LONGITUDINAL STUDY OF ANTIMICROBIAL RESISTANCE AMONG *ESCHERICHIA COLI* ISOLATED FROM INTEGRATED MULTI-SITE COHORTS OF HUMANS AND SWINE

A Dissertation

by

WALID QASIM MOHAMMAD ALALI

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

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Biomedical Sciences

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Approved by:

Chair of Committee,	H. Morgan Scott
Committee Members,	Bo Norby
	D. Bruce Lawhorn
	Roger B. Harvey
	Suresh Pillai
Head of Department,	Evelyn Castiglioni

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ABSTRACT

Longitudinal Study of Antimicrobial Resistance among *Escherichia coli* Isolated from Integrated Multi-site Cohorts of Humans and Swine. (December 2007)

Walid Qasim Mohammad Alali, D.V.M., Jordan University of Science and Technology; M.S., Kansas State University

Chair of Advisory Committee: Dr. H. Morgan Scott

Many studies have attempted to link antimicrobial use in food animal agriculture with an increased risk of antimicrobial-resistant (AR) bacterial levels in humans. Our data arise from longitudinal aggregated fecal samples in a 3-year cohort study of vertically integrated populations of human workers and consumers, and swine. Human and swine *E. coli* isolates (N = 2130 and 3485, respectively) were tested for antimicrobial susceptibility using the Sensititre™ broth microdilution system. The associations between AR prevalence for each antimicrobial agent, multi-drug resistant *E. coli*, or multivariate AR *E. coli*, and the risk factors (host species, production type (swine), vocation (human swine worker versus non-worker), and season) in the study were assessed using generalized estimating equations (GEE), GLM with multinomial distribution, or GEE in a multivariate model using a SAS® macro to adjust for the correlated AR phenotypes. There were significant ($p < 0.05$) differences in AR isolates: 1) between host-species with swine at higher risk for ceftiofur, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfisoxazole, and tetracycline. The prevalence of ciprofloxacin, nalidixic acid, and trimethoprim/sulfamethoxazole resistance were higher

among human isolates, 2) swine production group was significantly associated with AR with purchased boars, nursery piglets, and breeding boars at a higher risk of resistance to streptomycin and tetracycline, and 3) human swine worker cohorts exhibited an elevated tetracycline prevalence, but lowered sulfisoxazole prevalence when compared to non-workers. High variability among seasonal samples over the 3-year period was observed. There were significant differences in multiple resistance isolates between host species, with swine at higher risk than humans of carrying multi-resistant strains; however, no significant differences in multiple resistance isolates within humans by vocation or within swine by production group. The odds-ratios, adjusted for multivariate dependence of individual AR phenotypes, were increased relative to unadjusted odds-ratios among 1) swine as compared to human for tetracycline (OR = 21.8 vs. 19.6), and 2) increased significantly among swine-workers as compared to non-workers only for tetracycline (OR = 1.4 vs. 1.3). Occupational exposure to swine-rearing facilities appears to be associated with an increased relative odds for the prevalence of tetracycline resistance compared to non-workers.

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I would like to acknowledge the USDA-CSREES-NRICGP Section 32.1 (Epidemiologic Approaches to Food Safety) Grant #2003-35212-13298, for funding the research project that also paid for my stipend and tuition throughout my Ph.D. studies. Also, thanks to the USDA-ARS-Southern Plains Agricultural Research Center (College Station, Texas) for providing the lab space and allowing us to share their equipment and expertise to analyze our samples.

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CHAPTER I

INTRODUCTION

1.1. Background

Antimicrobial agents, which include antibiotics, rank among the greatest discoveries in the history of human kind. They are essential in the treatment of infectious diseases as well as in disease prophylaxis in human and veterinary medicine. Antimicrobial resistance (AR) emergence, dissemination, propagation, and maintenance among bacteria have become a world wide health concern, especially in human and veterinary medicine (Anderson, 1999; Levy and Marshall, 2004; WHO, 2001). The misuse and intensive use of therapeutic forms of antimicrobial agents in human medicine and companion animals, as well as the therapeutic use, prophylactic use, and subtherapeutic use for growth promotion in food animals have substantially increased selective pressures on bacteria; favoring the propagation and maintenance of AR bacteria (Levy, 2002). The lack of response to first line of medications (i.e., antimicrobial agents), the need for second or third line of treatment (more potent agents), the increased cost of individual treatment, the spread of nosocomial infections, and the development of multi-drug resistant pathogenic strains are serious health problems attributed to the AR bacteria dilemma (Kollef and Fraser, 2001). Nosocomial infections, which arise in hospital settings, have been reported in 5 – 6% of hospitalized patients (Horan et al., 1986). Increasing numbers of these infections are caused by AR bacteria that circulate in many hospital settings in different parts of the world. The frequency of AR bacteria generally varies between the hospital environment and the outside community due to the

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This dissertation follows the style of Preventive Veterinary Medicine.

differences in the antimicrobial selective pressures and the availability of sensitive and resistant strains (Lipsitch, 2001).

Antimicrobial resistance can develop in commensal (normal microflora) and pathogenic bacteria both in human and animal gastrointestinal tracts as well as in the environment. It has been suggested that AR genes existed in microorganisms long before antimicrobial use began (Levy, 2002). Antimicrobial resistance genes are known to reside on several genetic structures in bacterial cells such as the genome, plasmids, transposons, and integrons (Barbosa and Levy, 2000; Levy, 2002). Mutations in the bacterial genetic material (i.e., DNA) that lead to AR, as well as the exchange of AR genes (conjugation, transformation, transduction) among bacteria (same or different species), have a significant role in the transmission dynamics of AR bacteria within and among human and animal populations. Furthermore, cross-resistance and co-selection of AR genes (Courvalin and Trieu-Cuot, 2001), and persistence of these genes in human and animal populations, as well as in the environment, even after banning the use of antimicrobial agents (e.g., in Denmark (Aarestrup et al., 2001)), have added to the propagation and maintenance of AR bacteria in the community.

Many authors have attempted to link antimicrobial use in food animal agriculture with an increased risk of AR bacterial levels in humans (Stobberingh et al., 1999; White et al., 2001; Winokur et al., 2001). These authors have speculated that AR bacteria in animals could transfer to human populations mainly through direct contact (e.g., occupational exposure) and indirect contact with animals (e.g., consumption of contaminated food products of animal origin). However, those studies were based on

historical (typically cross-sectional) data lacking a temporal component to better establish cause-effect relationships. Moreover, it has been suggested that antimicrobial use on farms is not the only risk factor that affects resistance levels in animals (Kummerer, 2003; Singer et al., 2006).

The need for empirical identification and quantification of AR genes in animal populations, in addition to tracking the spread of AR bacteria to human and animal populations, is imperative (Singer et al., 2003). Advances in this area will facilitate the assessment of transmission risk and dynamics of AR bacteria among human and animal cohorts. In several AR studies, assessments of human exposure to AR bacteria from animals, and food products of animal origin, in relation to resistance levels in human populations have lacked the control of several essential factors. These include: 1) open study populations with limited or no control over the in- and out-migration of subjects (humans or animals) in the study areas, 2) human travel and trade (animals and food products), which serve as a source for AR bacteria that can be introduced into susceptible populations, and 3) lack of temporal components, as in cohort studies, which require follow-up of individual or groups of subjects (i.e., humans and animals) over a period of time (Aubry-Damon et al., 2004; Bates et al., 1994; Nijsten et al., 1994; Nijsten et al., 1996b; Stobberingh et al., 1999; Van den Bogaard et al., 2001; Van den Bogaard et al., 2002).

Our study design and study population have effectively controlled many of the limitations that plagued previous AR studies. Our agri-food study model was a multi-site housing and vertically integrated swine and human population system in Texas.

There was a very limited in- and out- migration of humans to and from the system. Swine were moved from farrow-finish on to grower/finisher units, then were slaughtered and processed within the system and fed back to humans in the system. The research project was a 3-year longitudinal study (January 2004 – January 2007) and samples were collected monthly at the group-level.

1.2. Study objectives

The objectives of this 3-year longitudinal study were 1) to examine the relationship between the prevalence of AR commensal *E. coli* isolated from human wastewater and swine fecal samples of group-level cohorts of human and swine and potential risk factors: host species (swine versus human), swine production type (e.g., breeding/gestation, farrowing, nursery, grower, finisher), human vocation (swine worker versus non-worker), human consumer versus non-consumer, and season, in a multi-site housing, vertically integrated swine and human population agri-food system, and 2) to compare the quantities of the ceftiofur resistant gene (bla_{CMY2}) standardized to a bacterial reference gene (*rpoB*) in the total community DNA extracted from the human wastewater and swine fecal samples in relation to the risk factors in the study.

CHAPTER II

LITERATURE REVIEW

2.1. Antimicrobial-resistant enteric bacteria in agri-food systems and risks to public health

The majority of the existing AR research has focused on: 1) the relationship between antimicrobial use and resistance levels in human and animal populations, 2) the transmission of resistant bacteria from animals to humans, and 3) the clinical implications of resistance levels in both species. In general, AR bacteria can be transmitted to humans from several different sources: 1) AR bacteria from slaughtered animals may contaminate the carcass meat and other animal food products, which then could enter the food chain, 2) humans and animals shed AR enteric bacteria into fecal matter such as sewage, which in turn may contaminate agriculture crops or water supplies, and then may enter the food chain, 3) wild animals, such as rodents and birds, that acquire and shed resistant bacteria, may contaminate animal feed, agriculture crops, and water supplies, 4) human direct contact (i.e., occupational exposure) with animals (pets and food animals) that are shedding AR bacteria, or 5) food handlers (rather than consumers) may acquire resistant organisms through exposure to contaminated animal food products (Phillips et al., 2004).

Vancomycin-resistant Enterococci bacteria (VRE) were isolated from a wastewater treatment plant that drained from a hospital, an urban community, and from both (mixed-influent) in Oxford, England (Bates et al., 1994). The number of human wastewater samples was not specified in the study. The authors of that study isolated VRE bacteria

from fecal samples collected from the following "non-human" sources: 1) pigs (n = 36) from a facility at the University of Oxford, 2) poultry, cattle, sheep, pigs, and horses from a farm 16 km outside the city (total n = 16), and 3) fresh (n = 3) and frozen (n = 2) chicken carcasses samples from local retail stores. *Enterococcus faecium* isolates were tested for vancomycin susceptibility using kanamycin aesulin azide (KAA) selective agar supplemented with vancomycin (40 mg/L). The VRE isolates were screened for the *vanA* gene using PCR and then ribotyped to test for genetic similarity based on the rRNA gene. There were 14, 12, and 9 vancomycin-resistant *Enterococcus faecium* isolates obtained from the hospital, community, and the mixed-human drainage, respectively. Fifteen of the pig fecal samples, all the fresh chicken samples, and 1 sample each from turkey, live chicken, and pony samples also had vancomycin-resistant *E. faecium* isolates. The authors reported that some of the clinical human isolates were genetically similar (i.e., had indistinguishable bands on ribotyping) to some of the non-human strains. The authors suggested that animals may have acted as VRE reservoirs due to the selection pressure applied by the use of certain antimicrobial agents (i.e., growth-promoters), which in turn may have increased the risk of AR bacteria transmission to humans via the food chain. Their study failed to report the historical antimicrobial use in the "non-human" (i.e., live animals) populations. Thus, the claim of association between level of VRE isolates and antimicrobial use cannot be established. Additionally, the ribotyping results provided no information on the VRE isolates dynamics of transmission; that is, though the genes were indistinguishable, the direction of spread (from animal to human, or vice versa) remains unknown.

The prevalences of AR *E. coli* among individual human fecal samples were estimated for 3 human populations in The Netherlands (Nijsten et al., 1994). The populations and number of fecal samples collected were as follows: farm workers (i.e., pig farmers, n = 290), abattoir workers (n = 316), and with suburban residents serving as a control group (n = 160). Information was also collected on recent antimicrobial use and hospital visits for the first 2 populations. Fecal samples were cultivated on selective media supplemented with and without antibiotics. Overall, differences were noticed among the three populations with pig farmers exhibiting the highest AR prevalence among enteric bacteria across all antimicrobial agents when compared to abattoir workers and suburban residents. Suburban residents had the lowest overall AR prevalence. There was no significant difference between: 1) the level of resistance among those abattoir workers with or without direct contact with pig fecal contents or pig carcasses, 2) abattoir workers with or without exposure to domestic animals, or 3) among the study populations that had a recent use of antibiotic or not, or that had a recent stay at the hospital or not. The authors suggested that contact with livestock or pig carcasses increased the risk of AR bacteria transmission to humans. By examining the control group we find that 1) the selection of study subjects was anonymous, and 2) no information on the exposure status of the suburban residents to pets and other livestock was provided. The lack of information on: 1) food consumption (e.g., pork), 2) exposure to different animal species, and 3) movement (i.e., in- and out-migration) of individuals in the 3 populations, could possibly bias the observed AR prevalence

differences in the study. Furthermore, since this was a cross-sectional study, a cause and effect relationship could not be definitively established.

To assess the risk of AR bacterial transmission to humans through direct exposure to animals, Nijsten et al., (1996b) compared the prevalence of AR *E. coli* isolated from pig farmers and their reared pigs. Individual fecal samples were collected from the farmers (total n = 290), and composite fecal samples were collected from either 3 breeding sows or finisher pigs per farm (total n = 291). The authors did not specify the sampling method for farmers; that is, whether it was random or convenient. The selection of 2 swine production groups (mature pigs or finisher pigs) does not fully represent the risk of farmer exposure to other swine groups (e.g., farrowing sows and piglets, nursery piglets, grower pigs) that could bias the association between exposure to pigs and the resistance levels in farmers. Ninety-two % (266/290) and 98% (285/291) of the fecal samples had *E. coli* growth on unsupplemented agar from farmers and pigs, respectively. The prevalence of both single and multiple AR *E. coli* isolates from pig feces was significantly ($p \leq 0.05$) higher than those from farmers. When *E. coli* resistance profiles were compared within the same farm, only 4% (10) of the isolates were similar. It was suggested by the authors that the AR *E. coli* isolated from farmers and their pigs were different. In other words, pigs were not considered as an important source of 'direct' transmission for AR *E. coli* to farmers. There was no statistical analysis conducted to examine the within and between farm variability in the study since the data were collected at the individual-level. Furthermore, the authors did not control for the farm-level effect. This study represents another open-population based research project.

Therefore, there was no control over the farmer population exposure to additional risk factors such as meat consumption, exposure to outside travel, pets, and other farm animals.

Further, to investigate the potential transmission of fecal AR *E. coli* isolated from pigs and their owners (Nijsten et al., 1996a), plasmid analysis of selected isolates as performed by *in-vitro* conjugation of donor and recipient strains. The 'selected' *E. coli* isolates (donors) were represented in 2 groups: 1) 10 pairs of isolates with similar resistance phenotypes obtained from farmers and their pigs on the same farm, and 2) 13 randomly chosen *E. coli* isolate pairs with different resistant phenotypes obtained from fecal samples of farmers and their pigs from another farm. The nalidixic acid-resistant *E. coli* K12 strain was used as a recipient bacterium. This strain was susceptible to all other antimicrobial agents used in the study. The frequencies of plasmid transfer were calculated as the proportion of transconjugants in the colony-forming unit (CFU) of the donor strains on a selective media treated with antibiotics. There were 6 isolates from farmers, and 6 from their pigs, each subjected to conjugation with susceptible control isolates obtained from pigs (other than the study population) and humans (i.e., community residents other than the pig farmers). The authors hypothesized that the first group would have more similar phenotypes among the *E. coli* (recipient isolates) than the second group. There was a higher overall transfer rate from pig isolates than from farmer isolates. There was no evidence of transfer of resistance plasmids between pig and farmer isolates. The authors concluded that there was no common transfer of resistance plasmids between fecal *E. coli* isolates from farmers and their pigs. In their

study, Nijsten et al. (1996a) assumed that resistance genes reside only on plasmids. It has been demonstrated that resistance genes can be located on other genetic elements, such as transposons and integrons (Hall and Collis, 1998). Therefore, plasmid analysis alone does not account for resistance genes in other cellular locations. This could bias the quantification of the AR actual phenotypes that transferred from the donor to the recipient cells.

The AR levels among commensal bacterial flora isolated from pig farmers (n = 113, exposed) and non-farmers (n = 113, control) were compared by Aubry-Damon et al. (2004). The study populations were selected via an agricultural health-insurance service in France. One farmer was randomly chosen per farm, and one non-farmer (control) was selected for each farmer and matched for sex, age, and county of residence. Data on antimicrobial use and hospital visits in the past 6 months were collected for both groups. Nasal, pharyngeal, and fecal samples were obtained from the study participants, cultured for commensal bacteria and tested for antimicrobial susceptibility using a disk diffusion method. Historical antimicrobial use was not significantly different ($p < 0.01$) between farmers and non-farmers. The prevalence of *Staphylococcus aureus* isolates from the nasal and pharyngeal samples was significantly ($p < 0.01$) higher in pig farmers than non-farmers. The prevalences of fecal *E. coli* isolates resistant to tetracycline, streptomycin, and nalidixic acid were significantly ($p < 0.01$) higher in farmers when compared to non-farmers. The authors suggested that direct exposure of farmers to pigs was associated with carriage of higher levels of AR commensal bacteria. Farmer exposures to AR bacteria and to antimicrobial agents were explained as follows: direct

contact with pigs and their feces (given that pigs were shedding AR bacteria), farmers' exposure to antimicrobial agents in the work environment (for example, antibiotic residues and dust), and farmers could have received more antimicrobial agents due to medical reasons (occupational hazard) as compared to non-farmers. This study has been classified as "cross-sectional" although the 2 groups were selected based on exposure, which is not typical for this type of study design, especially when matching was applied based on exposure. Typically, in cross-sectional studies individuals are selected and then their exposure and outcome status at a single point of time are assessed. The non-exposed individuals' selection in this study could have been improved if the authors had chosen subjects that lived in a similar environment as the farmers, yet were not exposed to the pigs or to the farmers themselves. The consumption of food products from animal origin, and consequently the risk of acquiring AR bacteria through food consumption in the 2 groups, could have been different; however, this was not assessed in the study. Matching of farmers and non-farmers by age, sex, and county of residence do not truly control for this difference. Furthermore, the exposure of both farmers and non-farmers to other farm animals and pets, travel, and food consumption were not assessed in the study.

Antimicrobial resistance phenotypes and genotypes for *Enterococcus faecalis* and *Enterococcus faecium* isolated from humans, broiler chicken and pigs were compared by Aarestrup et al. (2000). Stool samples (n = 254) were obtained from healthy humans with no recent history of antimicrobial use or hospital visits. These stool samples were collected as a component of diagnostic samples submitted for diarrheal pathogen

detection. The animal isolates (n = 2372) were obtained from the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) antimicrobial surveillance program in 1998. All *E. faecalis* and *E. faecium* isolates for the first 9 months of the surveillance program were assessed. One isolate per human sample and all isolates from broilers and pigs for both bacterial species were tested for antimicrobial susceptibility using Mueller-Hinton-II agar plates with 2-fold serial dilutions to different antimicrobial agents. Minimum inhibitory concentration (MIC) was defined as the lowest concentration with no visible growth on the agar plate. The presence or absence of genes conferring resistance to aminoglycosides, chloramphenicol, macrolides, and tetracycline among *E. faecalis* and *E. faecium* isolates from human, broilers, and pigs was determined using polymerase chain reaction (PCR). One VRE isolate was recovered out of 38 human samples tested using selective enrichment media treated with 20 μg/ml vancomycin. The prevalence of resistant *E. faecalis* isolates for chloramphenicol, kanamycin and streptomycin from humans (14%, 18%, and 20%, respectively) was higher than those isolated from broilers (2%, 2%, and 10%, respectively) and pigs (4%, 24%, and 39%, respectively). However, prevalence of *E. faecalis* isolates resistant to bacitracin, erythromycin and tetracycline from pigs (11%, 85%, and 68%, respectively) and broilers (60%, 44%, and 59%, respectively) was higher than those isolated from humans (17%, 22%, and 37%, respectively). The prevalence of *E. faecium* isolates resistant to kanamycin from pigs (18%) was higher than humans (12%), and broilers (1%), but higher for vancomycin, erythromycin, quinupristin/dalfopristin, virginiamycin, and tetracycline in broiler (10%, 74%, 79%,

75%, and 32%, respectively) and pig (17%, 81%, 60%, 85%, and 63%, respectively) isolates than in human isolates (0%, 20%, 11%, 45%, and 12%, respectively). Similar single resistance, and resistance to 2 antimicrobial agent, phenotypes were detected among the human, broiler and pig *E. faecalis* or *E. faecium* isolates. All chloramphenicol resistant isolates from the 3 populations harbored the chloramphenicol resistance gene: *cat-*$_{pIP501}$, while the tested tetracycline resistance genes were detected in some but not all tetracycline phenotypic resistance isolates. Similar genotypes were detected among human, broiler and pig isolates. Thus, the authors suggested that transmission of AR *Enterococci* bacteria and AR genes had taken place between humans, broilers, and pigs based on the similarity in phenotypes and genotypes among *E. faecalis* and *E. faecium* isolates. However, the similarity of the isolate phenotypes and genotypes from both human, and animals do not conclusively demonstrate AR bacterial transmission. In addition, similarity among phenotypes and genotypes could have happened simply by chance, since none of the reported unique phenotypes and genotype was followed over time. Clinical human isolates from the DANMAP program do not represent the 'true' status of AR commensal bacteria among 'healthy' humans and food animals and therefore results are susceptible to bias.

