0001
4	0.1294	0.0536	0.4192	0.7369	0.1943	0.2707	0.0642	0.1302	0.2066	0.0184	0.0011	0.0438	0.6238	0.3438	0.0003
5	0.5447	0.0048	0.0860	0.5618	0.7002	0.0448	0.0061	0.0159	0.0303	0.0012	0.0174	0.0037	0.1607	0.9748	0.0061
6	0.0104	0.3827	0.7949	0.1615	0.0189	0.9721	0.4283	0.6525	0.8432	0.1912	<.0001	0.3370	0.5635	0.0452	<.0001
7	<.0001	0.2215	0.0194	0.0006	<.0001	0.0404	0.1926	0.1006	0.0587	0.4307	<.0001	0.2561	0.0082	<.0001	<.0001
8	0.0006	0.9644	0.2396	0.0213	0.0012	0.3775	0.8999	0.6397	0.4713	0.6949	<.0001	0.9658	0.1359	0.0038	<.0001
9	0.0007	0.9918	0.2622	0.0245	0.0015	0.4078	0.9436	0.6795	0.5057	0.6548	<.0001	0.9220	0.1508	0.0045	<.0001
10	0.0024	0.6987	0.4556	0.0601	0.0048	0.6514	0.7596	0.9709	0.7725	0.4104	<.0001	0.6351	0.2878	0.0132	<.0001

Dependent Variable: Pc

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
11	0.0621	0.1138	0.6497	0.4902	0.0993	0.4541	0.1332	0.2452	0.3624	0.0443	0.0003	0.0953	0.8916	0.1940	<.0001
12	0.1524	0.0439	0.3714	0.8028	0.2254	0.2351	0.0529	0.1099	0.1774	0.0147	0.0015	0.0357	0.5641	0.3894	0.0004
13	0.5863	0.0040	0.0755	0.5211	0.7463	0.0388	0.0051	0.0135	0.0260	0.0010	0.0204	0.0030	0.1433	0.9761	0.0072
14	0.9590	0.0006	0.0183	0.2169	0.7854	0.0082	0.0008	0.0023	0.0051	0.0001	0.0827	0.0004	0.0404	0.5317	0.0351
15	0.5716	<.0001	0.0043	0.0812	0.4318	0.0017	0.0001	0.0004	0.0010	<.0001	0.2200	<.0001	0.0108	0.2551	0.1092
16	0.1610	<.0001	0.0003	0.0104	0.1052	<.0001	<.0001	<.0001	<.0001	<.0001	0.6972	<.0001	0.0008	0.0491	0.4427
17	0.0009	0.9308	0.2959	0.0297	0.0019	0.4523	0.9954	0.7363	0.5558	0.6006	<.0001	0.8616	0.1735	0.0057	<.0001
18	0.2848	0.0178	0.2090	0.9086	0.3954	0.1216	0.0219	0.0503	0.0875	0.0052	0.0047	0.0141	0.3471	0.6192	0.0014
19	0.6731	0.0027	0.0577	0.4450	0.8406	0.0288	0.0035	0.0096	0.0190	0.0006	0.0279	0.0021	0.1131	0.8788	0.0102
20	0.9677	0.0006	0.0188	0.2210	0.7938	0.0084	0.0008	0.0024	0.0052	0.0001	0.0808	0.0004	0.0414	0.5388	0.0342
21	0.7774	0.0003	0.0098	0.1432	0.6146	0.0041	0.0003	0.0011	0.0025	<.0001	0.1318	0.0002	0.0229	0.3919	0.0600
22	0.2695	0.0195	0.2218	0.9362	0.3765	0.1301	0.0240	0.0545	0.0940	0.0058	0.0042	0.0154	0.3652	0.5949	0.0013
23	0.5618	0.0044	0.0815	0.5447	0.7193	0.0422	0.0057	0.0148	0.0285	0.0011	0.0186	0.0034	0.1533	0.9953	0.0065
24	0.8936	0.0004	0.0147	0.1880	0.7229	0.0064	0.0006	0.0018	0.0040	<.0001	0.0981	0.0003	0.0331	0.4791	0.0427
25	0.0008	0.9562	0.2815	0.0274	0.0017	0.4334	0.9791	0.7124	0.5346	0.6230	<.0001	0.8867	0.1638	0.0051	<.0001
26	0.0710	<.0001	<.0001	0.0031	0.0432	<.0001	<.0001	<.0001	<.0001	<.0001	0.9842	<.0001	0.0002	0.0179	0.7193
27	0.0061	0.4933	0.6539	0.1126	0.0114	0.8780	0.5457	0.7934	0.9926	0.2627	<.0001	0.4399	0.4438	0.0287	<.0001
28	0.2505	0.0219	0.2392	0.9722	0.3526	0.1418	0.0268	0.0603	0.1031	0.0066	0.0037	0.0174	0.3895	0.5640	0.0011
29	0.9661	0.0006	0.0187	0.2202	0.7922	0.0084	0.0008	0.0024	0.0052	0.0001	0.0812	0.0004	0.0412	0.5375	0.0344
30	0.0308	<.0001	<.0001	0.0010	0.0176	<.0001	<.0001	<.0001	<.0001	<.0001	0.7033	<.0001	<.0001	0.0066	0.9986
31	0.9404	0.0018	0.0366	0.3014	0.8951	0.0183	0.0024	0.0062	0.0122	0.0005	0.0809	0.0014	0.0721	0.6437	0.0363
32	0.0008	0.8663	0.2202	0.0226	0.0016	0.3412	0.8071	0.5725	0.4236	0.8101	<.0001	0.9311	0.1284	0.0046	<.0001
33	0.0123	0.4768	0.7274	0.1572	0.0214	0.9420	0.5249	0.7522	0.9366	0.2634	<.0001	0.4277	0.5184	0.0478	<.0001
34	0.5025	0.0110	0.1326	0.6608	0.6425	0.0757	0.0136	0.0310	0.0542	0.0033	0.0199	0.0088	0.2266	0.8936	0.0075
35	0.6306	0.0003	0.0085	0.1131	0.4919	0.0038	0.0004	0.0011	0.0024	<.0001	0.2319	0.0002	0.0192	0.3093	0.1219
36	0.0052	0.6814	0.5159	0.0868	0.0095	0.7083	0.7378	0.9885	0.8244	0.4131	<.0001	0.6224	0.3441	0.0230	<.0001
37	<.0001	0.4392	0.0681	0.0042	0.0002	0.1205	0.3959	0.2429	0.1609	0.7148	<.0001	0.4891	0.0343	0.0007	<.0001
38	0.0015	0.9846	0.2985	0.0360	0.0030	0.4444	0.9549	0.7063	0.5399	0.6687	<.0001	0.9194	0.1819	0.0080	<.0001
39	0.0002	0.5433	0.0967	0.0068	0.0004	0.1650	0.4943	0.3160	0.2159	0.8420	<.0001	0.5989	0.0508	0.0011	<.0001
40	0.0014	0.9947	0.2867	0.0339	0.0027	0.4291	0.9342	0.6871	0.5229	0.6877	<.0001	0.9400	0.1736	0.0074	<.0001
41	0.0034	0.7874	0.4294	0.0642	0.0063	0.6066	0.8463	0.8993	0.7167	0.4978	<.0001	0.7252	0.2773	0.0159	<.0001
42	0.0179	0.3960	0.8329	0.2012	0.0302	0.9482	0.4394	0.6502	0.8280	0.2094	<.0001	0.3522	0.6113	0.0652	<.0001
43	0.0026	0.8555	0.3802	0.0527	0.0049	0.5469	0.9154	0.8304	0.6524	0.5549	<.0001	0.7918	0.2406	0.0126	<.0001
44	0.0262	0.3187	0.9505	0.2583	0.0432	0.8307	0.3568	0.5471	0.7143	0.1607	0.0001	0.2808	0.7192	0.0897	<.0001
45	0.1670	0.0651	0.4274	0.7822	0.2392	0.2854	0.0768	0.1460	0.2226	0.0248	0.0025	0.0543	0.6194	0.3964	0.0008
46		0.0014	0.0305	0.2680	0.8363	0.0150	0.0019	0.0050	0.0099	0.0004	0.0946	0.0011	0.0612	0.5912	0.0434
47	0.0014		0.2897	0.0344	0.0028	0.4330	0.9395	0.6920	0.5273	0.6828	<.0001	0.9347	0.1757	0.0076	<.0001
48	0.0305	0.2897		0.2852	0.0499	0.7826	0.3254	0.5067	0.6686	0.1432	0.0002	0.2542	0.7661	0.1019	<.0001
49	0.2680	0.0344	0.2852		0.3670	0.1794	0.0412	0.0843	0.1353	0.0120	0.0059	0.0281	0.4398	0.5671	0.0020
50	0.8363	0.0028	0.0499	0.3670		0.0257	0.0035	0.0091	0.0174	0.0007	0.0608	0.0022	0.0952	0.7409	0.0263
51	0.0150	0.4330	0.7826	0.1794	0.0257		0.4787	0.6976	0.8790	0.2338	<.0001	0.3867	0.5666	0.0565	<.0001
52	0.0019	0.9395	0.3254	0.0412	0.0035	0.4787		0.7487	0.5779	0.6281	<.0001	0.8747	0.2009	0.0094	<.0001