The hypothesis of whether the ingestion of meat contaminated with AR *Enterococci* can colonize the human intestine was tested in a randomized experimental trial by Sorensen et al. (2001). Eighteen healthy human individuals agreed to participate and received no antimicrobial agents for at least one month prior the start of the study. These persons were randomly divided into 3 treatment groups: Group 1: subjects

ingested a prepared mixture of 2 strains of glycopeptide-resistant *E. faecium* (10^7 CFU) isolated from retail chickens, and these strains also carried the vancomycin-resistance gene (*vanA*); Group 2: subjects ingested one streptogramin-resistant *E. faecium* strain (10^7 CFU) isolated from a pig carcass that also carried the *vatD* gene (another vancomycin resistance gene); Group 3: subjects ingested one strain of *E. faecium* susceptible to both glycopeptide and streptogramins (10^7 CFU) isolated from retail chicken. All strains were obtained from the Danish Veterinary Laboratory and the bacterial suspension was added to 250-ml of whole milk before being given to the subjects, to yield a final total dose of 10^7 bacteria. Those strains that were obtained from the laboratory were not tested for antimicrobial susceptibility before preparing the inoculum (e.g., culture of the strains on antibiotic-supplemented media) in order to reconfirm their resistance to the glycopeptide and streptogramins. Some of these strains could have dropped their resistance plasmids/genes due to the subculture process (Smith and Bidochka, 1998). This is a small sample size (6 subjects per treatment group) with which to detect a statistically significant difference when it exists (low power). Stool samples from the participants were collected 2 days before ingestion, daily for one week, and then again at 14 and 35 days post-ingestion. Samples were cultured on bile esculin azide (BE) agar plated treated with vancomycin, virginiamycin, erythromycin, or azteronam, based on the day of sampling. The authors did not plate all the stool samples on the same antibiotic supplemented agar, which makes it difficult to compare resistance levels to individual antimicrobial agents among the 3 groups at once, and over time. The obtained isolates were typed using pulsed field gel electrophoresis (PFGE) to determine

their genetic similarity. There were no glycopeptide-resistant or streptogramin-resistant *E. faecium* strains isolated from the subjects before the ingestion. The authors could have sampled the subjects multiple times before ingestion (in order to determine possible intermittent shedding) to affirm that subjects were actually free of resistant *E. faecium* strains. Group 1 subjects shed glycopeptide-resistant *E. faecium* during the 1st week, with peak concentrations on days 2 and 3 ($2x10^4$ to $1x10^8$ CFU/g) while none were detectable from subjects at days 14 and 35. Group 2 subjects had streptogramin-resistant *E. faecium* detected during the 1st week (peaks on days 2, 3, 4 and 5 with 10^4 CFU/g), from one subject at day 14, and none at day 35. One subject in group 2 shed glycopeptide-resistant *E. faecium* on days 5 and 6 ($8x10^3$ CFU/g), but this strain had a different PFGE pattern than the ingested strains. Group 3 subjects had no glycopeptide-resistant or streptogramin-resistant *E. faecium* isolated. PFGE showed that the *E. faecium* strains from groups 1 and 2 were similar to the strains that had been ingested (other than the single isolate from one subject in group 2, as noted above). The authors suggested that AR *Enterococci* isolates from retail chicken and pork can colonize, survive, and pass through the human intestines for up to 14 days after ingestion. Based on these findings, they recommended that antimicrobial agents be discontinued as growth promoters in animal feed.

In another study,in order to provide evidence of VRE bacteria transmission from animals to humans, fecal samples were collected from: turkeys (n = 81) and their farmers (n = 81), turkey slaughter-house workers (n = 100), and suburban residents (n = 200) in the Netherlands (Stobberingh et al., 1999). The last group was randomly selected from a

telephone directory of people living in the same province as the farms. The study participants were asked to complete a questionnaire concerning recent hospital visits, antimicrobial agents used by themselves and in their animals, and whether they owned pet animals or not. Samples were cultured on Kenner fecal (KF) *Streptococcus* agar (with and without vancomycin), then antimicrobial susceptibility to several agents was determined using the broth microdilution method in Iso-Sensitest Broth (CM473; Oxoid, UK). Vancomycin-resistant *Enterococci* strains were screened for the presence of *vanA*, *vanB*, and *vanC* genes using PCR. VRE strains were typed using PFGE to assess their genetic similarity. In addition, *vanA*-containing transposons in these isolates were characterized using restricted fragment length polymorphism (RFLP) analysis. DNA sequencing was performed on the PCR products. There were no differences between the isolates from farms that used avoparcin and those that did not. Avoparcin is a glycopeptide that has demonstrated cross-resistance with vancomycin in The Netherlands. There was a total of 283 *Enterococci* isolates in general, and 59 VRE isolates from all the samples tested. The VRE isolates were mainly *E. faecium* strains, followed by *E. faecalis*. The percentages of VRE isolates that harbored the *vanA* gene from turkeys, turkey farmers, turkey slaughter-house workers, and suburban residents were 35.6, 22.0, 16.9, and 23.7, respectively. The PFGE analysis demonstrated heterogeneity among the VRE isolates, although a *vanA* transposon was identical in 3 turkeys and those turkey farmers' VRE isolates arising from the same farm. Based on PFGE results demonstrating that turkeys and turkey farmers have different enterococcal strains, the authors have suggested that it is less likely for turkey isolates to colonize the

human gut for extended periods of time. However, they reported that VRE isolates were capable of being transmitted from turkeys to the turkey farmers because of the *vanA* transposon detection in strains isolated from both species from the same farm. The authors failed to state that animal isolates are not the only source of AR bacteria. Other sources (e.g., wild animals, pets, food, and other humans) that shed, or are contaminated with isolates harboring the *vanA* gene and/or the transposon can pass the gene/*Enterococci* strains to farm animals and humans.

Van den Bogaard et al. (2001) reported the prevalence and degree of resistance among fecal *E. coli* isolated from: 1) turkeys (n = 47), turkey farmers (n = 47), and turkey slaughter-house workers (n = 47), 2) broilers (n = 50), broiler farmers (n = 51), and broiler slaughter-house workers (n = 46), and 3) laying-hens (n = 25) and laying-hen farmers (n = 25). One-time individual fecal samples from human subjects and composite fecal samples from poultry were collected. Questionnaires were used to collect information from human subjects on antimicrobial use and hospital visits in the previous 3 months, as well as on contact with other animals. *Escherichia coli* bacteria were cultured on Levine agar (Becton Dickenson BV, Etten-Leur, The Netherlands) and tested for antimicrobial susceptibility by randomly choosing one colony and testing it using Iso-Sensitest broth microdilution. Ciprofloxacin-resistant *E. coli* isolates were genotyped using PFGE to assess their genetic similarity. None of the study subjects had received any antimicrobial agents, except for 4 broiler farmers and 4 broiler slaughter-house workers. Hospital visits were reported only for 2 broiler slaughter-house workers and one laying-hen farmer. The highest overall AR prevalence among poultry was for

turkey isolates, then broilers, and lowest for laying-hen isolates. This finding was explained by the relatively high levels of antimicrobial use in turkeys and broilers compared to relatively little use in laying-hens. Among human populations, turkey farmer isolates had the highest overall AR prevalence and the lowest prevalence was for laying-hen farmers. The resistance levels were similar among turkey and broiler slaughter-house workers. Similar AR phenotypes were observed among turkeys, turkey farmers, and turkey slaughter-house workers. The most common multi-resistant AR pattern was oxytetracycline- amoxicillin- streptomycin- sulphamethoxazole-trimethoprim. The authors did not specify whether the poultry sent to the slaughter house were coming from the participating farms or from any other farms (i.e., slaughter house workers could have been exposed to a variety of sources of poultry). The PFGE analysis showed heterogeneous patterns of ciprofloxacin-resistant *E. coli* isolates. There were 5 similar patterns found in *E. coli* isolates from turkey and turkey farmers from the same farm, and in one broiler and broiler farmers from different farms. The authors hypothesized that more similar patterns would have been detected if more than 1 ciprofloxacin-resistant *E. coli* colony per sample had been selected. They concluded that AR bacterial transmission occurred from animals to humans based on the isolates' clonal patterns. However, the authors did not address the source of AR bacteria nor did they determine their temporal relations. The authors did not control for the risk of AR bacteria due to consumption of food products of animal origin.

The prevalence of resistance of fecal *Enterococci* isolated from: 1) broilers (n = 51), broiler farmers (n = 51) and broiler slaughter-house workers (n = 46), 2) laying-hens (n

= 26) and laying-hen farmers (n = 26) in The Netherlands was further examined by Van den Bogaard et al. (2002). Individual fecal samples from humans and composite fecal samples from poultry were collected at a single point in time. Questionnaires were used to collect information regarding human antimicrobial use and hospital visits in the previous 3 months, as well as contact with other animals. This study was conducted approximately 6 months after the suspension of the use of avoparcin in Europe (an antimicrobial agent similar to vancomycin). *Enterococci* strains were isolated on KF-*Streptococcus* agar with, or without, vancomycin and were tested for antimicrobial susceptibility by randomly choosing one colony and then testing it using Iso-Sensitest broth microdilution. VRE that were recovered from the same farm (poultry and farmer) were later subjected to PFGE typing, *vanA* transposon analysis using restriction fragment length polymorphism (RFLP), DNA sequencing of the transposon gene PCR product, and by hybridization to test for the presence of *vanA*, *vanB*, and *vanC* genes. The *Enterococci* recovery percentages ranged from 89 – 96% per sample. Eighty-four VRE isolates were recovered with, and without, enrichment. Seventy-three VRE isolates were hybridized and showed the presence of *vanA* gene only. The majority of isolates that harbored the *vanA* gene were *E. faecium* (n = 41), and to a lesser extent *E. faecalis* (n = 5), *E. hirae* (n = 16), and *E. durans* (n = 9). The overall prevalence of AR was highest among *Enterococci* isolated from broiler fecal samples. The prevalence of resistance to vancomycin was significantly higher among broiler farmers' isolates than either laying-hen farmers or broiler slaughter-house workers. The authors did not collect information on food exposure (e.g., meat consumption) of the sampled populations.

Only 2 farms out of the 10 had *Enterococci* isolates in which VRE bacteria from broilers and farmers at the same farm were identical based on the PFGE analysis. Three out of eight *vanA* transposon types, detected in the VRE isolates from broilers and farmers, were identical. On the basis of this study, the authors suggested that farmer exposure to poultry was a risk factor for AR bacteria dissemination and human gut colonization, specifically *Enterococci* strains. The evidence of AR bacteria transmission from animals to humans claimed by the authors is clearly circumstantial (cross-sectional) and lacks a temporal component. Additionally, their study lacks the temporal tracking of resistance markers among and within both species.

2.2. Assessing antimicrobial resistance using phenotypes

Measuring the phenotypic resistance of commensal and pathogenic bacteria is the most common method of assessing AR in human and animal populations. Antimicrobial resistance phenotypes are the *in vitro* characteristics of bacterial isolate resistance against the action of one or more antimicrobial agents. These characteristics may be measured using several different methods, such as broth microdilution and disk diffusion (Wheat, 2001). The MIC values or the zone of inhibition are usually further categorized into resistant, intermediate, or susceptible based on pre-determined breakpoints. Resistance profiles for a bacterial isolate can range from pansusceptible through resistance to multiple antimicrobial agents. Several cross-sectional studies have focused on estimating the prevalence of single and multi-drug resistance of specific commensal or pathogenic bacteria in human and/or animal populations. Thereafter, several of those

studies also tried to assess the association between the AR phenotypic estimates and other risk factors.

As part of a surveillance program in Spain to monitor AR bacteria in livestock, 220 samples (one sample/pig) were collected from the colon of pigs (Teshager et al., 2000). Collection was performed at 4 different slaughter houses over a period of 3 months. *Escherichia coli* bacteria were isolated on Drigalski agar (each agar plate represented one sample) and 3-4 colonies were picked from each plate and then mixed before they were tested for antimicrobial susceptibility using a broth microdilution method. The MIC values were interpreted as breakpoints according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999) guidelines. The mixing approach of 3-4 colonies from an agar plate for antimicrobial susceptibility testing can be problematic if the number of different resistance phenotypes per sample is greater than 1. Hence, the phenotypic variability was not assessed in that study. Based on this antimicrobial susceptibility testing method, samples would be more likely to be positive (i.e., resistant: if at least 1 colony out of 4 was resistant, the sample would be classified positive) than to be negative (i.e., susceptible: all 4 colonies would need to be susceptible, to be classified as susceptible) introducing a differential misclassification bias. Two hundred and five *E. coli* strains were recovered (93% recovery rate). Groups of isolates were highly resistant to tetracycline (95.6%), sulfamethazine (87.8%), trimethoprim (83.4%), demonstrated low resistance to cephalothin (16.1%), neomycin (10.7%), gentamicin (4.4%), ciprofloxacin (8.3%), and cefoxitin (1.5%), and exhibited total susceptibility to ceftazidime and cefotaxime. This study failed to report the sampling method of the pigs

(e.g., random, systematic, or convenient) at the slaughter houses, and the relationship between sources of the pigs (i.e., farms), their antimicrobial use, and their levels of resistance.

In an earlier study, Dunlop et al. (1998a) estimated the levels and patterns of AR *E. coli* isolated from finisher pigs (i.e., those within one month of slaughter). Twenty composite fecal samples were collected from floors to represent different swine pens at each of 34 farrow-to-finish farms in Ontario, Canada. Farm samples (n = 68) were collected on 2 occasions over a 4-month period of the study. A total of 8119 *E. coli* were isolated on MacConkey agar and tested for antimicrobial susceptibility using the hydrophobic grid membrane filter (HGMF) method. Briefly, an *E. coli* suspension was passed through a grid membrane filter, the filter was placed on a selective media treated with a specific antimicrobial agent, and then colonies were counted using the HGMF MI-100 Interpreter® electronic counter. If the percentage of resistant colonies on the filter exceeded 50% of the total colonies that grew on the filter, then the tested *E. coli* isolate was classified as resistant. The authors did not perform a confirmatory test as to whether isolates obtained from MacConkey agar were *E. coli* or not. The overall prevalences of *E. coli* single-resistant to ampicillin, gentamicin, sulfisoxazole, and tetracycline in pig samples were 29%, 0.6%, 38%, and 71% respectively. Within-farm variation in the percentages of resistance was higher for all the antimicrobial agents tested as compared to between-farm variation. This was explained by the differences in antimicrobial usage at the individual level. However, it might have been expected that the variation in antimicrobial use is larger between farms compared to within farms. The

authors compared their prevalence results to other studies that used disk and broth microdilution susceptibility methods. Those methods are based on zones of inhibition or turbidity (i.e., growth), and not colony counts. In addition, in disk and broth microdilution methods, isolates are commonly classified as resistant or susceptible, based on NCCLS (1999) standards. The frequencies of resistance among *E. coli* isolates that were differentiated based on their resistance to other antimicrobial agents was assessed using an unadjusted relative risk (RR). A strong association or 'resistance traits' linkage' between gentamicin and sulfisoxazole (RR = 34) resistance levels was not further explained in the Dunlop et al. (1998a) study. This high correlation may be possible if, for example; co-selection between gentamicin and sulfisoxazole existed in the isolates.

The prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from beef cattle feedlots in the US were determined by Dargatz et al. (2003). Between 1999 and 2000, 10,417 fecal samples were collected from cattle in 422 pens in 73 feedlots in 12 states. Those samples were selectively cultured for *Salmonella* and at least 3 colonies/ positive sample were picked and further characterized using serotyping. These isolates were then tested for antimicrobial susceptibility using a custom panel of 17 antimicrobial drugs; thereafter, MIC values were determined and interpreted as susceptible, intermediate, or resistant according to NCCLS (1999) breakpoints. Of the 713 *Salmonella* isolates from 654 fecal samples, 35.9% were resistant to tetracycline, 11.1% to streptomycin, 10.4% to ampicillin, 10.4% to chloramphenicol. Less than 10% of the isolates were resistant to cefoxitin, ceftiofur, ceftriaxone, cephalothin, kanamycin,

nalidixic acid, sulphamethoxazole, and trimethoprim/sulphamethoxazole. None of the isolates were resistant to ciprofloxacin or gentamicin. The authors reported that resistant isolates were clustered by pen and by feedlot. The clustering was based on the similarity of the antibiograms (i.e., phenotypes) of *Salmonella* serotypes isolated from the same feedlots or pens. However, clustering of isolates based on the similarity of phenotypes without a performing a formal statistical test to assess clustering in the data, and claiming the isolates are clonal without any genotypic analysis, is a suboptimal analytical approach. In addition, there was no attempt to model or adjust for the hierarchical multi-level nesting and dependency in the data (i.e., cattle/pen/feedlot/state).

In a repeated (time-series) cross-sectional study, AR patterns of *E. coli* were compared from farm animals and environmental samples collected every 3 months over a 12-month period (Sayah et al., 2005). Thirty-one farms were sampled in Michigan and the antimicrobial use history for these farms was determined by questionnaires administered during the farm visits. Fecal samples were collected *per rectum* from livestock (dairy and beef cattle, swine, horses, sheep and goats) and poultry (cloacal swabs), while fecal droppings from deer and wild geese, environmental samples (water runoff from the farms, lagoons and manure piles), and sewage samples (human fecal matter from septic tanks) rounded out the collection. A total of 2,522 samples was collected from the 31 farms and *E. coli* were isolated using enrichment (Tryptic Soy Broth (TSB)) and culture methods (MacConkey agar) and then confirmed using biochemical tests (indole, methyl red, Voges-Proskauer, and Simmons citrate). Confirmed *E. coli* isolates (n = 1,286) were tested for antimicrobial susceptibility using a

disk diffusion method. The diameter of zone of inhibition (measured as a continuous outcome) was used as is, or else was classified into resistant or susceptible according to NCCLS (1999) breakpoints. Overall, the most frequent antimicrobial agent used on farms was penicillin (86%), followed by chlortetracycline (30%), sulfamethazine (16%), and oxytetracycline (14%). The highest AR in all samples was for tetracycline (27.3%), followed by cephalothin (22.7%), sulfisoxazole (13.3%) and streptomycin (13.1%). Interestingly, *E. coli* isolates resistant to cephalothin were present in all samples, while *E. coli* isolates resistant to tetracycline were present in all samples except in river water. The highest multi-drug resistant (i.e., up to 10 – 12 different antimicrobial agents) *E. coli* isolates were found in swine samples (fecal and farm environment) and the lowest were found in deer and wild geese (i.e., pansusceptible and single-resistant). In addition, swine exhibited the highest likelihood of shedding *E. coli* isolates resistant to tetracycline, sulfisoxazole, streptomycin, and ampicillin when compared to all other animals in this study. Resistance patterns were similar between *E. coli* isolated from animal fecal samples and the farm environment. Similar, but not identical, phenotypes were observed in swine and poultry isolates for tetracycline and streptomycin (high resistance levels) and sulfisoxazole, and cephalothin (low resistance levels). Fifty-three percent of *E. coli* isolates were pansusceptible, 34% were resistant to 1 or 2 antimicrobial agents, and 13% were resistant to 3 or more. Based on the similarity in resistance patterns among farm animals and the environment, the authors concluded that livestock served as an environmental reservoir for AR bacteria. However, in their study, common resistance phenotypes were examined by farm (isolates from farms with diverse

animal species) and by environment. The authors could have combined both farm and environmental AR phenotypic data and examined the data set for common resistance phenotypes to better assess common sources of resistance. They suggested that resistance phenotypes could be utilized to determine the source of AR bacteria, and that this is a preferable method to molecular approaches which are more costly and require more experience to perform. It might be possible to determine the source if we track very unique phenotypes over time; however, culture methods have several disadvantages such as: missing unculturable bacterial cells, measuring gene expression versus presence, and selection bias associated with testing a colony or even 5 out of a sample that contains literally millions of bacterial types, and the length of time some bacterial isolates take to grow. Molecular methods are a preferred method in many laboratories because of the rapid, reliable assessment of AR genes, and ability to track specific genes over time. In this repeated cross-sectional study, the sampled study subjects (animals and environment in this study) at a single time-point were not described in the text. When repeated inclusion occurs, time factors can be accounted for in the analysis (Dohoo et al., 2003). The seasonal (i.e., samples were collected every 3 months) effects were not assessed in the study by Sayah et al. (2005).

2.3. Assessing antimicrobial resistance using genotypes

Antimicrobial resistance genotype is defined by qualitatively or quantitatively measuring genetic marker(s) that code(s) for antimicrobial resistance on a bacterial plasmid, genome, transposon, and/or integron. Those genotypes are characterized using an array of molecular methods. The most common molecular techniques used in AR

research are gel-based PCR, real-time PCR, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), PFGE, DNA sequencing, and hybridization-based methods including microarrays (Woodford and Sundsfjord, 2005). Genetic methods are known to have advantages over the conventional phenotypic methods for assessing antimicrobial resistance; however, disadvantages for these methods also exist (Cockerill, 1999). Some of the advantages are: 1) genetic methods detect or quantify resistant gene(s) and not simply the expression of the gene (i.e., phenotypes) *in vitro*, 2) genetic methods can be applied directly to the sample (e.g., total community DNA) without the need to isolate the target organism, and 3) some genotypic analyses can be performed in a shorter time compared to than phenotypic analyses, especially when growing the organism takes a long time or else it cannot be cultured. Some of the disadvantages of molecular methods are: 1) the sensitivity of the molecular assays can be an issue, especially when the target gene is below the detection limit, 2) false positives (especially with cross-amplification of other gene(s), contamination of the template, or primer-dimer formation), and 3) detecting or quantifying resistance given that multiple genes can code for resistance to one antimicrobial agent, such as for tetracycline (Cockerill, 1999).

As one example, transposon (Tn) 1546, that is present in most of vancomycin resistant *E. faecium* (VREF) isolates, was characterized by the *vanX* gene using PCR to determine its base pair variation (Jensen, 1998). A total of 271 VREF isolates from human, pigs and poultry were analyzed for G and T type fragments of the *vanX* gene. All poultry isolates were G type and almost all pig isolates were T type. However, human isolates had both types. The authors suggested that G type *E. faecium* isolates

from humans arose from poultry and T type arose from pigs. Therefore, they concluded that horizontal transfer of VREF was from animals to humans, and was not likely to be animal-to-animal (i.e., poultry-to-pigs). However, poultry and pigs could simply be carriers of host-specific strains and not the only reservoir or source of VREF containing *vanX* gene isolates. Other sources (e.g., other animal species) can possibly carry this resistant organism and pass it to humans directly (e.g., through contact) or indirectly (e.g., through contaminated food and water). Therefore, the similarity in the gene types in animal and human isolates does not provide clear evidence of transmission of VREF *vanX* gene and evidence that poultry and pigs were the reservoir of the isolates.

The frequency of multiple tetracycline resistance genes in *E. coli* isolated from different animal and human populations was determined by Bryan et al. (2004). Fecal samples were obtained from humans, cats, cows, deer, turkeys, ducks, sheep, geese, dogs, pigs, horses, chicken, and goats. The samples were collected from a state fair, microbiology students at the University of Minnesota, a wildlife management area, and several poultry farms across the state of Minnesota. A total of 1263 *E. coli* isolates were obtained from 87 fecal samples. Isolates were grown on media supplemented with tetracycline at concentrations ranging from 5 – 233 μg/ml. The highest resistance (≥ 233 μg/ml) to tetracycline was found in *E. coli* strains isolated from pigs (61%), chickens (29%), and turkeys (29%). Isolates with MIC ≥ 93 μg/ml (classified as highly resistant, n = 325) were tested for 14 different tetracycline resistant genes using a multiplex PCR. Ninety-seven of the isolates tested had at least 1 of the 14 resistant genes and the most common gene detected was *tetB* (63%) followed by *tetA* (35%). More than half of the

tested pig and human *E. coli* isolates contained *tetB* gene. The external validity for this study is questionable. The subjects' sampling is not representative (i.e., selection bias) of the general population (both humans and animals). For example, the state fair animals do not represent the target population of farm animals. Furthermore, the state fair animals are exposed to different sources of resistant bacteria, such as humans that tend them and the different environmental conditions they are exposed to. Also, the antimicrobial use in these animals could be very different from farm animals. The authors did not compare the genes' frequencies with colonies cultivated on agar that were not supplemented with antibiotic. On the other hand, the authors used multiplex PCR to test for several genes in the isolates, which reduces selection bias. However, one of the major drawbacks of multiplex PCR is the complicated optimization and designing of multiple primers where the possibility of formation and identification of primer-dimers and non-specific products can become a problem (Rachlin et al., 2005).

The dissemination of tetracycline genes among *Enterococci* strains isolated, and total community DNA extracted, from 2 swine farm lagoons and downstream groundwater was examined by Chee-Sanford et al. (2001). The 2 swine farms were grower-finishers and farrow-nursery operations, respectively. Groundwater samples were collected from 20 wells downstream of the lagoons or the swine operations. Also, lagoon samples were collected from both swine farm sites. Tetracycline-resistant *Enterococci* strains were isolated from groundwater and lagoon samples on agar treated with 20 μg/ml tetracycline. Several tetracycline resistant genes (O, Q, W, M, B(p), S, otrA, and T) were amplified from the resistant isolates (genomic DNA) and from total

community DNA samples using PCR. Denaturing gradient gel electrophoresis (DGGE) was performed on the amplified PCR products to determine their genetic fingerprints. *Enteroccoci* isolates were recovered in high numbers ($4 \times 10^3 - 3 \times 10^4$ CFU/ml) from lagoons, and in low numbers (1.3×10^2) from groundwater. All of the tetracycline resistance genes were detected in the lagoon samples, but few in the groundwater. Identical *tet*(M) genes, based on the DGGE analysis, were found in lagoon and groundwater samples. The authors suggested that tetracycline resistance genes were mobile within the environment and could be disseminated to bacteria in human drinking water; consequently increasing the levels of AR in human populations. The authors did not identify the number of samples per lagoon, and thus we assume their interpretations are based on the very few results per sampling site. A larger sample size is needed to capture the variability within and between samples as it relates to the tetracycline resistance gene diversity. Resistant isolates carrying the gene *tet*(M) could have been present in the soil due to contamination from 1) wild animals (e.g., droppings) and hence reached the groundwater wells, or 2) contamination from sewage treatment plants that reached the wells (human source). Based on the study findings, different tetracycline resistance genes appear to exist in lagoons, and to a lesser extent in groundwater, but no concrete evidence was provided to support the hypothesis of tetracycline-resistant *Enterococci* disseminating from lagoons to groundwater.

Donaldson et al. (2006) isolated commensal *E. coli* from the feces of healthy dairy heifer calves and tested them for: 1) antimicrobial susceptibility to an array of antimicrobial agents including ceftiofur, 2) the presence of the bla_{CMY2} resistance gene,

and 3) genetic similarity using PFGE analysis. Fecal samples (n = 20) were collected from healthy calves (age 1 – 9 weeks) every 4 weeks for a 5-month period on one Holstein dairy farm in Pennsylvania. Newborn calves delivered between April and August 2003 were included in the study. Calves on this farm often received ceftiofur sodium (Naxcel®) for scours and respiratory infection treatment. Only those calves born in April 2003 were fed medicated milk replacer (supplenented with tetracycline and neomycin); thereafter, only non-medicated milk replacer was fed. Fecal samples were cultivated on MacConkey agar with and without ceftiofur supplementation (8 µg/ml) and *E. coli* were isolated. Susceptibility testing also was performed using a disk diffusion method and values interpreted using NCCLS guidelines. Eighty-eight % of the sampled calves (n = 96) harbored ceftiofur-resistant *E. coli* isolates and the mean of the ceftiofur resistant *E. coli* CFU counts was highest in June. Ceftiofur-resistant *E. coli* (n = 122) were genotyped using a PFGE method to assess DNA similarity of the isolates. Also, these 122 isolates were screened for the bla_{CMY2} resistant gene using PCR. There was no significant difference in the frequency of isolating ceftiofur resistant *E. coli* over time (i.e., months). The 122 ceftiofur resistant *E. coli* isolates showed 27 distinct patterns based on the PFGE analysis. However, 63.1% of the isolates belonged to one group based on cluster analysis. A total of 117 isolates (96%) contained the bla_{CMY2} resistance gene based on PCR. All of the 122 *E. coli* isolates were reported as multi-drug resistant to 3 or more antimicrobial agents. In addition, it was reported in their study that calves as young as 1-day old were found to be shedding multi-drug resistant *E. coli* isolates. The authors concluded that young healthy calves acquired resistant *E. coli* shortly after

birth, likely from such disparate sources such as: the maternity pen, calf-to-calf contact, exposure to the farm workers and, contaminated drinking water. In addition, commensal AR *E. coli* isolates from healthy calves apparently can disseminate into the dairy farm environment, as suggested by the authors. It would be better if they had identified and followed cohorts of newborn calves (grouped around the same age) to start with, from different farms, and reported the *E. coli* resistance levels (ceftiofur resistance and bla_{CMY2}) changes over time; both within as well as among calf cohorts. Furthermore, the authors could have sampled the calves' maternity pen (e.g., cow feces, pen environment, and workers) before and after delivery to identify potential sources of AR *E. coli* that might help to colonize the calves' intestines. The authors did not test the effect of actual treatment records of injectable ceftiofur on the level of ceftiofur resistant *E. coli* and the presence of the bla_{CMY2} gene.

Gentamicin-resistant *E. coli* isolates (n = 78) were obtained from laboratory submitted cases of bovine (n = 51) and porcine (n = 27) diarrhea in Denmark and screened for the presence of gentamicin resistance genes and class 1 integrons using PCR (Sandvang and Aarestrup, 2000). Assessment of the genetic similarity of the isolates was performed by PFGE analysis. The 78 isolates were selected from a strain collection at the Danish Veterinary Laboratory. Antimicrobial susceptibility testing of *E. coli* isolates to 15 antimicrobial agents, including gentamicin (40 µg (assumed to be per ml)), was performed by a tablet diffusion test using Neo-Sensitabs® on Mueller-Hinton II agar. All of the gentamicin-resistant *E. coli* isolates were susceptible to amikacin, 7.6% were resistant to apramycin, 33% to neomycin, 26% to netilmicin, 90%

to kanamycin, and 100% to tobramycin. None of the gentamicin-resistant isolates harbored the *acc(6)-Ib* gene, 76% of the gentamicin-resistant isolates harbored the *ant(2")-Ia* gene (98% of cattle gentamicin-resistant isolates and 37% from swine gentamicin-resistant isolates), 14% of these isolates harbored the *aac(3)-IIa* (mainly from swine gentamicin-resistant isolates), and 8% of these isolates harbored the *acc(3)-IV* gene (only in swine gentamicin-resistant isolates). The PFGE analysis showed 45 distinct patterns overall; 24 of the 51 bovine isolates had an identical pattern, and 2 of the 25 porcine had identical PFGE pattern. Twenty percent (n = 16) of the 78 isolates were positive for class 1 integron presence. The resulting PCR products were sequenced to determine the gene cassettes. The *ant(2")-Ia* gene cassette was found in low frequency (12% ,or 2 of the 16). It is important to note that gentamicin has been never approved for use in animal agriculture in Denmark, and that other related antimicrobial agents (e.g., tobramycin and kanamycin) are used in very small quantities in human medicine. Therefore, the authors have speculated that gentamicin resistant *E. coli* isolates may have entered the Danish farms with either imported pigs or else are due to co-selection with other antimicrobial agents. In addition, the authors concluded that integrons did not seem to be a frequent gentamicin resistance gene carrier. Clinical isolates obtained from the Danish Veterinary Laboratory are not representative of the 'true' status of AR commensal bacteria in 'healthy' livestock in Denmark. Therefore, selection bias may be an issue when making inferences about gentamicin resistance levels in the general healthy livestock population.

Three tetracycline resistance genes *tet*(O), *tet*(W), and *tet*(Q) were quantified in total community DNA extracted from feedlot lagoons using real-time PCR by Smith et al. (2004). Eighteen lagoon samples were collected over a 2-month period from 7 lagoons in the midwestern United States. Total community DNA was extracted from a 2-ml aliquot of each of the 500-ml samples using the QIAGEN DNA Stool Mini-Kit®. The leftover lagoon sample was used to quantify: 1) tetracycline in the lagoon sample using an ELISA technique, 2) total suspended solids, 3) volatile suspended solids (VSS), and finally 4) 1-ml of the sample was directly plated on several tetracycline treated (0 – 16 μg/ml concentration) agar plates. Quantitative real-time PCR (qPCR) was performed by building standard curves from plasmids that carried the genes of interest, using a TaqMan assay, and reporting gene copy numbers. Gene copy numbers were normalized (i.e., standardized) for the lagoon sample-to-sample variation using the VSS amounts (mg) in those samples. Samples were categorized into above or below the median tetracycline level (1.95 μg/liter). Without normalization, the total resistance genes, as well as the individual *tet*(O) and *tet*(W) genes, were significantly higher for the above-median tetracycline samples than the below-median ones. However, the authors did not present gene copy number comparisons between above and below the median tetracycline levels when normalized to VSS. Instead, the authors showed that the linear relationship between resistance gene numbers and the tetracycline levels in the lagoon sample was weaker when normalized to VSS ($r^2 = 0.15$) than without normalization ($r^2 = 0.5$). They mentioned that the goal of normalizing the data was to account for the difference in cattle numbers and distance from the lagoons at each feedlot. Therefore,

the weak relationship stated above was due to the small proportion of tetracycline resistant bacteria in the organic matter present in the lagoon samples. However, the goal of normalization (i.e., standardization) of samples, whether using the VSS method, total community DNA concentration, 16S rDNA or rpoB (an RNA polymerase beta-subunit) copy numbers, is to account for the differences in the background bacterial populations that contain the resistant bacteria and their resistance gene(s). The problem with the VSS method is that different lagoon samples may have different bacterial population sizes, but yet the same organic matter content, and thus the VSS quantity would be the same. The linear relationship between the total copy numbers of the resistance genes (log-transformed) for each lagoon, and the log of the percentage of colonies that grew on plates treated with 2 µg/ml tetracycline compared to 0 µg/ml, was significantly correlated ($r^2 = 0.22$, $p = 0.022$). The authors concluded that the qPCR method can replace the conventional phenotypic method of measuring resistance, with the advantage of quantifying resistance genes in all organisms rather than simply the cultivatable ones. In contrast, while the conventional phenotypic method can measure bacterial susceptibility to many antimicrobial agents at one time, it could take much more time and labor to test all known resistance genes for all antimicrobial agents using real-time PCR (i.e., qPCR). Therefore, while qPCR techniques have several useful applications (e.g., monitoring specific resistance gene levels in the environment) in assessing AR levels, they will never entirely replace the need for the phenotypic analysis, at least in the near future (Woodford and Sundsfjord, 2005).

Fecal commensal *E. coli* isolates (n = 181) obtained from healthy adults were characterized for the presence of integrons and their association with the isolate phenotypes by Shurnik et al. (2005). The adult participants did not receive any antibiotics for 1 month prior to sample collection. The study subjects represented 3 different groups: Wayampi Amerindians living in French Guyana, pig farmers, and bank or insurance workers; the latter 2 groups were from western France. There were 25 participants per group and 4-5 isolates obtained from each subject. Total genomic DNA was extracted from the pure *E. coli* isolates (1 isolate per distinct phenotype) and then tested for *intI1*, *intI2*, and *intI3* genes by real-time PCR using the SYBR® Green (Stratagene, Inc., La Jolla, CA, USA) assay. The PCR-products were sequenced to differentiate the gene cassettes on each of the integrons. There was a total of 27 (15%) integron positive isolates. From those, 20 isolates contained the class 1 integron, 6 isolates had class 2, 1 isolate had both classes, and none of the isolates had the class 3 integron. Isolates positive for integrons had at least one resistance gene. There was a significantly higher number of integron-positive *E. coli* isolates from pig farmers than from either Amerindians or bank-insurance workers. Two of the 18 class 1 integrons had the *dfr* gene cassettes, 8 integrons had *aadA*, and 8 integrons had both gene cassettes. Four of the class 2 integrons had *dfr-1*, *sat*, *aadA*, and *orfX* genes and 2 had *sat*, *aadA*, and *orfX*. The authors suggested that the higher prevalence of *E. coli* isolates harboring integrons in pig farmers, as compared to the other 2 groups, was due to the farmers' exposure to animals. The prevalence of *E. coli* integron positive isolates was likely higher in the bank workers than Amerindians because of the higher probability of

exposure to antimicrobial agents in bank workers compared to Amerindians. The higher prevalence of integrons in pig farmers could be a result of not only working with pigs, but also that they are more likely to be exposed to other animal species and antibiotics themselves (medicated feed) when compared to the other 2 groups. Furthermore, the exposure to antimicrobial agents could be different in the 3 populations (work and living standard-related). For example, the Amerindians group may have completely different exposures (at different risk of acquiring AR bacteria from animals and food as well as differences in their antimicrobial use) than the other 2 groups because of living in a developing region of French Guyana.

2.4. Risk factor studies as related to antimicrobial resistance in animal agriculture

Multiple studies have examined various antimicrobial use practices as they relate to levels of AR bacteria in food-producing animals at both the individual and the group-level. Collecting and presenting antimicrobial-use data in a ready-to-use format is difficult. It requires collecting a huge amount of information on antimicrobial product used (amount and route of administration), number of animals treated, duration of use, and changes related to season (amount and type of antimicrobial, as well as illness in the animals) (Shryock, 1999). However, antimicrobial use alone does not generally explain the spread and persistence of AR bacteria in animal populations where the antimicrobial use is discontinued or else is very limited. Other risk factors such as antimicrobial residues in the environment (e.g., soil, water, wastewater, and lagoons), wild animals that shed and contaminate animal feed and water with AR bacteria, and AR gene

dissemination in different human and animal populations also have an impact on the AR levels on farms and consequently on the food chain (Kummerer, 2003).

Mathew et al. (1999) have studied the relationship between multi-drug resistance patterns and the antimicrobial use, as well as pig age, on 10 swine farms in Tennessee. The farms were classified into low antimicrobial use (LOW, n = 3, if no subtherapeutic antibiotics or if tetracycline alone was used for short periods in subtherapeutic doses) and high antimicrobial use (HIGH, n = 7, routine use of subtherapeutic antibiotics in feed and/or injectable antibiotics). Rectal swabs from 5 sows per farm in addition to 5 rectal swabs from piglets per sow were collected. Additional samples were collected from those sampled piglets at 35 and 63 days of age. *Escherichia coli* were isolated on MacConkey agar, confirmed using biochemical analysis (API20), and then tested for antimicrobial susceptibility using the disk diffusion method for the following antimicrobial agents: apramycin, carbadox, gentamicin, neomycin, and oxytetracycline. The overall prevalence of multi-drug resistance was higher on HIGH farms than LOW farms across pig production sampling time points. Among LOW farms, the incidence of multi-drug resistance in pigs across the production (i.e., growth) stages did not differ. However, *E. coli* multi-drug resistance was significantly ($p < 0.001$) higher for pigs 35 days of age in HIGH farms compared to other pig production stages. When the multi-drug resistance profile included both neomycin and oxytetracycline, *E. coli* multi-drug resistance was highest at 63 days of age. The authors have suggested that age was a risk factor affecting resistance levels in swine, mainly in nursery pigs where subtherapeutic antibiotic use is high. The authors did not examine the effect of size of the farm (i.e.,

number of pigs) on the multi-drug resistance. Furthermore, the time variable was not controlled for in the analysis. They could have utilized generalized linear models (GLM) and adjusted for farm or time dependency using a generalized estimating equation (GEE) for the multinomial outcome (multi-drug resistance).

The relationship between prevalence of resistant *E. coli* and antimicrobial use at the group-level on 34 Canadian swine farms was assessed by Dunlop et al. (1998b). The 34 farrow-to-finish farms were classified into 4 groups: 1) farms with no antimicrobial supplementation in the post-weaning ration, 2) farms with antimicrobial agents supplemented to the weaning-pig ration, 3) farms with antimicrobial agents supplemented to the weaning-pig ration, and also supplemented with gram-positive antimicrobial agents in the grower-finisher ration, 4) same as (3) except that tetracycline was added to the grower-finisher ration instead of the gram-positive antimicrobial agents. Fecal samples (n = 20) were collected from finisher pigs 1 month before slaughter on each of the study farms. The *E. coli* isolation and susceptibility testing was described earlier in this chapter (see Dunlop et al., 1998a). The prevalence of resistance to each antimicrobial agent was regressed on the antimicrobial use data at both the group and individual pig level. Other risk factors in the study (e.g., farm size, farm management factor (e.g., number of sows, type of grower-finisher housing)) were controlled for by using logistic regression models. In general, in-feed medicated rations were significantly associated with an increase in the prevalence of *E. coli* resistant to each antimicrobial agent in this study, when compared to individual therapeutic antimicrobial agents also used in pigs. Tetracycline supplementation to the grower-

finisher pigs was associated significantly with an increased prevalence of tetracycline resistant *E. coli*. The prevalences of ampicillin, sulfisoxazole, and spectinomycin resistance to *E. coli* were significantly higher on farms with tetracycline supplementation in their grower-finisher pigs than on farms without this supplementation. The addition of gram positive antimicrobial agents (typically, either tylosin or salinomycin) to the grower-finishers rations was associated with an increased prevalence of spectinomycin-resistant *E. coli*. The individual treatment of piglets with gentamicin (this agent was not used in the feed ration) was associated with a slightly increased prevalence of gentamicin-resistant *E. coli* in finisher pigs. The prevalences of *E. coli* resistance to ampicillin, gentamicin, spectinomycin, sulfisoxazole, and tetracycline, in post-weaning pigs on the farms (n = 5) that had no antimicrobial use, were 7.1%, 1.0%, 16.9%, 24.0%, and 47.1%, respectively. The authors concluded, on the basis of this study, that medicated feed rations in swine farms have a larger effect on increasing prevalence of resistance *E. coli* when compared to individual antimicrobial treatment. In addition, they suggested that antimicrobial use on swine farms was associated with an increased level of *E. coli* resistance in finisher pigs. The authors could have sampled the finisher pigs in the slaughter-house lairage pens to assess the AR bacteria levels right before slaughter, and compared them to finisher pigs. They also could have sampled other swine production groups (e.g., piglets, nursery, and growers) to compare resistance levels to finishing pigs.

The prevalence of AR *Salmonella* and other AR Gram-negative fecal bacteria was compared between grower-finisher pigs that had medicated feed (subtherapeutic levels

of tetracycline) and those that had non-medicated feed (Funk et al., 2006). In their field trial, 3 swine farms were selected (convenience sampling). Selected barns (n = 22) within each farm containing pigs 10 – 24 weeks of age received treatment (subtherapeutic tetracycline), while pigs in other barns received no treatment. Individual fecal samples (n = 96) were collected *per rectum* from finisher pigs at each barn, cultured for *Salmonella* and other Gram-negative fecal bacteria, and tested for antimicrobial susceptibility. All *Salmonella* positive isolates 0.7% (n = 15, out of 2112 cultured fecal samples) were susceptible to amikacin, ciprofloxacin, and nalidixic acid. However, they were variably resistant to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. There were 76,521 fecal Gram-negative isolates obtained from 846 pigs in 22 barns. These isolates were tested for susceptibility to 4 antimicrobial agents: ampicillin, ceftiofur, gentamicin, and tetracycline. Resistance to tetracycline and ceftriaxone were reported in 97.5% and 0.7% of all the isolates from treated pigs, versus 84.3% and 0.3% from the non-treated, respectively. There was a significant association between the prevalence of fecal Gram-negative resistance to ampicillin (OR = 1.35), tetracycline (OR = 7.2), and ceftriaxone (OR = 2.36) and being one of the treated pigs, when compared to being untreated. However, the authors did not control for the actual individual pig treatments (e.g., ceftiofur) in the statistical models, which may further bias the ceftriaxone resistance levels.

Langlois et al. (1988) examined the relationship between AR fecal coliforms isolated from antibiotic-free pigs and 2 risk factors (age and housing type). The study population consisted of pigs in a single herd that had not been exposed to antibiotics for 126 months. The herd was composed of gestation gilts and sows (which were housed on pasture), as well as farrowing and finisher pigs that were housed in pens. Over a 20-month period, at 12 separate occasions, individual pig fecal samples were randomly collected *per rectum* by production stage in order to represent various age groups. Pig age groups were later classified for analytical purposes into: weaning (≤ 2 months), growers (2 – 6 month), developing (> 6 – 11 months), young adults (>11 – 24 months), and adult pigs (> 24 months). Fecal coliforms (n = 2,072) were isolated from the samples on MacConkey agar and 5 colonies per plate were tested for antimicrobial susceptibility to 13 antimicrobial agents using the disk diffusion method. The greatest percentages of fecal coliforms resistant to tetracycline (68%), sulfisoxazole (44.5%) and streptomycin (29.7%) were in weaning pigs, while ampicillin (13%) resistance was greatest in the growers. The fecal coliform resistance prevalences of tetracycline, sulfisoxazole, streptomycin, and kanamycin as well as multidrug resistance to 2 or more antimicrobial agents were significantly ($p < 0.05$) higher in weaning pigs compared to the other swine age groups. However, ampicillin resistance prevalence was significantly ($p < 0.05$) higher in the growers. In general, the authors indicated that resistance levels in fecal coliforms decreased as the pigs aged in an antibiotic-free herd. The prevalences of ampicillin and tetracycline fecal coliforms were significantly ($p < 0.05$) higher in finisher pens compared to farrowing pens and pasture, while sulfisoxazole resistance

was higher in farrowing pens compared to finishers and on-pasture pigs. The overall highest fecal coliform resistance was to tetracycline (63.5%). The authors concluded that there must be other risk factors that contributed to the increase and persistence of resistance levels in fecal coliforms in pigs beyond current antimicrobial use. In this study, the authors did not account for the effect of density (e.g., number of pigs per square meter in the pen) or the effect of mixing behavior (contact rate) among different age groups on the fecal coliform AR levels. Moreover, there was no information collected regarding the pigs that may have been introduced or imported into the herd. Imported pigs may carry and shed more AR bacteria and thus may be more likely to disseminate AR bacteria to other pigs in the herd. Furthermore, since sampling was performed over a 20-month period, the authors should have also examined the resistance level in relation to seasonal (time) changes.