Dependent Variable: Pc

i/j	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
53	0.0050	0.6920	0.5067	0.0843	0.0091	0.6976	0.7487		0.8132	0.4213	<.0001	0.6327	0.3368	0.0222	<.0001
54	0.0099	0.5273	0.6686	0.1353	0.0174	0.8790	0.5779	0.8132		0.2986	<.0001	0.4753	0.4684	0.0397	<.0001
55	0.0004	0.6828	0.1432	0.0120	0.0007	0.2338	0.6281	0.4213	0.2986		<.0001	0.7438	0.0788	0.0022	<.0001
56	0.0946	<.0001	0.0002	0.0059	0.0608	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.0005	0.0279	0.7228
57	0.0011	0.9347	0.2542	0.0281	0.0022	0.3867	0.8747	0.6327	0.4753	0.7438	<.0001		0.1513	0.0060	<.0001
58	0.0612	0.1757	0.7661	0.4398	0.0952	0.5666	0.2009	0.3368	0.4684	0.0788	0.0005	0.1513		0.1797	0.0001
59	0.5912	0.0076	0.1019	0.5671	0.7409	0.0565	0.0094	0.0222	0.0397	0.0022	0.0279	0.0060	0.1797		0.0110
60	0.0434	<.0001	<.0001	0.0020	0.0263	<.0001	<.0001	<.0001	<.0001	<.0001	0.7228	<.0001	0.0001	0.0110	