The AR bacteria levels were assessed in 2 non-antibiotic exposed small rodent populations (2 different species) in England (Gilliver et al., 1999). The first population (n = 38) was close to woodlands, gardens and a lake, while the second population (n = 70) was nearby to pastures where heifers were usually kept. Fecal droppings were collected, microbiologically cultured, and bacterial isolates were tested for antimicrobial susceptibility. Overall, high resistance levels (90% of the isolates) to amoxicillin, amoxicillin/clavulanic acid, and cefuroxime were reported. Depending on the bacterial species, 14-76% of coliforms were resistance to tetracycline, 0-67% to trimethoprim, and sensitive to chloramphenicol. The authors suggested that the level of AR fecal coliforms in the wild rodents was a source of resistant bacteria that disseminated to

livestock through contaminated pasture, feed and water. Interestingly, the authors did not assess the exposure of wild rodents to contaminated food and water with AR bacteria to determine the source of resistance. Also, the mixing effect of susceptible and resistant (AR bacteria carrier) rodents was not evaluated. This was a one-time point sampling (cross-sectional) study where cause and effect could not be established. Thus, without studying the temporal variability in the fecal coliforms resistance levels, the study conclusions should be interpreted with caution.

The proportion of AR *Salmonella* isolated from dairy farms as related to risk factors (antibiotic use on conventional vs. organic farms, herd size, and the U.S. state in which farms were located) was assessed by Ray et al. (2006). Fecal (n = 24,762) and environmental (n = 5,056) samples were collected from organic (n = 26) and conventional (n = 69) dairy farms from 4 states, with herds containing at least 30 milking cows. Organic farms had very limited or no antimicrobial usage for at least 3 years prior to the sample collection. The collection period started in August 2000 and ended in October 2001. Conventional farms typically had both therapeutic and subtherapeutic antimicrobial use. The number of fecal samples (i.e., sample size) was proportional to the size of the herd and represented: 1) preweaned calves that were fed medicated and non-medicated milk replacers, 2) cows after calving, 3) cows to be culled, and 4) sick cows. Environmental samples were collected from different parts of the dairy farms (e.g., calving and sick pen floors, feed bunks, lagoons, bird droppings, bulk tank milk and milk pipe filters). Samples were cultured for *Salmonella* and these isolates (n = 1243) were tested for antimicrobial susceptibility to 14 antimicrobial agents

using a broth microdilution method. The MIC values were reported and interpreted according to NCCLS (1999). Isolates that showed resistance to 4 or more antimicrobial agents were classified as multi-drug resistant. The relationship between the proportion of AR *Salmonella* farms or herds (farms were classified as having resistance if at least 1 isolate was resistant to one antimicrobial agent) and the risk factors for each antimicrobial agent were assessed using logistic regression and proportional hazards (PH) models (farm-level analysis). In the PH model, if at least one isolate for each antimicrobial agent from each farm had an MIC value equal to the highest MIC value tested, then the farm was considered right censored. In general, there were greater proportions of resistant isolates to most of the individual antimicrobial agents, as well as multi-drug resistance, from the conventional farms when compared to the organic farms, except for amoxicillin/clavulanic acid, nalidixic acid, and trimethoprim/sulfamethoxazole. However, the last 2 antimicrobial agents had only a single resistant isolate in each farm type. There was significantly higher resistance only to streptomycin among conventional farms when compared to organic farms using the logistic regression model. Similarly significant associations were reported for streptomycin and sulfamethoxazole but this time based on the PH model. Herd size was significantly ($p < 0.05$) associated with an increase risk of resistance for both types of farm for: amoxicillin/clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, gentamicin, streptomycin, sulfamethoxazole, and tetracycline. The association between herd-level multi-drug resistance isolates and the various risk factors was not significant ($p > 0.05$). The authors suggested that, based on the lack of

significant differences in resistance proportions for almost all the antimicrobial agents tested between both type of farms (conventional and organic), that on-farm antibiotic use was not the only factor driving resistance levels in dairy farms. The authors did not examine the differences in the AR prevalence in the study cohorts within farms in their analysis; for example, to examine whether the pre-weaned calves AR *Salmonella* prevalence was different when compared to sick or culled cows. The authors could have performed the analysis differently by applying a multi-level hierarchal model (state, farm, herd, and cattle cohort or cattle environment sample) using, for instance, generalized linear latent and mixed models (GLLAMM) to adjust for the dependence in the data (data were collected at the individual cattle level, and thereafter aggregated as described above). Also, in this study the authors collected environment samples, but did not assess the effect of the isolated AR *Salmonella* on the resistance levels on the study dairy farms and how they were related to antimicrobial use on farm.

2.5 Summary of the literature review

Antimicrobial resistance studies have largely been aimed at examining the relationship between AR bacteria levels (or AR genes (proportion or quantity)) in humans and animals and a variety of potential risk factors. The risk factors were generally: antimicrobial use (therapeutic and subtherapeutic), farm size, housing, animal species, stage of production, and environment. Most of the studies based their findings on the commonality of resistance phenotypes, genotypes, or both, among specific commensal or pathogenic AR bacteria. None of the studies controlled for their study subjects' exposure to extraneous sources of AR bacteria (e.g., animal species other than

the study subjects, food from unknown sources, contaminated water) that could potentially bias the study results. Also, and to the best of our knowledge, there have been no studies that addressed the AR transmission risk among human and animal populations longitudinally and in a “controlled” semi-closed agri-food system.

CHAPTER III

MATERIALS AND METHODS

3.1. Study design

Our study agri-food system consisted of multi-site housing of vertically integrated populations of human workers and consumers, and swine. Humans were housed in 19 purposively chosen and separate geographical locations (units) across the state of Texas. Twelve of these units had swine operations.

3.1.1. Human population

The human population consisted of a total of approximately 39,000 male individuals spread over the 19 units. Based on occupational exposure (i.e., vocation) to swine and other factors, these individuals were classified as either swine workers, non-swine workers, swine slaughter-plant workers, or non-swine workers and non-consumers, based on the opportunity to consume the pork produced within the system. There were 12 units where both swine workers and non-workers resided, 6 units with only non-swine workers, and only 1 unit with a swine slaughter-plant facility (Fig. 1, A-D).

Approximately 86% of the human population consisted of non-swine workers. There also existed a non-consumer and non-worker cohort, which resided outside the agri-food system (at some of the units); this cohort was sampled in order to represent the general population as a 'negative control' group. There was moderate movement of 'new residents' into the system via a centralized unit, but restricted movement out of the system (Fig. 2).

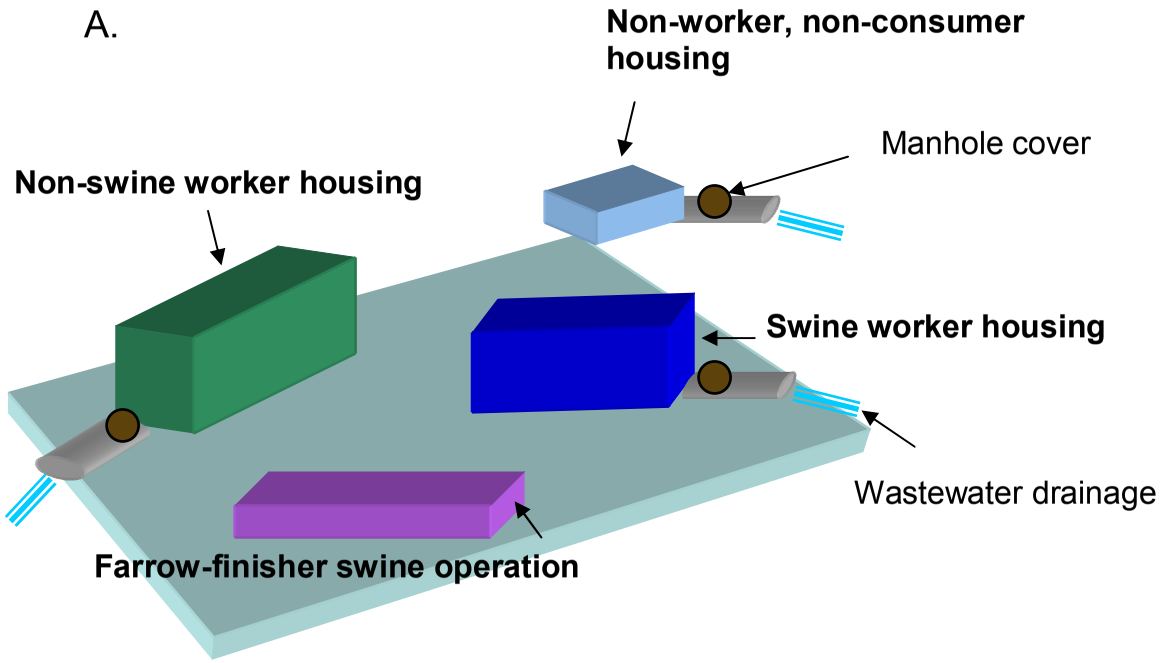

Fig. 1. Schematic presentation of the 4 general types of units in the agri-food system. A. Schematic of units (n = 5) that were composed of human population housing with farrow-to-finish swine operations.

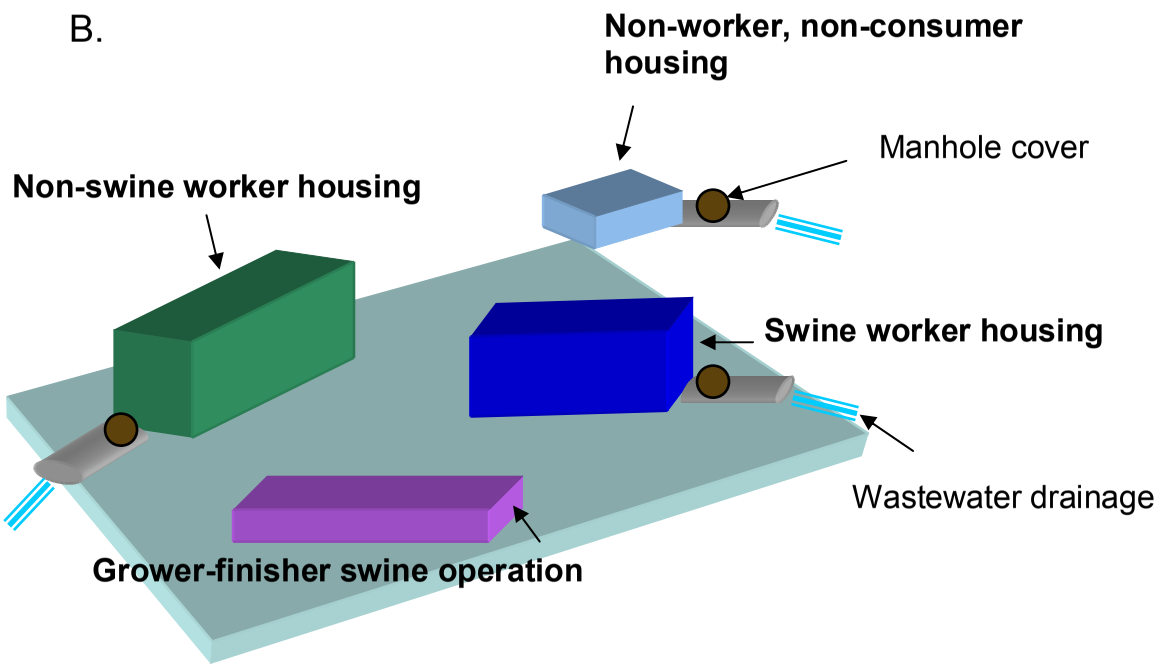

Fig. 1. (Continued). B. Schematic of units (n = 7) that were composed of human population housing with grower-to-finisher swine operations.

C.

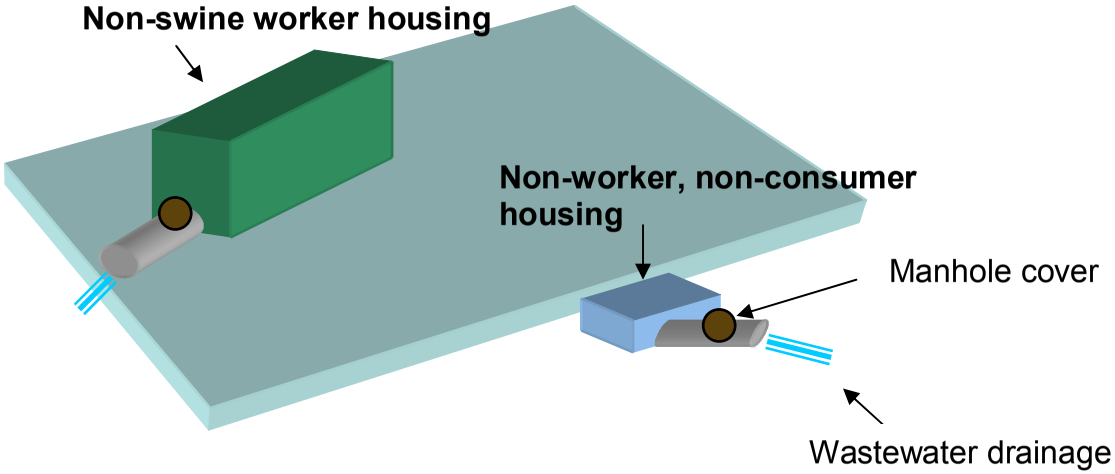

Fig. 1. (Continued). C. Schematic of units (n = 6) that were composed of human population housing without swine operations

D.

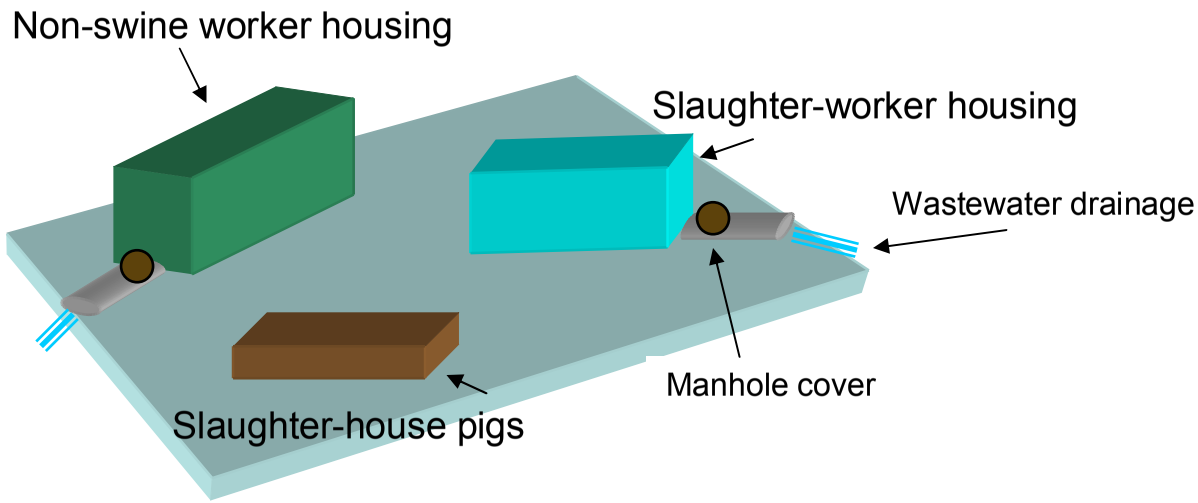

Fig. 1. (Continued). D. Schematic of a single unit (n = 1) that was composed of human population housing with a slaughter-plant facility.

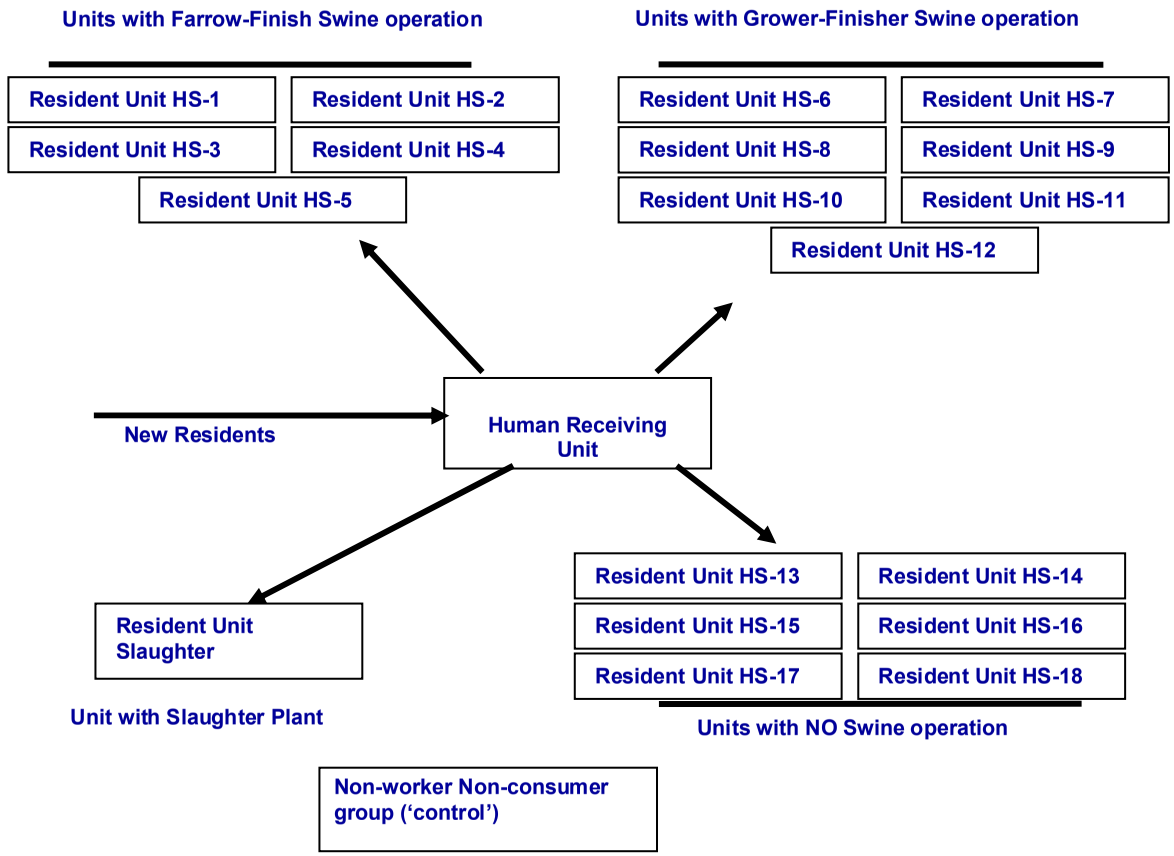

Fig. 2. Schematic diagram for the flow of the human population in the system. HU: are human units. The 'control': non-worker non-consumer group resides outside the system.

3.1.2. Swine population

The swine population consisted of approximately 26,000 – 28,000 pigs during any given month, located across 12 operations. These operations were comprised of 5 farrow-to-finish swine facilities, and 8 additional grower-to-finisher facilities. There were occasional (i.e., roughly every 4 months) movements of pigs into the system (purchased purebred and mixed breed boars) into a single quarantine swine operation where pigs were held for 4 weeks prior moving to other operations. In addition, there were outside gilts introduced into the system during the study period. However, there was little or no movement out of the system since all the pigs were slaughtered and consumed within the system, except for very minor numbers of slow-growing swine.

The swine population flowed vertically through farrow-to-finish units (farrowing barns to the hot nursery, to the cold nursery, to grower, and last to finisher barns) or to grower-to-finisher units, then sent to slaughter where pork products were processed and fed back (consumed) to the human population within the system (Fig. 3). When pigs arrived at the slaughter plant, they were held in holding pens overnight before they were slaughtered. For the purpose of data analysis, the swine population was categorized into 7 production groups: 1) farrowing crate pigs (included farrowing sows and their piglets), 2) nursery piglets (included both hot and cold nursery piglets), 3) breeding/gestation females (included breeding gilts, pregnant sows and gilts), 4) breeding boars, 5) isolation boars (boars at the quarantine facility), 6) grower-to-finisher pigs, and 7) slaughtered pigs (pigs at the holding pens).

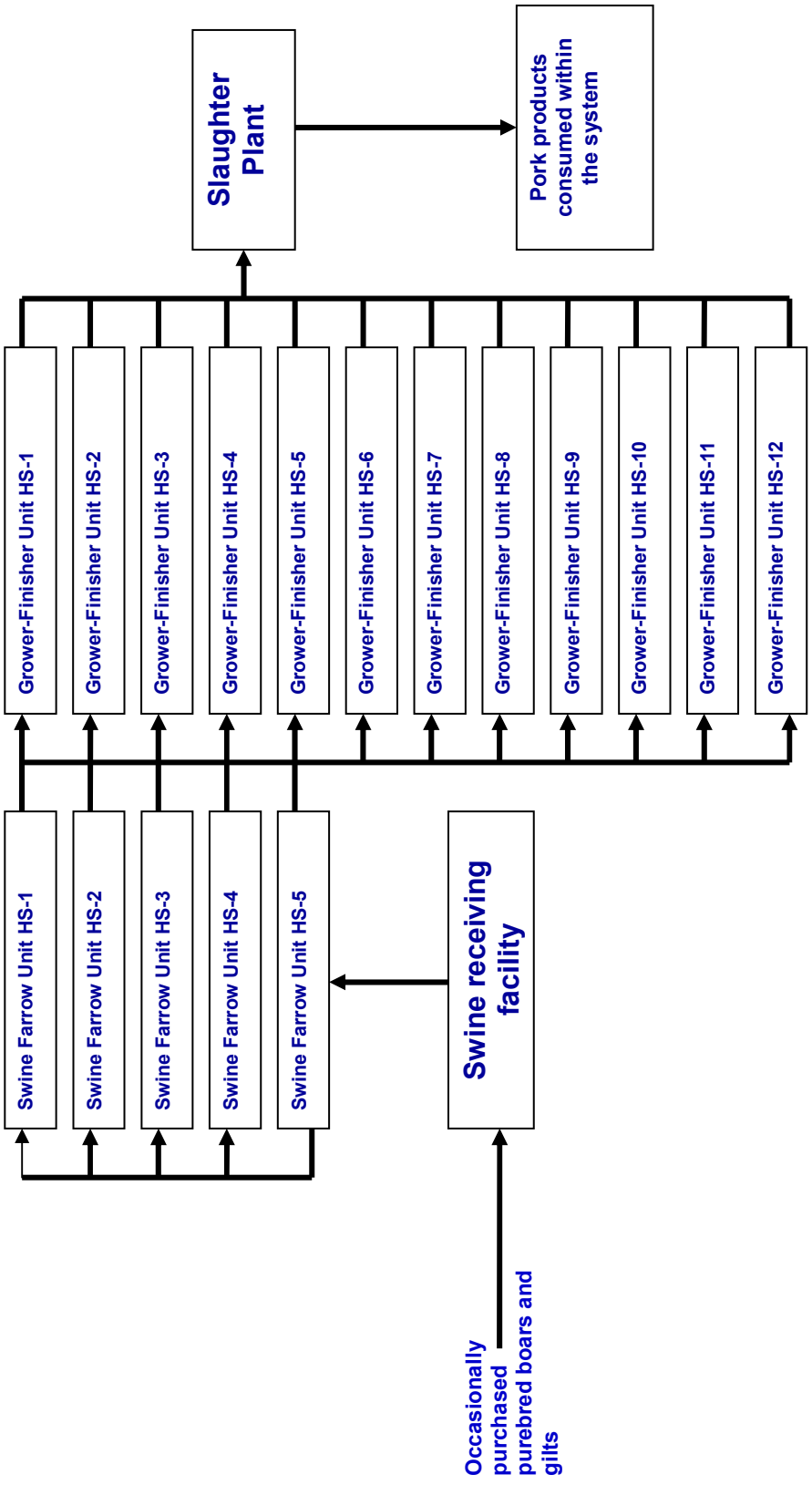

Fig. 3. Schematic diagram for the flow of the swine population in the system. SU: are swine units.

3.2. Sampling scheme

3.2.1. Human population

Multiple composite wastewater grab samples (approximately 50 ml each) were collected from all 19 human units. Those samples were collected monthly over a period of 36 months (February 2004 – January 2007) by trained personnel at each unit location. Typically, at each unit with a swine operation, 3 swine-worker wastewater (manhole) samples (consumers), 3 non-swine worker (both consumers, and non-consumers) wastewater (manhole) samples, and 1 mixed influent (draining from both of the groups) sample were collected. At those units without a swine operation, 4 wastewater samples (3 non-swine workers (both consumers, and non-consumers) from manholes and 1 mixed influent sample were collected. At the single unit with a slaughter-plant, 7 wastewater samples were collected, representing 3 non-worker manholes (consumers), 3 slaughter-plant worker manholes (consumers), and 1 mixed influent. The specific sampling locations were chosen purposively to differentiate the occupational human cohorts (swine-workers (consumers), non-swine workers (consumers), slaughter-plant workers (consumers), and the non-worker, non-consumer cohorts. Typically, the number of wastewater samples collected per month was 116. Sample pick-up and shipping from each unit was performed by a privately licensed and contracted agency. Samples were shipped to the USDA-ARS-Southern Plains Agricultural Research Center (USDA-ARS-SPARC) (College Station, Texas) for further analysis.

3.2.2. Swine population

Composite fresh fecal floor samples (approximately 50 g each) and barn-wash/pre-lagoon influent samples (approximately 50 ml) were collected from the 12 swine operation pens and the slaughter-plant holding pens by a swine specialist veterinarian. Those samples were likewise collected monthly for a period of 36 months (January 2004 – December 2006). Samples were kept at 4ºC overnight until they were shipped to the USDA-ARS-SPARC laboratory. To effectively sample all of the swine pens, a tri-monthly sampling scheme was carried out to sample different pen areas of the same swine facility each month.

A composite fecal sample (50 g) was composed of equal portions of fecal pats from multiple pens. Barn-wash/pre-lagoon samples were obtained from certain collection points that drained off the fecal sampled pens. The collected samples at each farm represented the different swine production groups described earlier. Furthermore, composite fecal samples from the slaughter-plant holding pens, kill floor influents, and pork trim samples from the slaughter-plant unit also were collected. Approximately one-third of the total pork consumed by the human population was from imported pork trim (personal communication with swine specialist veterinarian). Grab samples were collected from each of these imports (pork trim) and evaluated for AR *E. coli* isolates.

Typically, the total number (from all locations) of composite fecal samples per month was 140, and the number of barn-wash/pre-lagoon influent samples was 35. The number of monthly swine samples was variable over the study period due to changes in the number of pigs at different production stages in the operations over the study period.

3.3. Phenotypic analysis of antimicrobial resistance

3.3.1. Microbiological isolation of E. coli

Upon arrival at the laboratory, 5 aliquots of each of the human and swine samples were frozen at -72ºC both without (n=2), and with (n=3), glycerol at a ratio of 3:1 (sample: glycerol) for later analysis. At the time of microbiological analysis, frozen human wastewater samples were thawed completely, vortexed or mixed with a sterile loop, and then a 1-ml aliquot of wastewater sample was added to 9 ml of tryptic soy broth (TSB) (Becton, Dickinson and Company, Sparks, MD)) for enrichment. This mixture was then incubated for 18 hr at 37ºC, streaked onto a selective medium of CHROM agar-*E. coli*™ (DRG International, Mountainside, NJ) and incubated further at 37ºC for 18-24 hours. No enrichment step was used when swine fecal samples were cultured (i.e., directly streaked onto CHROM agar). For pork, approximately 5 g was dissected out of the pork trim sample, mixed for 1 minute in a stomacher with peptone water (10-ml), and then streaked with a sterile loop onto CHROM agar as described above.

A single typical *E. coli* colony (blue color with smooth surface) was randomly selected, streaked onto a blood agar plate, and then incubated (18 hr, 37ºC). Based on a pilot study for this research project (Scott et al., 2005), CHROM agar-E.coli™ selective media was noted to be highly specific and yielded >98% *E. coli* colonies. Furthermore, a biochemical test strip (API 20E, BioMerieux Inc.) was used regularly as quality control to confirm the isolates as *E. coli*. *Escherichia coli* isolates were transferred onto

glycerol coated beads (Key Scientific, Round rock, TX) and stored at -72ºC for future retrospective analyses.

3.3.2. Determination of antimicrobial susceptibility for E. coli *isolates*

A typical *E. coli* isolate was picked from the blood agar as described earlier, and the minimum inhibitory concentration (MIC) was determined for different antimicrobial agents by broth microdilution (CDC, 2003) using the Sensititre™ automated system according to the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH). The MIC values were interpreted as either resistant or susceptible (which included intermediate MIC classifications) based on NCCLS break points (1999). To assess the variable number of different resistant phenotypes in the samples, 5 *E. coli* colonies were selected randomly from human and swine samples during one month only (February, 2005). The mean number of different phenotypes for human and swine for that month were 2.25 and 1.98 (out of 5), respectively and the median was 2 for both human and swine. The antimicrobial agents that were used in the NARMS (2003) panels and their break points are provided in Table 1. Quality control (QC) organisms from the American Type Culture Collection (ATCC, Manassas, VA) strains *E. coli* 25922 and 35218, *Enterococcus faecalis* 51299, and *Pseudomonas aeruginosa* 27853 were evaluated on approximately every 200 NARMS custom panels, or with each new serial numbered batch, used in the laboratory to ensure panel laboratory quality.

3.3.3. Sample phenotypic analysis scheme

Samples were analyzed on a quarterly basis over the 3-year study period. All human (n = 1241) and swine (n = 1847) samples collected during the first 12 months of the

Table 1

Tabulated ranges of dilutions and breakpoints for determination of *E. coli* resistance to 15 antimicrobial agents using a broth microdilution method (Sensititre™, TREK Diagnostic Systems Inc., Cleveland, OH) on the NARMS gram negative bacteria panel.

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

[a] Sulfisoxazole was added to the new NARMS 2003 panel and sulfamethoxazole and cephalothin antimicrobial agents were dropped from its predecessor. Sulfisoxazole had the same cut points as sulfamethoxazole on the previous NARMS panel.

study were phenotypically analyzed. These data were later collapsed into 4 seasons (or quarters) based on: 1) winter: February – April, 2) spring: May – July, 3) summer: August – October, and 4) autumn: November – January). Thereafter, only quarterly phenotypic analysis was performed on the other 24 months of sampling (human, n = 812, and swine, n = 1303). Quarterly sampling and analysis was conducted because the highest variability was observed between seasons as compared to between months within season based on the first 12 months of data analysis.

3.4. Genotypic analysis

3.4.1. Total community DNA extraction

Total community DNA was extracted from diluted swine fecal matter, and from concentrated human wastewater samples, using the UltraClean™ Fecal and Soil DNA kits (Mo Bio®, Solana Beach, CA), respectively, according to the manufacturer's instructions. Glycerol-free samples were thawed completely at room temperature before extraction was performed. Extracted community DNA samples (~50 µl) were kept at -20ºC.

3.4.2. Antimicrobial resistance gene quantification using real-time PCR (qPCR)

Absolute quantification is based on comparison of an unknown sample to a standard dilution series with predetermined and known concentrations of a gene. The resulting amplification plots from the samples of unknown concentration are compared to the results from the standard curve and the number of the target gene copies is estimated from the curve (Wittwer and Kusukawa, 2004). In our study, the target gene was *b*-lactamase cephamycins resistance (bla_{CMY-2}) and the house-keeping (i.e., reference) gene

for standardizing the sample-to-sample variation in bacterial content was the RNA polymerase beta subunit (*rpoB*).

The target gene was quantified using the following 3 steps: 1) real-time PCR amplification to create PCR-products (i.e., standard template) in order to build the standard curve, 2) nested real-time PCR amplification to build a standard curve from the PCR-products, and 3) Ct value determination (the cycle number at which the fluorescence intensity reaches a set cycle threshold value) for the amplified bla_{CMY-2} gene in the unknown total community DNA samples. The sequence of the primers is shown in Table 2. Fig. 4 shows a schematic of the bla_{CMY-2}gene and the position of the primers used in this study.

3.4.2.1. Quantification standards (PCR product standard)

An *E. coli* isolate that harbors a plasmid-mediated AmpC B-lactamases bla_{CMY-2} gene (Odeh et al., 2002) was obtained from the University of Illinois, Chicago. This isolate was streaked on sheep blood agar and incubated for 18 hours at 37ºC. Total DNA was extracted from a randomly picked colony, then suspended in 500 ml of Sigma water (Sigma-Aldrich, St. Louis, Mo.), heated at 95ºC for 10 min to lyse the bacterial cell wall, centrifuged for 2 min to pellet the cell debris, and then the sample template was stored at -20ºC.

Table 2

Oligonucleotides used as primers for real-time PCR amplification of *bla*$_{CMY-2}$ and *rpoB* genes.

Gene name	Amplification step	Primer name	Primer sequence	Expected product size (bp)
bla$_{CMY-2}$	Initial amplification	585F	5'- CAGACGCGTCCTGCAACCATTAAA -3'	
		1038R	5'- TACGTAGCTGCCAAATCCACCAGT -3'	454
	Nested amplification	675F	5'- AGGGAAGCCCGTACACGTT -3'	
		738R	5'- GCTGGATTTCACGCCATAGG -3'	64
rpoB	Initial amplification	1519F	5' - TGC CTC AGG ATA TGA TCA ACG CCA - 3'	
		1991R	5' - GAA CAA GCT GGA TTC GCC TTT GCT - 3'	473
	Nested amplification	1789F	5' - ACG CAC AGA CTA ACG AAT ACG GCT - 3'	
		1852R	5' - ACA ACA CCG TCG GTC ACT TTA CGA - 3'	64

Primer pairs were designed using Primer Express® software (Applied Biosystems, Foster City, CA).

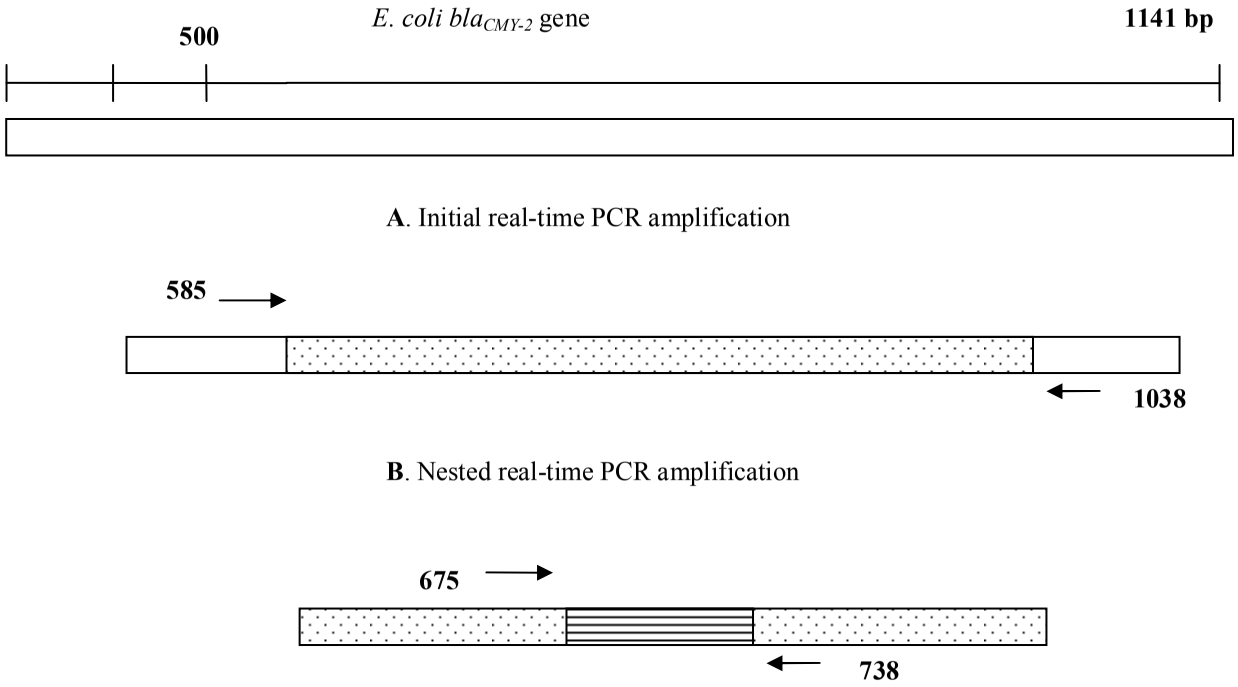

Fig. 4. An outline of the quantitative PCR (qPCR) assay. The target was the *bla*$_{CMY-2}$ gene with numbering corresponding to the *E. coli bla*$_{CMY-2}$ gene (Gene bank accession number AB212086). (A) Primers used for initial real-time PCR amplification of 454-bp PCR segment from the *E. coli bla*$_{CMY-2}$ gene. (B) Nested real-time PCR (qPCR) used to generate standard curves and amplify *bla*$_{CMY-2}$ gene in the unknown samples.

The amplification targeted the *bla*$_{CMY-2}$ gene using primers 585F and 1038R, producing 454-bp PCR products (i.e., amplicons). The PCR reaction was performed on a total volume of 50 µl using the Brilliant® SYBR® Green qPCR Master Mix (Stratagene, La Jolla, CA) in a real-time PCR system (Stratagene Mx3000P™, Stratagene, La Jolla, CA). Each reaction included 25 µl 2 x SYBR Green qPCR master mix, 17.75 µl of Sigma water (Sigma-Aldrich, St. Louis, MO), 0.75 µl of each primer (2.7 µM), and 0.75 µl of the reference dye (30 nM). The reaction conditions for amplification of DNA were 95ºC for 10 min, 35 cycles of 95ºC for 10 s, 55ºC for 45 s, and 84ºC for 20 s. To determine the specificity of amplification, analysis of the product melting curve was performed after the last cycle. A negative control was included in the run. The PCR products were then loaded into 1% agarose gel electrophoresis to re-confirm that only one product was synthesized per reaction. The PCR products were purified (i.e., extracted) from the gel using QIAquick® Gel Extraction Kit (Valencia, CA), quantified and checked for purity by a NanoDrop® ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE) at a wavelength of 260 and 280 nm. The concentration of DNA was calculated according to the formula ng DNA/µl = optical density at 260 nm × 50 × dilution factor and the number of PCR products was then calculated.

Ten-fold serial dilutions were performed on the PCR-products and 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 copies of the gene per reaction were used to construct the standard curve. The dilutions were performed in water first, then an aliquot from each dilution was

added to a wastewater, swine fecal matter total community DNA free of *bla*$_{CMY-2}$ to act as a background matrix.

3.4.2.2. Standard curve methodology

Amplification via nested PCR reactions was performed to generate a standard curve (gene copy numbers versus Ct values) using the prepared serial dilutions of the PCR-products as described above. The amplicon size was 64 bp using primers 675F and 738R (see Fig. 4). The PCR reaction was performed in triplicates on a total volume of 50 µl using the Brilliant® SYBR® Green qPCR Master Mix (Stratagene, La Jolla, CA).

Each reaction included 25 µl 2 × SYBR Green qPCR master mix, 17.75 µl of Sigma water (Sigma-Aldrich, St. Louis, Mo.), 0.75 µl of each primer (2.5 µM), and 0.75 µl of the reference dye (30 nM). The reaction conditions for amplification of DNA were 95ºC for 10 min, 40 cycles of 95ºC for 10 s, 52ºC for 30 s, and 79ºC for 17 s. To determine the specificity of amplification, analysis of the product melting curve was performed after the last cycle. Negative and positive controls were included in each real-time PCR run.

3.4.2.3. Gene quantification in total community DNA samples

The Ct value for the amplified *bla*$_{CMY-2}$ gene sequence (62 bp obtained using the primers 675F and 738R) in the unknown community DNA samples was determined as follows. The reaction mix and amplification conditions were the same as for the standard curve. The gene copy numbers in the community DNA samples were determined based on the comparison of the obtained Ct values to the standard curve. A standard curve was constructed with every real-time qPCR run aimed at quantifying the

target gene in the unknown community DNA samples. The *bla*$_{CMY-2}$ gene copy numbers per wastewater or fecal sample were then standardized using the reference gene (*rpoB*). The reference gene was also quantified using the same methodology as the target gene except for: 1) the primer sequences (see Table 2), 2) the concentration of each primer which was 2.8 µM and 2.3 µM for generating PCR-products and building the standard curve as well as determining the Ct in the community DNA samples, respectively, 3) the amplification reaction conditions which were: 95ºC for 10 min, 35 cycles of 95ºC for 15 s, 55ºC for 60 s, 72ºC for 15 s for generating the PCR-products, and 95ºC for 10 min, 40 cycles of 95ºC for 10 s, 53ºC for 30 s, 76ºC for 30 s for building the standard curve and determining the Ct value in the community DNA samples.

3.4.3. Preliminary genotypic analysis

Total community DNA extracted from human wastewater and swine fecal matter samples (n = 24) from the month of February 2004 were examined for *bla*$_{CMY-2}$ and *rpoB* gene quantities. These quantities were expressed in copies/µl and as a ratio (*rpoB*: *bla*$_{CMY-2}$) using the qPCR method as described earlier. The community DNA samples were selected to represent different human vocation cohorts and swine production groups in one sampling month. Samples were run in triplicates along with standard curves.

3.5. Statistical analysis

3.5.1. Descriptive statistics

The 15 antimicrobial resistance outcomes (binary) – as well as multi-drug resistance totals (multinomial) – were cross-tabulated with each of the risk factor categories: host

species (swine versus human), swine production type (e.g., breeding/gestation, breeding boars, farrowing, nursery, grower-finisher, isolation boar, slaughter holding pens), human vocation (swine worker versus non-worker), human consumer versus non-consumer, and season. Initially, the proportion of bacteria resistant to each of the antimicrobial agents was compared across levels of each risk factor using either two-sided 2 × 2 Fisher's exact test or 2 × n likelihood-ratio chi-square test, as appropriate, in STATA™ ver. 9.2 (College Station, TX). Multi-drug resistance was assessed for each risk factor similarly as the sum of resistance (out of 15 agents, upper (6+) categories were collapsed) across all isolates using an m × n likelihood-ratio chi-square test.

3.5.2. Multivariable analysis of risk factors for E. coli *resistance to individual antimicrobial agents*

The association between the AR *E. coli* isolate phenotypes and the risk factors in the study was assessed using a generalized linear model (GLM), with binomial error distribution, logit link function, and adjusted for dependency within unit location using generalized estimated equations (GEE) in STATA™ ver 9.2. GEE is a marginal (i.e., population-averaged) model that assumes that the relationship between the outcome (binary) and the predictors (risk factors) is the same for all subjects across clusters (Carriere and Bouyer, 2002; Dohoo et al., 2003; Hanley et al., 2003).

3.5.3. Multivariable analysis of risk factors for E. coli *resistance to multiple antimicrobial agents*

The ordinal response (multi-drug resistance from 0 to 15 antimicrobial agents; 6+ collapsed into a single upper category) of *E. coli* phenotypes in relation to the risk factors was assessed using a GLM model, with a multinomial distribution and a cumulative logit link function, and adjusted for dependency using GEE within unit location in SAS® ver 9.1 (PROC GENMOD, SAS Institute, Inc., Cary, NC).

3.5.4. Multivariate analysis (dependence among multiple binary outcomes)

3.5.4.1. Cluster analysis techniques approach

Our objective was to assess the application of cluster analysis techniques, applied to an AR phenotype data set, by identifying clusters with similar (i.e., related) AR phenotypes in order to better describe the resistance patterns among bacterial isolates. The further objective of the analysis was to examine *E. coli* membership in the obtained clusters (ordered from low-to-high resistance clusters) in relation to the study risk factors.

Antimicrobial resistance phenotypic data from the first season of longitudinal study were used to demonstrate and assess cluster analysis techniques. The data set we worked with included 504 *E. coli* isolates that were obtained from human wastewater samples and were subsequently tested for antimicrobial susceptibility. This data set contained outcomes for 15 antimicrobial agents on NARMS 2001 panels that included cephalothin and where amikacin was not considered in the analysis as the MIC values were not interpretable (Scott et al., 2005). That is because the amikacin breakpoint fell

several dilutions beyond the range provided on the NARMS panels making the binary classification (susceptible or resistant) uninterpretable at ≥ 4.

These data were in two forms: 1) ordinal data in the form of MIC values, and 2) binary scale (i.e., susceptible or resistant) which represented the dichotomized interpretation for 15 antimicrobial agents.

Squared-Euclidean distance for dissimilarity was used for both types of data. Although squared Euclidean distance has been suggested to be most useful for continuous data (Everitt et al., 2001), we employed this measure using hierarchical methods on both types of outcomes (ordinal and binary data). Cluster methods are typically descriptive, rather than analytical, and to use squared-Euclidean distance to classify the cases would be proper so long as it resulted in biologically meaningful clusters.

Six hierarchical agglomerative clustering methods were applied to the *E. coli* phenotypes in order to determine their cluster memberships. These methods were single linkage, complete linkage, average linkage, centroid linkage, median linkage, and Ward's minimum variance. Furthermore, the k-means method was applied to both types of data using the squared-Euclidean distance proximity measure. The MIC values were used in three ways: 1) their original form, 2) standardized with a mean of 1 and a standard deviation of 1, and 3) natural log transformed. The cluster analysis was performed using SAS® ver. 9.1 (SAS Institute, Cary, NC) and SPSS® (for Windows ver. 12.0.1, SPSS Inc., Chicago, IL).

Cluster analyses typically provide more than one cluster solution. Therefore, to obtain the best solution (aiming for parsimony), we attempted to minimize the within-cluster variability, maximize between-cluster variability, all the while keeping the number of clusters to a minimum. In addition, we paid particular attention to whether the clusters' memberships reflected the underlying biological relationship among resistance phenotypes (i.e., many of the antimicrobial agents are of the same class).

The final number of clusters in SAS® was determined using the squared multiple correlation (R^2), cubic clustering criterion (CCC) (Sarle, 1983), Pseudo-F (Calinski and Harabasz, 1974), and pseudo-T^2 (PST2) (Duda and Hart, 1973) statistics. R^2, which is the proportion of variance accounted for by the clusters, was evaluated and plotted in a dendogram. The CCC is calculated by looking at the difference between the observed and expected R^2. Values of CCC greater than 2 or 3 indicate good clusters (i.e., clusters are well separated); values between 0 and 2 indicate probable clusters and require further inspection, and large negative values may indicate outliers that should be removed before clustering is reattempted. Pseudo-F and pseudo-T^2 statistics indicate possible point(s) suggesting the appropriate number of clusters.

At each stage of clustering, minimum within cluster sum of squares (WCSS), maximum between cluster sum of squares (BCSS) and variance ratio criterion (VRC) [i.e., pseudo-F] are calculated as:

$$VRC = \frac{BSCC}{k-1} / \frac{WCSS}{n-k} \qquad (1)$$

k= number of clusters; n = number of cases.

Therefore, based on these calculations for different *k*, it is recommended to choose the value of *k* that has absolute or local maximum. In other words, choose the first value of pseudo-F from a monotonic decreasing series (Calinski and Harabasz, 1974). The ratio index (2) proposed by Duda and Hart (1973) can be transformed into the pseudo-T^2 statistic.

We then looked for the number of clusters that could be identified by a small value of pseudo-T^2 that was immediately followed by a large value.

$$\frac{J_e(2)}{J_e(1)} \qquad (2)$$

$J_e(2)$ is the within cluster sum of squares error when data are split into two clusters. $J_e(1)$ is the sum of square error before splitting the data. If $J_e(2) < J_e(1)$, then the one cluster hypothesis is rejected in favor of the two cluster solution.

3.5.4.2. Multivariate model of correlated dependence

The multiple binary AR outcomes (n = 15) for *E. coli* isolates were simultaneously assessed in relation to the risk factors using a GEE model fitted in a multivariate model using a SAS® macro to adjust for dependence among resistance phenotypes and dependence within unit location. This SAS® macro was adapted from Shelton et al. (2004) and modified to perform the analysis on our data. Briefly, the macro accounts for the correlations (i.e., dependence) among the binary outcomes in relation to each covariate (i.e., predictor). The macro creates an outcome vector matrix (NY × 1), design matrix (NY × CN) to allow separate covariate effects for each of the correlated AR phenotypes, and a vector matrix for the covariate effects (C × 1); where N = number of

observations, Y = number of outcomes, and C = number of covariates. These formed matrices are stored into a new dataset which is then used in SAS PROC GENMOD to fit the multivariate GEE model.

The antimicrobial agent odds-ratios (unadjusted versus adjusted for dependence among resistance phenotypes) were examined for: 1) host species (swine versus human (referent)), and 2) human swine workers, isolation boars, breeding boars, farrowing sows and piglets, breeding/gestation females, grower-finisher pigs, and nursery pigs, as compared to the human non-worker cohort (referent).

CHAPTER IV

RESULTS

4.1. Descriptive statistics

There were 5559 (2130 human and 3429 swine) commensal *E. coli* isolates that arose from the wastewater and fecal matter samples over the 3-year study period. Due to scheduling conflicts, not all units were sampled for wastewater collection every month. The total number of human wastewater and swine fecal samples collected, the number of isolates retained and tested for antimicrobial susceptibility by unit over the 3-year period, as well as the proportion of the isolates cultivated relative to the number of samples collected is shown in the tables on pages 76 and 78. The 15 antimicrobial resistance outcomes (binary: susceptible or resistant) for *E. coli* isolates were cross-tabulated first by host species and by unit as shown in the table on page 80. The proportion of resistant bacteria among human isolates across the 19 units differed significantly ($p < 0.05$) for the following antimicrobial agents: ampicillin ($p = 0.029$), chloramphenicol ($p = 0.023$), ciprofloxacin ($p = 0.008$), nalidixic acid ($p < 0.001$), streptomycin ($p = 0.021$), and tetracycline ($p < 0.001$). The proportion of resistant bacteria among swine isolates across only those 12 units with swine operations and the slaughter-house pigs differed significantly ($p < 0.05$) for the following antimicrobial agents: amoxicillin/clavulanic acid ($p < 0.001$), ampicillin ($p < 0.001$), cefoxitin ($p < 0.001$), chloramphenicol ($p < 0.001$), gentamicin ($p < 0.001$), kanamycin ($p < 0.001$), streptomycin ($p < 0.001$), sulfisoxazole ($p < 0.001$), and tetracycline ($p<0.001$). Next, the phenotypes were cross-tabulated by human vocation cohort (i.e., non-workers and

non-consumers, swine workers, non-swine workers, influent mixture, slaughter-plant workers) as shown in the table on page 84. Only tetracycline resistance differed significantly ($p = 0.025$) among human exposure (i.e., vocation) cohort isolates with the highest prevalence found in slaughter-plant workers (25.9%), versus swine-workers (23.1%), non-swine-workers (17.6%), non-workers and non-consumers (14.3%), and influent mixture (22.8%). The same approach was used to cross-tabulate by swine production group (i.e., slaughter-plant holding pens (slaughtered pigs), breeding boars, isolation/quarantine boars, breeding sows, farrowing sows and piglets, growers and finishers, and nursery piglets) as shown in the table on page 86. In general, the isolation (i.e., purchased) boars showed higher levels of resistance ($p < 0.001$) than swine-rearing and slaughtered pigs for the following antimicrobial agents: ampicillin, chloramphenicol, kanamycin, sulfisoxazole, and tetracycline. In contrast, nursery piglets showed higher level of resistance ($p < 0.001$) than isolation boars, other swine-rearing and slaughtered pigs for the following antimicrobial agents: cefoxitin, ceftiofur, gentamicin, and streptomycin. There were 12 *E. coli* bacteria isolated from the 160 (7.5%) pork trim samples, 11 of which were resistance to at least 1 antimicrobial agent. Among these, the frequency of multi-resistance phenotypes were as follows: pansusceptible (n = 1), single-resistant (n = 4), and resistant to 3 antimicrobial agents (n = 7), with the most common phenotypes being: tetracycline and ampicillin-streptomycin- tetracycline. Results were also cross-tabulated by consecutive seasons (winter, spring, summer, and autumn) throughout the 3-year period as shown in the table on page 87 and season-collapsed (winter, spring, summer, and autumn) over the 3-year period as shown in

Table 3

Total number of human wastewater samples (planned, received, received-labeled properly, cultured, samples grew on chrome agar plate, at least one isolate retained and tested for antimicrobial susceptibility per plate, and total isolates retained and tested for antimicrobial susceptibility) and their proportion by unit.

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
Total	Planned samples	144	144	144	252	252	252	252	252	144	252
	Received samples	128	126	140	224	244	249	208	237	139	206
	Received-labeled samples	96	104	140	224	237	247	176	222	109	185
	Cultured samples	76	79	78	124	131	138	112	128	75	112
	Samples grew on chrome agar plate	58	54	58	102	108	115	104	105	65	92
	At least one isolate retained and tested for antimicrobial susceptibility per plate	57	54	58	99	108	115	104	102	65	92
	Total isolates retained and tested for antimicrobial susceptibility	74	75	64	108	134	144	134	146	85	122
Overall proportions	Received/ planned samples	88.89	87.50	97.22	88.89	96.83	98.81	82.54	94.05	96.53	81.75
	Received-labeled samples/proposed samples	66.67	72.22	97.22	88.89	94.05	98.02	69.84	88.10	75.69	73.41
	Cultured samples/received-labeled samples	79.17	75.96	55.71	55.36	55.27	55.87	63.64	57.66	68.81	60.54
	Samples grew on chrome agar/cultured samples	76.32	68.35	74.36	82.26	82.44	83.33	92.86	82.03	86.67	82.14
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	75.00	68.35	74.36	79.84	82.44	83.33	92.86	79.69	86.67	82.14

Table 3 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
Total	Planned samples	144	252	144	252	252	180	252	252	252	4068
	Received samples	143	238	148	220	181	185	237	251	231	3735
	Received-labeled samples	134	220	148	214	172	165	215	250	228	3486
	Cultured samples	89	127	80	114	96	104	124	138	128	2053
	Samples grew on chrome agar plate	78	100	70	99	99	88	105	123	103	1726
	At least one isolate retained and tested for antimicrobial susceptibility per plate	78	100	69	98	99	89	104	123	100	1714
	Total isolates retained and tested for antimicrobial susceptibility	82	128	77	120	126	118	145	136	112	2130
Overall proportions	Received/ planned samples	99.31	94.44	102.78	87.30	71.83	102.78	94.05	99.60	91.67	91.81
	Received-labeled samples/proposed samples	93.06	87.30	102.78	84.92	68.25	91.67	85.32	99.21	90.48	85.69
	Cultured samples/received-labeled samples	66.42	57.73	54.05	53.27	55.81	63.03	57.67	55.20	56.14	58.89
	Samples grew on chrome agar/cultured samples	87.64	78.74	87.50	86.84	103.13	84.62	84.68	89.13	80.47	84.07
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	87.64	78.74	86.25	85.96	103.13	85.58	83.87	89.13	78.13	83.49

Unit Legend: H-Intake = human isolates from a single intake facility, HU (1, 4, 5, 6, 7, 8, 10, 11, 15, 17, 18) = human and swine isolates from 11 separate units housing both host species, HU (3, 9, 12, 13, 16) = human isolates from 5 separate units housing no swine operations, H-Slaugh = human isolates from a single facility with a swine slaughter-plant, HU-19/S-Isolation = human isolates from 1 unit housing and swine isolates from a single isolation/quarantine unit. This unit is the sole facility that receives purebred replacement stock (boars) supplying the other swine facilities.

Table 4

Total number of swine fecal samples (received, cultured, grew on chrome agar plate, at least one isolate retained and tested for antimicrobial susceptibility per plate, and total isolates retained and tested for antimicrobial susceptibility) and their proportion by unit.

		Swine fecal matter samples						
	Number of :	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
Total	Received samples	678	207	620	217	845	646	693
	Cultured samples	372	116	343	127	432	353	369
	Samples grew on chrome agar plate	372	116	343	127	429	353	367
	At least one isolate retained and tested for antimicrobial susceptibility per plate	370	114	343	127	427	353	366
	Total isolates retained and tested for antimicrobial susceptibility	431	135	391	154	489	392	418
Overall proportions	Cultured samples/received samples	54.87	56.04	55.32	58.53	51.12	54.64	53.25
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	99.31	100.00	99.46
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	99.46	98.28	100.00	100.00	98.84	100.00	99.19

Table 4 (Continued)

		Swine fecal matter samples						
	Number of :	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Intake)	Total
Total	Received samples	227	458	210	191	226	477	5695
	Cultured samples	129	261	119	110	124	295	3150
	Samples grew on chrome agar plate	128	179	119	110	124	288	3055
	At least one isolate retained and tested for antimicrobial susceptibility per plate	127	110	119	110	124	362	3052
	Total isolates retained and tested for antimicrobial susceptibility	149	115	138	120	147	406	3485[a]
Overall proportions	Cultured samples/received samples	56.83	56.99	56.67	57.59	54.87	61.84	55.31
	Samples grew on chrome agar/cultured samples	99.22	68.58	100.00	100.00	100.00	97.63	96.98
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	98.45	42.15	100.00	100.00	100.00	122.71	96.89

[a] Kill floor influents and pork trim AR outcomes were excluded from the analysis because of the small sample size and the low number of positive isolates.

Table 5

Phenotypic resistance to each of 15 antimicrobial agents for commensal enteric *E. coli* isolates from human and swine fecal-matter samples taken at 19 different housing units in a multi-site integrated farrow-to-plate agri-food system. Frequency and percentage of resistant *E. coli* isolates are presented cross-tabulated by host species and unit. Isolate comparisons are across all human vocation cohorts, swine production groups, and seasons.

			Sampled by unit[c] (n=5559 overall sample isolates)										
			Frequency of resistant *E. coli* (%)										
Antimicrobial agent	Host Species (S=Swine, H=Human)	Odds Ratio[a] (p-value)[b]	HU-1 n=505	H-intake n=75	HU-3 n=64	HU-4 n=243	HU-5 n=524	HU-6 n=296	HU-7 n=616	HU-8 n=538	HU-9 n=85	HU-10 n=539	*p*-value[d]
Amikacin	S (n=3429)	-	0 (0.0)	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	4 (1.0)	0.154
	H (n=2130)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Amoxicillin /Clavulanic Acid	S (n=3429)	0.80	27 (6.3)	-	-	1 (0.7)	4 (1.0)	2 (1.3)	21 (4.36)	4 (1.0)	-	4 (1.0)	<0.001
	H (n=2130)	(0.265)	1 (1.4)	4 (5.3)	3 (4.7)	5 (4.6)	7 (5.2)	2 (1.4)	2 (1.5)	2 (1.4)	2 (2.4)	3 (2.5)	0.35
Ampicillin	S (n=3429)	1.13	87 (20.2)	-	-	7 (5.2)	101 (25.9)	14 (9.2)	128 (26.6)	51 (13.0)	-	80 (19.2)	<0.001
	H (n=2130)	(0.119)	10 (13.5)	15 (20.0)	15 (23.4)	22 (20.4)	29 (21.6)	24 (16.7)	20 (14.9)	24 (16.4)	7 (8.2)	15 (12.3)	0.029
Cefoxitin	S (n=3429)	0.76	25 (5.8)	-	-	2 (1.5)	3 (0.8)	2 (1.3)	15 (3.1)	6 (1.5)	-	3 (0.7)	<0.001
	H (n=2130)	(0.187)	2 (2.7)	4 (5.3)	2 (3.1)	7 (6.5)	8 (6.0)	2 (1.4)	3 (2.2)	2 (1.4)	2 (2.4)	2 (1.6)	0.081
Ceftiofur	S (n=3429)	6.68	28 (6.5)	-	-	0 (0.0)	9 (2.3)	4 (2.6)	19 (3.9)	2 (0.5)	-	2 (0.5)	<0.001
	H (n=2130)	(<0.001)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)	1 (0.7)	0 (0.0)	2 (1.4)	1 (1.2)	0 (0.0)	0.482
Ceftriaxone	S (n=3429)	-	2 (0.5)	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	0 (0.0)	0.761
	H (n=2130)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Chloramphenicol	S (n=3429)	2.02	31 (7.2)	-	-	0 (0.0)	6 (1.5)	6 (4.0)	30 (6.2)	8 (2.0)	-	12 (2.9)	<0.001
	H (n=2130)	(<0.001)	1 (1.4)	0 (0.0)	1 (1.6)	1 (0.9)	2 (1.5)	3 (2.1)	6 (4.5)	0 (0.0)	3 (3.5)	0 (0.0)	0.023
Ciprofloxacin	S (n=3429)	0.07	0 (0.0)	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	-	0 (0.0)	0.976
	H (n=2130)	(0.003)	0 (0.0)	3 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	0 (0.0)	0.008
Gentamicin	S (n=3429)	4.19	6 (1.4)	-	-	1 (0.7)	9 (2.3)	0 (0.0)	0 (0.0)	1 (0.3)	-	37 (8.9)	<0.001
	H (n=2130)	(0.002)	1 (1.4)	3 (4.0)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)	0.066

Table 5 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Odds Ratio[a] (p-value)[b]	Sampled by unit[c] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) HU-1 n=505	H-intake n=75	HU-3 n=64	HU-4 n=243	HU-5 n=524	HU-6 n=296	HU-7 n=616	HU-8 n=538	HU-9 n=85	HU-10 n=539	*p*-value[d]
Kanamycin	S (n=3429)	10.01	22 (5.1)	-	-	3 (2.2)	46 (11.8)	11 (7.2)	45 (9.3)	36 (9.2)	-	80 (19.2)	<0.001
	H (n=2130)	(<0.001)	1 (1.4)	1 (1.3)	2 (3.1)	1 (0.9)	1 (0.8)	0 (0.0)	1 (0.8)	1 (0.7)	3 (3.5)	0 (0.0)	0.092
Nalidixic Acid	S (n=3429)	0.29	0 (0.0)	-	-	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.3)	-	2 (0.5)	0.555
	H (n=2130)	(<0.001)	5 (6.8)	10 (13.3)	2 (3.1)	0 (0.0)	1 (0.8)	1 (0.7)	1 (0.8)	3 (2.0)	7 (8.2)	1 (0.8)	<0.001
Streptomycin	S (n=3429)	3.46	115 (26.7)	-	-	30 (22.2)	132 (33.9)	22 (14.5)	150 (31.1)	106 (27.0)	-	121 (29.0)	<0.001
	H (n=2130)	(<0.001)	5 (6.8)	4 (5.3)	6 (9.4)	9 (8.3)	17 (12.7)	8 (5.6)	13 (9.7)	10 (6.9)	6 (7.1)	10 (8.2)	0.021
Sulfisoxazole	S (n=3429)	1.99	104 (24.1)	-	-	24 (17.8)	106 (27.2)	39 (22.7)	130 (27.0)	95 (24.2)	-	93 (22.3)	<0.001
	H (n=2130)	(<0.001)	11 (14.9)	9 (12.0)	12 (18.8)	13 (12.0)	22 (16.4)	18 (12.5)	20 (14.9)	27 (18.5)	17 (20.0)	12 (9.8)	0.17
Tetracycline	S (n=3429)	19.58	383 (88.9)	-	-	104 (77.0)	325 (83.3)	142 (93.4)	459 (95.2)	319 (81.4)	-	350 (83.9)	<0.001
	H (n=2130)	(<0.001)	12 (16.2)	11 (14.7)	12 (18.8)	18 (16.7)	30 (22.4)	22 (15.3)	39 (29.1)	26 (17.8)	10 (11.8)	28 (23.0)	<0.001
Trimethoprim / Sulfamethoxazole	S (n=3429)	0.15	8 (1.9)	-	-	1 (0.7)	1 (0.3)	2 (1.3)	6 (1.2)	9 (2.3)	-	4 (0.96)	0.074
	H (n=2130)	(<0.001)	6 (8.1)	4 (5.3)	6 (9.4)	5 (4.6)	13 (9.7)	9 (6.3)	9 (6.7)	9 (6.2)	9 (10.6)	5 (4.1)	0.266

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by unit[c] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) HU-11 n=231	HU-12 n=128	HU-13 n=77	H-Slaugh n=193	HU-15 n=263	HU-16 n=118	HU-17 n=265	HU-18 n=283	HU-19 (S-Isolation) n=516	*p*-value[d]
Amikacin	S (n=3429)	0 (0.0)	-	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0.154
	H (n=2130)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Amoxicillin /Clavulanic Acid	S (n=3429)	2 (1.3)	-	-	1 (1.4)	1 (0.7)	-	1 (0.8)	2 (1.4)	7 (1.7)	<0.001
	H (n=2130)	2 (2.4)	4 (3.1)	1 (1.3)	3 (2.5)	0 (0.0)	4 (3.4)	4 (2.8)	4 (2.9)	1 (0.89)	0.35

Table 5 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by unit[c] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) HU-11 n=231	HU-12 n=128	HU-13 n=77	H-Slaugh n=193	HU-15 n=263	HU-16 n=118	HU-17 n=265	HU-18 n=283	HU-19 (S-Isolation) n=516	*p*-value[d]
Ampicillin	S (n=3429)	12 (8.0)	-	-	7 (9.6)	13 (9.5)	-	15 (12.5)	7 (4.8)	167 (41.4)	<0.001
	H (n=2130)	15 (18.3)	20 (15.6)	13 (16.9)	11 (9.2)	26 (20.6)	28 (23.7)	22 (15.2)	19 (14.0)	10 (8.9)	0.029
Cefoxitin	S (n=3429)	2 (1.3)	-	-	1 (1.4)	2 (1.5)	-	0 (0.0)	2 (1.4)	6 (1.5)	<0.001
	H (n=2130)	1 (1.2)	1 (0.8)	2 (2.6)	1 (0.8)	0 (0.0)	3 (2.5)	4 (2.8)	2 (1.5)	1 (0.9)	0.081
Chloramphenicol	S (n=3429)	1 (0.7)	-	-	2 (2.7)	3 (2.2)	-	4 (3.3)	3 (2.0)	43 (10.6)	<0.001
	H (n=2130)	4 (4.9)	2 (1.6)	0 (0.0)	2 (1.7)	2 (1.6)	1 (0.9)	4 (2.8)	7 (5.2)	1 (0.9)	0.023
Ciprofloxacin	S (n=3429)	0 (0.0)	-	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0.976
	H (n=2130)	1 (1.2)	2 (1.6)	2 (2.6)	0 (0.0)	0 (0.0)	3 (2.5)	0 (0.0)	2 (1.5)	0 (0.0)	0.008
Gentamicin	S (n=3429)	1 (0.7)	-	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
	H (n=2130)	0 (0.0)	2 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.066
Kanamycin	S (n=3429)	4 (2.7)	-	-	4 (5.5)	8 (5.8)	-	9 (7.5)	8 (5.4)	125 (30.9)	<0.001
	H (n=2130)	1 (1.2)	2 (1.6)	3 (3.9)	2 (1.7)	0 (0.0)	5 (4.2)	1 (0.7)	2 (1.5)	0 (0.0)	0.092
Nalidixic Acid	S (n=3429)	0 (0.0)	-	-	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0.555
	H (n=2130)	5 (6.1)	51 (39.8)	2 (2.6)	2 (1.7)	6 (4.8)	6 (5.1)	1 (0.7)	7 (5.2)	5 (6.1)	<0.001
Streptomycin	S (n=3429)	21 (14.0)	-	-	11 (15.0)	20 (14.6)	-	22 (18.3)	34 (23.1)	133 (32.9)	<0.001
	H (n=2130)	10 (12.2)	7 (5.5)	7 (9.1)	8 (6.7)	11 (8.7)	24 (20.3)	14 (9.6)	18 (13.2)	5 (4.5)	0.021
Sulfisoxazole	S (n=3429)	23 (15.4)	-	-	12 (16.4)	17 (12.4)	-	30 (25.0)	33 (22.5)	202 (50.0)	<0.001
	H (n=2130)	17 (20.7)	12 (9.4)	14 (18.2)	14 (18.7)	20 (15.9)	21 (17.8)	25 (17.2)	19 (14.0)	8 (7.1)	0.17
Tetracycline	S (n=3429)	96 (64.4)	-	-	46 (63.0)	100 (73.0)	-	109 (90.8)	98 (66.7)	390 (96.5)	<0.001
	H (n=2130)	14 (17.1)	15 (11.7)	9 (11.7)	34 (28.3)	31 (24.6)	38 (32.2)	20 (13.8)	31 (22.8)	16 (14.3)	<0.001
Trimethoprim / Sulfamethoxazole	S (n=3429)	0 (0.0)	-	-	1 (1.4)	0 (0.0)	-	0 (0.0)	1 (0.7)	6 (1.1)	0.074
	H (n=2130)	10 (12.2)	6 (4.7)	5 (6.5)	6 (5.0)	8 (6.4)	13 (11.0)	13 (9.0)	14 (10.3)	3 (2.7)	0.266

Table 5 (Continued)

[a] Odds-ratios are presented comparing the odds of each phenotype of antimicrobial resistance in swine versus human *E. coli* isolates.
[b] *p*-values are adjusted for the dependence of host species isolate response within each unit location by using the generalized estimating equation (GEE) statistic (STATA™ ver. 9.2, College Station, TX).
[c] Unit Legend: H-Intake = human isolates from a single intake facility, HU (1, 4, 5, 6, 7, 8, 10, 11, 15, 17, 18) = human and swine isolates from 11 separate units housing both host species, HU (3, 9, 12, 13, 16) = human isolates from 5 separate units housing no swine operations, H-Slaugh = human isolates from a single facility with a swine slaughter-plant, HU-19/S-Isolation = human isolates from 1 unit housing and swine isolates from a single isolation/quarantine unit. This unit is the sole facility that receives purebred replacement stock (boars) supplying the other swine facilities.
[d] *p*-values are based on a likelihood ratio chi-square test of the differences in risk across all units that were sampled. These data are presented and analyzed by host species.

Table 6

Phenotypic resistance of commensal *E. coli* isolates from human wastewater samples (n = 2130 isolates with vocation cohort properly identified). Frequencies and proportions are contrasted by human vocation cohort (i.e., non-workers and non-consumers, swine worker, non-swine worker, slaughter-plant worker, influent mixture) across all unit locations and seasons.

Antimicrobial	Human vocation cohort Frequency of resistant *E. coli* isolates (%)					*p*-value[a]
	Non-worker, non-consumer isolates (n=77)	Swine-worker isolates (n=536)	Non-swine worker isolates (n=1084)	Slaugher-plant workers isolates (n=58)	Influent (mixed isolates (n=232)	
Amikacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Amoxicillin /Clavulanic Acid	1 (1.3)	14 (2.6)	28 (2.6)	2 (3.5)	5 (2.2)	0.922
Ampicillin	15 (19.5)	85 (15.9)	180 (16.6)	5 (8.6)	38 (16.4)	0.459
Cefoxitin	3 (3.9)	10 (1.9)	27 (2.5)	1 (1.7)	6 (2.6)	0.824
Ceftiofur	0 (0.0)	3 (0.6)	2 (0.2)	0 (0.0)	0 (0.0)	0.468
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Chloramphenicol	1 (1.3)	15 (2.8)	19 (1.8)	1 (1.7)	2 (0.9)	0.404
Ciprofloxacin	1 (1.3)	5 (0.8)	9 (0.8)	0 (0.0)	0 (0.0)	0.294
Gentamicin	0 (0.0)	2 (0.4)	6 (0.5)	0 (0.0)	0 (0.0)	0.474
Kanamycin	2 (2.6)	7 (1.3)	17 (1.6)	0 (0.0)	0 (0.0)	0.063
Nalidixic Acid	1 (1.3)	37 (6.9)	58 (5.4)	1 (1.7)	13 (5.6)	0.099
Streptomycin	7 (9.1)	57 (10.6)	97 (9.0)	2 (3.4)	21 (9.1)	0.367
Sulfisoxazole	8 (10.4)	68 (12.7)	179 (16.5)	6 (10.3)	30 (12.9)	0.126
Tetracycline	11 (14.3)	124 (23.1)	191 (17.6)	15 (25.9)	53 (22.8)	0.025
Trimethoprim / Sulfamethoxazole	3 (3.9)	42 (7.8)	85 (7.8)	4 (6.9)	12 (5.2)	0.409

[a] *p*-values are based on a likelihood ratio chi-square test of the differences in risk between human vocation cohorts. These *p*-values are not adjusted for the dependence of responses within unit locations.

Table 7

Phenotypic resistance of commensal *E. coli* isolates among swine fecal samples (n = 3429 isolates with production group identified). Frequencies are contrasted by swine production group (i.e., slaughter-house holding pens, breeding boars, isolation /quarantine boars, breeding/gestation females, farrowing sows and piglets, growers and finishers, and nursery piglets) across all unit locations and seasons.

Antimicrobial agent	Swine production group Frequency of resistant *E. coli* isolates (%)							*p*-value[a]
	Slaughter-holding Pig samples (n=72)	Breeding boar samples (n=195)	Isolation boar samples (n=331)	Breeding/gestation females samples (n=131)	Farrowing sows & piglets samples (n=755)	Grower & finisher Pig samples (n=1576)	Nursery pig samples (n=368)	
Amikacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	3 (0.2)	0 (0.0)	0.784
Amoxicillin /Clavulanic Acid	1 (1.4)	1 (0.5)	6 (1.8)	1 (0.8)	30 (4.0)	13 (0.8)	25 (6.8)	< 0.0001
Ampicillin	7 (9.7)	49 (25.1)	159 (48.0)	16 (12.2)	196 (26.0)	176 (11.2)	86 (23.4)	< 0.001
Cefoxitin	1 (1.4)	0 (0.0)	5 (1.5)	1 (0.80	23 (3.0)	14 (0.9)	25 (6.8)	< 0.0001
Ceftiofur	1 (1.4)	1 (0.5)	3 (0.9)	0 (0.0)	24 (3.2)	28 (1.8)	26 (7.1)	< 0.0001
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)	0.177
Chloramphenicol	2 (1.5)	3 (1.5)	41 (12.4)	3 (2.3)	23 (3.0)	23 (3.0)	25 (6.8)	<0.0001

Table 7 (Continued)

Antimicrobial agent	Swine production group Frequency of resistant *E. coli* isolates (%)							*p*-value[a]
	Slaughter-Holding Pig Samples (n=72)	Boar Samples (n=195)	Intake Boar Samples (n=331)	Breeding/gestation females Samples (n=131)	Farrowing sows & piglets Samples (n=755)	Grower & Finisher Pig Samples (n=1576)	Nursery Pig Samples (n=368)	
Ciprofloxacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0.956
Gentamicin	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	9 (1.2)	13 (0.8)	32 (8.7)	<0.0001
Kanamycin	4 (5.6)	23 (11.8)	113 (34.1)	10 (7.6)	67 (8.9)	102 (6.5)	82 (22.3)	<0.0001
Nalidixic Acid	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	0 (0.0)	<0.0001
Streptomycin	11 (15.3)	55 (28.2)	115 (34.7)	21 (16.0)	232 (30.7)	308 (19.5)	175 (47.5)	<0.0001
Sulfisoxazole	12 (16.7)	30 (15.4)	179 (54.1)	18 (13.7)	179 (23.7)	325 (20.6)	165 (44.8)	<0.0001
Tetracycline	46 (63.9)	176 (90.3)	325 (98.2)	107 (81.7)	622 (82.4)	1310 (83.1)	335 (91.0)	<0.0001
Trimethoprim / Sulfamethoxazole	1 (1.4)	4 (2.0)	6 (1.8)	1 (0.8)	9 (1.2)	11 (0.7)	39 (1.1)	0.296

[a] *p*-values are based on a likelihood ratio chi-square test of the differences in risk between swine production groups. These *p*-values are not adjusted for the dependence of responses within unit locations.

Table 8

Phenotypic resistance of commensal *E. coli* isolates from human and swine fecal samples. Frequencies are contrasted by host species and season. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season[a] (n=5559 overall sample isolates)												
		Frequency of resistant *E. coli* (%)												
Antimicrobial agent	Host Species (S=Swine, H=Human)	Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006	*p*-value[b]
Amikacin	H (n=2130)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
	S (n=3429)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.57)	0 (0.00)	0 (0.00)	1 (0.74)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.275
Amoxicillin /Clavulanic Acid	H (n=2130)	11 (4.04)	12 (4.55)	8 (2.82)	9 (3.30)	4 (0.98)	3 (2.80)	1 (0.97)	1 (1.00)	0 (0.00)	2 (2.02)	1 (1.37)	2 (2.90)	0.067
	S (n=3429)	22 (4.18)	9 (2.27)	15 (3.71)	10 (2.53)	4 (0.77)	3 (1.96)	3 (1.28)	1 (0.74)	2 (1.38)	4 (2.09)	1 (0.67)	3 (1.69)	0.013
Ampicillin	H (n=2130)	57 (20.96)	42 (15.91)	43 (15.14)	60 (21.98)	81 (19.90)	12 (11.21)	14 (13.59)	12 (12.00)	6 (7.59)	6 (6.06)	5 (6.85)	7 (10.14)	(<0.001)
	S (n=3429)	104 (19.77)	68 (17.13)	92 (22.77)	69 (17.47)	82 (15.71)	23 (15.03)	90 (38.46)	23 (17.04)	20 (13.79)	41 (21.47)	34 (22.67)	43 (24.29)	(<0.001)
Cefoxitin	H (n=2130)	11 (4.04)	16 (6.06)	7 (2.46)	7 (2.56)	4 (0.98)	0 0.00	1 (0.97)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.37)	2 (2.90)	(<0.001)
	S (n=3429)	20 (3.80)	11 (2.77)	14 (3.47)	8 (2.03)	1 (0.19)	3 (1.96)	2 (0.85)	0 0.00	3 (2.07)	2 (1.05)	1 (0.67)	4 (2.26)	(<0.001)

Table 8 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season[a] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006	*p*-value[b]
Ceftiofur	H	1 (0.37)	0 (0.00)	0 (0.00)	0 (0.00)	5 (1.23)	1 (0.93)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.159
	S	19 (3.61)	22 (5.54)	15 (3.71)	13 (3.29)	1 (0.19)	3 (1.96)	1 (0.43)	0 (0.00)	2 (1.38)	2 (1.05)	1 (0.67)	4 (2.26)	(<0.001)
Ceftriaxone	H	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
	S	0 (0.00)	0 (0.00)	1 (0.25)	1 (0.25)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.884
Chloramphenicol	H	5 (1.84)	3 (1.14)	4 (1.41)	8 (2.93)	10 (2.46)	0 (0.00)	1 (0.97)	1 (1.00)	4 (5.06)	2 (2.02)	0 (0.00)	2 (2.90)	0.202
	S	31 (5.89)	13 (3.27)	22 (5.45)	17 (4.30)	10 (1.92)	4 (2.61)	15 (6.41)	3 (2.22)	8 (5.52)	7 (3.66)	10 (6.67)	9 (5.08)	0.024
Ciprofloxacin	H	3 (1.10)	2 (0.76)	0 (0.00)	1 (0.37)	5 (1.23)	0 (0.00)	2 (1.94)	1 (1.00)	1 (1.27)	0 (0.00)	0 (0.00)	0 (0.00)	0.278
	S	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.19)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.976
Gentamicin	H	4 (1.47)	0 (0.00)	0 (0.00)	2 (0.73)	2 (0.49)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.196
	S	11 (2.09)	10 (2.52)	11 (2.72)	9 (2.28)	6 (1.15)	1 (0.65)	1 (0.43)	4 (2.96)	0 (0.00)	1 (0.52)	0 (0.00)	1 (0.56)	0.008

Table 8 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season[a] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006	*p*-value[b]
Kanamycin	H	5 (1.84)	3 (1.14)	0 (0.00)	3 (1.10)	5 (1.23)	3 (2.80)	1 (0.97)	1 (1.00)	2 (2.53)	2 (2.02)	1 (1.37)	1 (1.45)	0.46
	S	64 (12.17)	32 (8.06)	44 (10.89)	43 (10.89)	43 (8.24)	7 (4.58)	53 (22.65)	15 (11.11)	13 (8.97)	39 (20.42)	19 (12.67)	29 (16.38)	(<0.001)
Nalidixic Acid	H	25 (9.19)	13 (4.92)	12 (4.23)	6 (2.20)	34 (8.35)	4 (3.74)	10 (9.71)	2 (2.00)	4 (5.06)	2 (2.02)	2 (2.74)	2 (2.90)	0.001
	S	0 (0.00)	1 (0.25)	0 (0.00)	1 (0.25)	1 (0.19)	0 (0.00)	0 (0.00)	1 (0.74)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.732
Streptomycin	H	25 (9.19)	19 (7.20)	28 (9.86)	28 (10.26)	52 (12.78)	3 (2.80)	11 (10.68)	10 (10.00)	3 (3.80)	3 (3.03)	4 (5.48)	6 (8.70)	0.007
	S	167 (31.75)	99 (24.94)	127 (31.44)	113 (28.61)	129 (24.71)	36 (23.53)	78 (33.33)	35 (25.93)	23 (15.86)	49 (25.65)	27 (18.00)	34 (19.21)	(<0.001)
Sulfisoxazole	H	32 (11.76)	25 (9.47)	28 (9.86)	37 (13.55)	84 (20.64)	7 (6.54)	11 (10.68)	65 (65.00)	6 (7.59)	5 (5.05)	4 (5.48)	7 (10.14)	(<0.001)
	S	114 (21.67)	108 (27.20)	153 (37.87)	149 (37.72)	76 (14.56)	32 (20.92)	96 (41.03)	30 (22.22)	25 (17.24)	41 (21.47)	36 (24.00)	48 (27.12)	(<0.001)
Tetracycline	H	47 (17.28)	40 (15.15)	64 (22.54)	56 (20.51)	100 (24.57)	10 (9.35)	34 (33.01)	19 (19.00)	9 (11.39)	18 (18.18)	7 (9.59)	12 (17.39)	(<0.001)
	S	427 (81.18)	322 (81.11)	357 (88.37)	351 (88.86)	436 (83.52)	143 (93.46)	207 (88.46)	113 (83.70)	122 (84.14)	164 (85.86)	126 (84.00)	153 (86.44)	(<0.001)

Table 8 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season[a] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%)												*p*-value[b]
		Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006	
Trimethoprim / Sulfamethoxazole	H	22 (8.09)	9 (3.41)	20 (7.04)	26 (9.52)	36 (8.85)	5 (4.67)	11 (10.68)	9 (9.00)	5 (6.33)	2 (2.02)	3 (4.11)	5 (7.25)	0.035
	S	2 (0.38)	4 (1.01)	8 (1.98)	9 (2.28)	5 (0.96)	0 (0.00)	3 (1.28)	1 (0.74)	1 (0.69)	1 (0.52)	1 (0.67)	4 (2.26)	0.133

[a] *p*-values are based on a likelihood ratio chi-square test of the differences in risk between seasons. These p-values are not adjusted for the dependence of responses within unit locations.
The 4 seasons or quarters were as follows: winter: February – April, spring: May – July, summer: August – October, and autumn: November – January.

Table 9. High variability was observed among seasonal samples over the 3-year period for both human and swine isolates across all units.

Sixty-two percent of the human *E. coli* isolates were pan-susceptible to the 15 antimicrobial agents on the NARMS panel, 20% were single-resistant, and 18% were resistant to 2 or more antimicrobial agents. In contrast, only 13% of the swine *E. coli* isolates were pan-susceptible, whereas 42% were single-resistant, and 45% were resistant to 2 or more antimicrobial agents (see table on page 97). The distribution of multi-drug resistant phenotypes (upper (6+) categories was not collapsed in Fig. 5 in order to show the maximum multi-drug resistance phenotypes for *E. coli* isolates from human and swine samples. The multi-drug resistance (multinomial) phenotypes for *E. coli* isolates were cross-tabulated first by season (winter, spring, summer, and autumn across all 3 years) and host species as shown in the table on page 99 and then season collapsed (winter, spring, summer, and autumn) over the 3-year period as shown in the table on page 101. The multi-drug resistant *E. coli* isolates from human samples differed significantly ($\chi^2 = 189.6$, $p < 0.001$) among seasons across all levels of multi-resistance. Likewise, the multi-drug resistant *E. coli* isolates from swine samples differed significantly ($\chi^2 = 206.3$, $p < 0.001$) among seasons across all levels of multi-resistance. High variability was observed among seasonal samples for multi-drug resistance phenotypes collapsed over the 3-year period for both human and swine samples.

Multi-level resistance, cross-tabulated by human vocation cohorts and swine production groups, is shown in the table on page 103, where: a) isolation boars showed the highest levels of resistance to multiple antimicrobial agents (1.21%) relative to

Table 9
Phenotypic resistance of commensal *E. coli* isolates from human and swine fecal samples. Frequencies are contrasted by host species and seasons-collapsed over 3 years. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season-collapsed[a] (n=5559 overall sample isolates)				
		Frequency of resistant *E. coli* (%)				
Antimicrobial agent	Host Species (S=Swine, H=Human)	Winter	Spring	Summer	Autumn	*p*-value[b]
Amikacin	H (n=2130)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
	S (n=3429)	3 (0.25)	0 (0.00)	0 (0.00)	1 (0.14)	0.172
Amoxicillin /Clavulanic Acid	H (n=2130)	15 (1.98)	17 (3.62)	10 (2.71)	12 (2.71)	0.347
	S (n=3429)	28 (2.35)	16 (2.16)	19 (2.41)	14 (1.98)	0.938
Ampicillin	H (n=2130)	144 (19.00)	60 (12.77)	62 (13.48)	79 (17.87)	0.007
	S (n=3429)	206 (17.27)	132 (17.81)	216 (27.41)	135 (19.09)	(<0.001)
Cefoxitin	H (n=2130)	15 (1.98)	16 (3.40)	9 (1.96)	9 (2.04)	0.393
	S (n=3429)	24 (2.01)	16 (2.16)	14 (2.16)	12 (1.70)	0.911
Ceftiofur	H (n=2130)	6 (0.79)	1 (0.21)	0 (0.00)	0 (0.00)	0.021
	S (n=3429)	22 (1.84)	27 (3.64)	17 (2.16)	17 (2.40)	0.105
Ceftriaxone	H (n=2130)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
	S (n=3429)	0 (0.00)	0 (0.00)	1 (0.13)	1 (0.14)	0.344
Chloramphenicol	H (n=2130)	19 (2.51)	5 (1.06)	5 (1.09)	11 (2.49)	0.106
	S (n=3429)	49 (4.11)	24 (3.24)	47 (5.96)	29 (4.10)	0.068

Table 9 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season-collapsed[a] (n=5559 overall sample isolates) Frequency of resistant *E. coli* (%) Winter	Spring	Summer	Autumn	*p*-value[b]
Ciprofloxacin	H (n=2130)	9 (1.19)	2 (0.43)	2 (0.43)	2 (0.45)	0.294
	S (n=3429)	1 (0.08)	0 (0.00)	0 (0.00)	0 (0.00)	0.549
Gentamicin	H (n=2130)	6 (0.79)	0 (0.00)	0 (0.00)	2 (0.45)	0.021
	S (n=3429)	17 (1.42)	12 (1.62)	12 (1.52)	14 (1.98)	0.831
Kanamycin	H (n=2130)	12 (1.58)	8 (1.70)	2 (0.43)	5 (1.13)	0.197
	S (n=3429)	120 (10.06)	78 (10.53)	116 (14.72)	87 (12.31)	0.012
Nalidixic Acid	H (n=2130)	63 (8.31)	19 (4.04)	24 (5.22)	10 (2.26)	(<0.001)
	S (n=3429)	1 (0.08)	1 (0.13)	0 (0.00)	2 (0.28)	0.365
Streptomycin	H (n=2130)	80 (10.55)	25 (5.32)	43 (9.35)	44 (9.95)	0.009
	S (n=3429)	319 (26.74)	184 (24.83)	232 (29.44)	182 (25.74)	0.2
Sulfisoxazole	H (n=2130)	122 (16.09)	37 (7.87)	43 (9.35)	109 (24.66)	(<0.001)
	S (n=3429)	215 (18.02)	181 (24.43)	285 (36.17)	227 (32.11)	(<0.001)
Tetracycline	H (n=2130)	156 (20.58)	68 (14.47)	105 (22.83)	87 (19.68)	0.008
	S (n=3429)	985 (82.56)	629 (84.89)	690 (87.56)	617 (87.27)	0.006
Trimethoprim / Sulfamethoxazole	H (n=2130)	63 (8.31)	16 (3.40)	34 (7.39)	40 (9.05)	0.001
	S (n=3429)	8 (0.67)	5 (0.67)	12 (1.52)	14 (1.98)	0.03

Table 9 (Continued)

[a] *p*-values are based on a likelihood ratio chi-square test of the differences in risk between seasons. These *p*-values are not adjusted for the dependence of responses within unit locations.

Table 10

Multi-drug resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal samples. Frequency and percentage of multi-drug resistant *E. coli* isolates are presented and contrasted by host species (upper multi-resistance categories collapsed into 6+).

	Sampled by host-species[a] (n=5559 overall sample isolates)	
	Frequency of multi-drug resistant *E. coli* (%)	
Multi-drug Resistance	Human	Swine
0	1,328	425
	(62.35)	(12.39)
1	423	1,433
	(19.86)	(41.79)
2	135	619
	(6.34)	(18.05)
3	84	493
	(3.94)	(14.38)
4	76	282
	(3.57)	(8.22)
5	46	94
	(2.16)	(2.74)
6+ [c]	38	83
	(1.78)	(2.42)

[a] Difference in the multi-drug resistant *E. coli* isolates between human and swine was significant ($\chi^2 = 1600$, $p < 0.001$) using the likelihood-ratio chi-square test

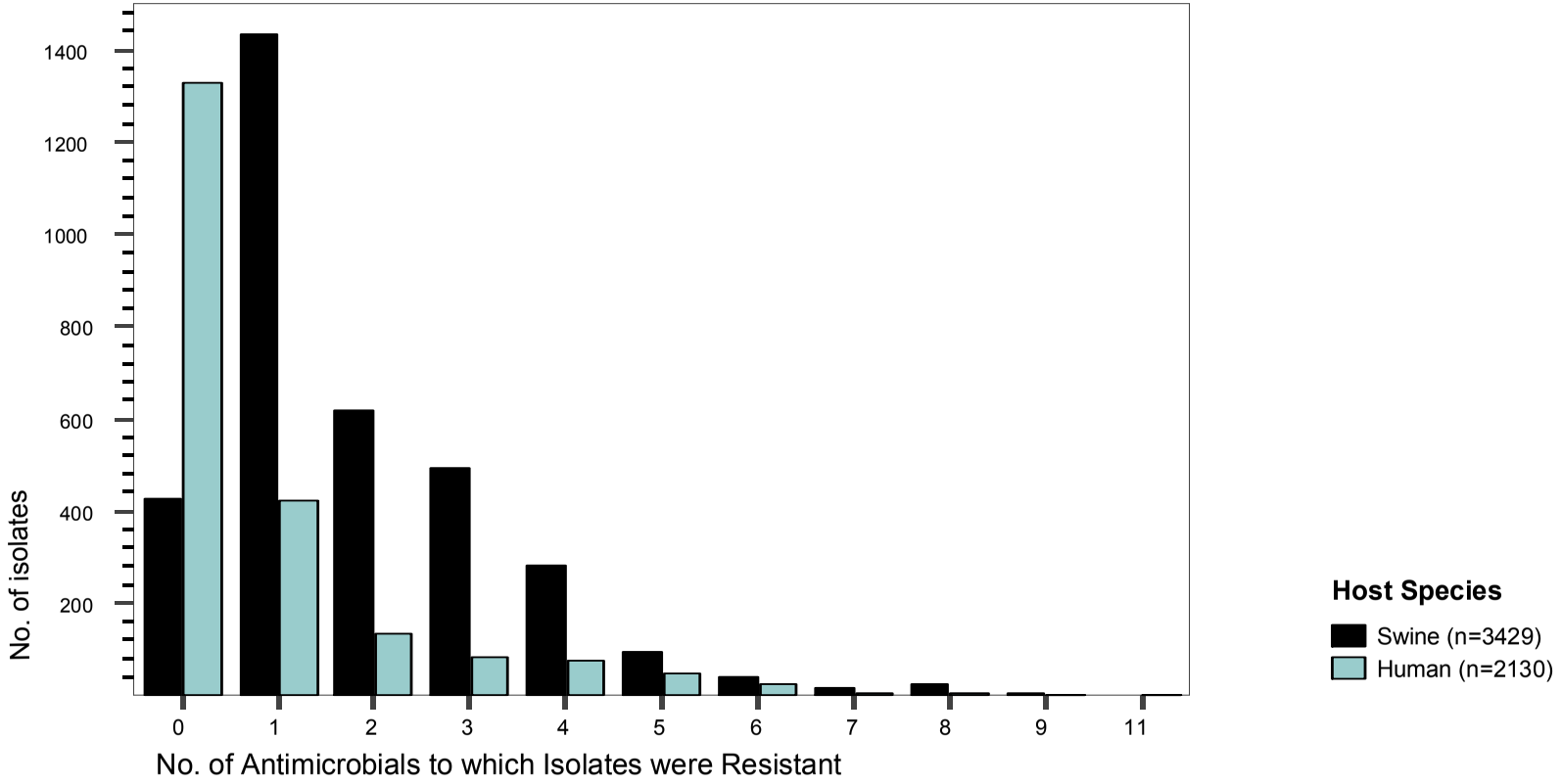

Fig. 5. Frequency bar chart illustrating the distribution of multi-drug resistance phenotypes (out of 15) exhibited by *E. coli* isolates from human and swine samples.

Table 11

Multi-drug resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal samples. Frequency and percentage of multi-drug resistant *E. coli* isolates are presented and contrasted by host species and season. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season[a] (n=5559 overall sample isolates)											
		Frequency of multi-drug resistant *E. coli* (%)											
Multi-drug Resistance	Host Species (S=Swine, H=Human)[b]	Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006
0	H=1328	164 (60.29)	176 (66.67)	183 (64.44)	171 (62.64)	218 (53.56)	84 (78.5)	61 (59.22)	26 (26)	61 (77.22)	74 (74.75)	60 (82.19)	50 (72.46)
	S=425	85 (16.16)	55 (13.85)	30 (7.43)	34 (8.61)	80 (15.33)	9 (5.88)	24 (10.26)	18 (13.33)	23 (15.86)	24 (12.57)	22 (14.67)	21 (11.86)
1	H=423	56 (20.59)	50 (18.94)	48 (16.90)	50 (18.32)	86 (21.13)	13 (12.15)	20 (19.42)	55 (55.00)	9 (11.39)	17 (17.17)	8 (10.96)	11 (15.94)
	S=1433	200 (38.02)	162 (40.81)	147 (36.39)	160 (40.51)	257 (49.23)	86 (56.21)	72 (30.77)	62 (45.93)	69 (47.59)	69 (36.13)	65 (43.33)	84 (47.46)
2	H=135	14 (5.15)	15 (5.68)	19 (6.69)	15 (5.49)	45 (11.06)	3 (2.80)	9 (8.74)	8 (8.00)	3 (3.80)	3 (3.03)	1 (1.37)	0 0.00
	S=619	88 (16.73)	78 (19.65)	102 (25.25)	79 (20.00)	78 (14.94)	25 (16.34)	34 (14.53)	17 (12.59)	26 (17.93)	45 (23.56)	28 (18.67)	19 (10.73)
3	H=84	12 (4.41)	8 (3.03)	18 (6.34)	13 (4.76)	17 (4.18)	2 (1.87)	4 (3.88)	2 (2.00)	3 (3.80)	3 (3.03)	0 0.00	2 (2.90)
	S=493	83 (15.78)	55 (13.85)	59 (14.60)	62 (15.70)	62 (11.88)	24 (15.69)	39 (16.67)	26 (19.26)	18 (12.41)	32 (16.75)	17 (11.33)	16 (9.04)
4	H=76	14 (5.15)	6 (2.27)	9 (3.17)	6 (2.20)	23 (5.65)	3 (2.80)	3 (2.91)	5 (5.00)	1 (1.27)	1 (1.01)	2 (2.74)	3 (4.35)
	S=282	36 (6.84)	34 (8.56)	30 (7.43)	36 (9.11)	34 (6.51)	5 (3.27)	41 (17.52)	8 (5.93)	6 (4.14)	13 (6.81)	12 (8.00)	27 (15.25)
5	H=46	8 (2.94)	4 (1.52)	4 (1.41)	11 (4.03)	11 (2.70)	1 (0.93)	1 (0.97)	2 (2.00)	0 (0.00)	0 (0.00)	2 (2.74)	2 (2.90)
	S=94	13 (2.47)	6 (1.51)	19 (4.70)	12 (3.04)	5 (0.96)	1 (0.65)	19 (8.12)	3 (2.22)	1 (0.69)	5 (2.62)	3 (2.00)	7 (3.95)

Table 11 (Continued)

Multi-drug Resistance	Host Species (S=Swine, H=Human)[b]	Sampled by season[a] (n=5559 overall sample isolates) Frequency of multi-drug resistant *E. coli* (%)											
		Winter 2004	Spring 2004	Summer 2004	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006
		4	5	3	7	7	1	5	2	2	1	0	1
	H=38	(1.47)	(1.89)	(1.06)	(2.56)	(1.72)	(0.93)	(4.85)	(2.00)	(2.53)	(1.01)	(0.00)	(1.45)
6+ [c]		21	7	17	12	6	3	5	1	2	3	3	3
	S=83	(3.99)	(1.76)	(4.21)	(3.04)	(1.15)	(1.96)	(2.14)	(0.74)	(1.38)	(1.57)	(2.00)	(1.69)

[a] Difference in the multi-drug resistant *E. coli* human isolates and seasons was significant ($\chi^2 = 189.6$, $p < 0.001$) using m × n likelihood-ratio chi-square test. Also, the difference in multi-drug resistant *E. coli* swine isolates and seasons was significant ($\chi^2 = 206.3$, $p < 0.001$) using m × n likelihood-ratio chi-square test. Both p-values are not adjusted for the dependence of responses within unit locations.

[b] Number of *E. coli* isolates for human and swine for each multi-drug resistance category.

[c] Multi-drug resistance greater than 6 antimicrobial agents (6+) was collapsed into a single upper category.

Table 12

Multi-drug resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal Samples. Frequency and percentage of multi-drug resistant *E. coli* isolates are presented and contrasted by host species and season-collapsed. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season-collaped[a] (n=5559 overall sample isolates)			
		Frequency of multi-drug resistant *E. coli* (%)			
Multi-drug Resistance	Host Species (S=Swine, H=Human)[b]	February	May	August	November
		433	334	304	247
	H=1328	(58.44)	(71.06)	(66.09)	(55.88)
0		188	88	76	73
	S=425	(15.76)	(11.88)	(9.64)	(10.33)
		151	80	76	116
	H=423	(19.92)	(17.02)	(16.52)	(26.24)
1		526	317	284	306
	S=1433	(44.09)	(42.78)	(36.04)	(43.28)
		62	21	29	23
	H=135	(8.18)	(4.47)	(6.30)	(5.20)
2		192	148	164	115
	S=619	(16.06)	(19.97)	(20.81)	(16.27)
		32	13	22	17
	H=84	(4.22)	(2.77)	(4.78)	(3.85)
3		163	111	115	104
	S=493	(13.66)	(14.98)	(14.59)	(14.71)
		38	10	14	14
	H=76	(5.01)	(2.13)	(3.04)	(3.17)
4		76	52	83	71
	S=282	(6.37)	(7.02)	(10.53)	(10.04)
		19	5	7	15
	H=46	(2.51)	(1.06)	(1.52)	(3.39)
5		19	12	41	22
	S=94	(1.59)	(1.64)	(5.20)	(3.11)
		13	7	8	10
	H=38	(1.72)	(1.49)	(1.74)	(2.26)
6+[c]		29	13	25	16
	S=83	(2.43)	(1.75)	(3.17)	(2.26)

Table 12 (Continued)

[a] Difference in the multi-drug resistant *E. coli* human isolates and seasons was significant ($\chi^2 = 50.51$, $p < 0.001$) using m × n likelihood-ratio chi-square test. Also, the difference in multi-drug resistant *E. coli* swine isolates and seasons was significant ($\chi^2 = 78.04$, $p < 0.001$) using m × n likelihood-ratio chi-square test. Both p-values are not adjusted for the dependence of responses within unit locations.
[b] Number of *E. coli* isolates for human and swine for each multi-drug resistance category.
[c] Multi-drug resistance greater than 6 antimicrobial agents (6+) was collapsed into a single upper category.

Table 13

Multi-drug resistance phenotypes of commensal *E. coli* isolates from human and swine fecal (human and swine cohorts properly identified). Frequency and percentage of multi-drug resistant *E. coli* isolates are presented and contrasted by human vocation cohorts and swine production cohorts. Isolate comparisons are across all unit locations and seasons.

	Sampled by human vocation and swine production group[a] (n=5559 overall sample isolates)										
	Frequency of multi-drug resistant *E. coli* (%)										
Multi-drug Resistance	Non-workers, non-consumers isolates (n=77)	Swine-worker isolates (n=594)	Non-swine worker isolates (n=1084)	Influent (Mixed) isolates (n=232)	Slaughter-holding pig isolates (n=72)	Breeding boar isolates (n=195)	Isolation boar isolates (n=331)	Breeding/gestation females isolates (n=131)	Farrowing sows & piglet isolates (n=755)	Grower & Finisher Pig isolates (n=1576)	Nursery Piglets isolates (n=368)
	52	354	681	142	24	15	4	20	110	233	18
0 (n=1653)	(67.53)	(59.6)	(62.82)	(61.21)	(33.33)	(7.69)	(1.21)	(15.27)	(14.57)	(14.78)	(4.89)
	14	133	209	47	29	85	67	69	283	780	120
1 (n=1836)	(18.18)	(22.39)	(19.28)	(20.26)	(40.28)	(43.59)	(20.24)	(52.67)	(37.48)	(49.49)	(32.61)
	2	43	57	21	10	46	73	24	127	284	55
2 (n=742)	(2.60)	(7.24)	(5.26)	(9.05)	(13.89)	(23.59)	(22.05)	(18.32)	(16.82)	(18.02)	(14.95)
	6	21	48	7	4	34	70	11	141	165	68
3 (n=575)	(7.79)	(3.54)	(4.43)	(3.02)	(5.56)	(17.44)	(21.15)	(8.40)	(18.68)	(10.47)	(18.48)
	0	16	46	8	3	10	75	7	60	74	53
4 (n=352)	(0.00)	(2.69)	(4.24)	(3.45)	(4.17)	(5.13)	(22.66)	(5.34)	(7.95)	(4.70)	(14.40)
	2.00	14	22	5	1	5	30	0	13	27	18
5 (n=137)	(2.60)	(2.36)	(2.03)	(2.16)	(1.39)	(2.56)	(9.06)	(0.00)	(1.72)	(1.71)	(4.89)
	1	13	21	2	1	0	12	0	21	13	36
6+ (n=120)[b]	(1.30)	(2.19)	(1.94)	(0.86)	(1.39)	(0.00)	(3.63)	(0.00)	(2.78)	(0.82)	(9.78)

[a] Difference in the multi-drug resistant *E. coli* human isolates across human vocation cohorts was not significant ($\chi^2 = 24.4$, $p = 0.142$) using m × n likelihood-ratio chi-square test. However, the difference in multi-drug resistant *E. coli* swine isolates and swine production group was significant ($\chi^2 = 495.2$, $p < 0.001$) using an m × n likelihood-ratio chi-square test. Both p-values are not adjusted for the dependence of responses within unit locations.

[b] Multi-drug resistance greater than 6 antimicrobial agents (6+) was collapsed into a single upper category.

swine-rearing, slaughtered pigs, and human vocation cohorts, b) breeding boars showed higher single-resistance levels (52.67%) and resistance to 2 or more antimicrobial agents (23.59%) relative to other swine and human cohorts, c) isolation boars showed higher level of resistance to 3, 4, and 5 antimicrobial agents (21.15%), (22.66%), and (9.06%), respectively relative to other swine and human cohorts, and d) nursery piglets showed higher level of resistance to (6+) antimicrobial agents (9.78%) relative to other swine and human cohorts. The multi-drug resistant *E. coli* isolates from human samples did not differ significantly ($\chi^2 = 24.4$, $p = 0.142$) among human vocation cohorts across all levels of multi-resistance. However, the multi-drug resistant *E. coli* isolates from swine samples differed significantly ($\chi^2 = 495.2$, $p < 0.001$) among swine production groups across all levels of multi-resistance.

4.2. Multivariable analysis of risk factors for E. coli *resistance to individual antimicrobial agents*

4.2.1. Comparison of E. coli *resistance to individual antimicrobial agents between host-species*

The relative odds of resistance were significantly increased ($p < 0.05$) among swine isolates as compared to human isolates for the following antimicrobial agents: tetracycline (OR = 19.58, 95% CI: 16.77 – 22.86), kanamycin (OR = 10.01, 95% CI: 5.51 – 18.23), ceftiofur (OR = 6.68, 95% CI: 2.77 – 16.10), gentamicin (OR = 4.19, 95% CI: 1.70 – 10.31), streptomycin (OR = 3.46, 95% CI: 2.89 – 4.15), chloramphenicol (OR = 2.02, 95% CI: 1.36 – 3.00), sulfisoxazole (OR = 1.99, 95% CI: 1.70 – 2.33). (Table 5, column 3). In contrast, the relative odds of resistance were significantly increased

($p < 0.05$) among human isolates as compared to swine isolates for the following antimicrobial agents: ciprofloxacin (OR = 18.94, 95% CI: 2.79 – 128.70), trimethoprim/sulfamethoxazole (OR = 6.67, 95% CI: 4.74 – 9.40), and nalidixic acid (OR = 3.45, 95% CI: 1.94 – 6.12) (see Table 5, column 3).

4.2.2. Comparison of E. coli *resistance to individual antimicrobial agents by swine production group*

The relative odds of resistance were significantly increased ($p < 0.05$) for isolation boars, nursery piglets, and breeding boars *E. coli* isolates as compared to the swine referent group (slaughtered pigs) for tetracycline (OR = 12.25 (95% CI: 2.41 – 62.15), 3.50 (95% CI: 1.08 – 11.27), and 3.20 (95% CI: 0.97 – 10.55), respectively), and streptomycin (OR = 2.90 (95% CI: 1.42 – 5.95), 4.96 (95% CI: 2.46 – 9.99), and 2.14 (95% CI: 1.02 – 4.50), respectively). Furthermore, the relative odds of resistance were significantly increased ($p < 0.05$) for isolation boars and nursery piglets *E. coli* isolates as compared to the swine referent group (slaughtered pigs) for sulfisoxazole (OR = 5.83 (95% CI: 3.01 – 11.34) and 4.06 (95% CI: 2.10 – 7.85)), respectively. Table 7 provides the proportion of resistant *E. coli* by swine production group for each antimicrobial agent.

4.2.3. Comparison of E. coli *resistance to individual antimicrobial agents by human vocation cohort*

The relative odds of resistance were significantly increased ($p < 0.05$) among swine-worker isolates for tetracycline (OR = 1.36, 95% CI: 0.41 – 1.57) as compared to swine non-workers; in contrast, the relative odds among workers were significantly lower ($p <$

0.05) for sulfisoxazole (OR = 0.73, 95% CI: 0.54 – 0.99). The relative odds of resistance did not differ significantly ($p > 0.05$) among the non-worker/non-consumer and slaughter-plant worker isolates as compared to swine non-workers; however, sample sizes were relatively small for these human categories. Furthermore, the relative odds of resistance did not differ significantly ($p > 0.05$) for pork consumer (i.e., swine workers, non-swine-workers, and slaughter-plant-workers) *E. coli* isolates as compared to the non-worker/non-consumer group.

4.2.4. Comparison of E. coli *resistance to individual antimicrobial agents by season*

There was no repeatable seasonal trend observed among the seasonal isolates (i.e., over all seasons, and collapsed by season) for both human and swine over the 3-year study period. This conclusion was based on the variability in the GEE model parameter estimates for the 15 antimicrobial resistance outcomes in relation to season (see tables on pages 107 and 116).

4.3. Multivariable analysis of risk factors for E. coli *resistance to multiple antimicrobial agents*

4.3.1. Comparison of E. coli *resistance to multiple antimicrobial agents by host-species*

The relative odds of multiple resistance were significantly increased ($p < 0.05$) among swine isolates (OR = 7.33, 95% CI: 6.46 – 8.31) as compared to human isolates across all levels of multi-resistance. Table 10 provides the proportion of multi-resistant *E. coli* by host-species cross-tabulated by each antimicrobial agent.

Table 14

Phenotypic resistance of commensal *E. coli* isolates from human and swine fecal samples. Odds ratios of resistant *E. coli* isolates are presented and contrasted by host species and season. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b])				
Antimicrobial agent	Host Species (S=Swine, H=Human)	Spring 2004 n=661	Summer 2004 n=688	Autumn 2004 n=688	Winter 2005 n=929	Spring 2005 n=260
Amikacin	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Amoxicillin /Clavulanic Acid	S (n=3429)	0.50 (0.11)	0.86 (0.67)	0.55 (0.16)	0.14 (0.005)	0.41 (0.19)
	H (n=2130)[c]	-	-	-	-	-
Ampicillin	S (n=3429)	0.88 (0.49)	1.2 (0.26)	0.84 (0.34)	0.83 (0.25)	0.73 (0.23)
	H (n=2130)	0.71 (0.12)	0.67 (0.07)	1.1 (0.78)	0.92 (0.69)	0.47 (0.03)

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Summer 2005 n=337	Autumn 2005 n=235	Winter 2006 n=224	Spring 2006 n=290	Summer 2006 n=223	Autumn 2006 n=246
Amikacin	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Amoxicillin /Clavulanic Acid	S (n=3429)	0.30 (0.08)	0.12 (0.11)	0.26 (0.12)	0.46 (0.20)	0.13 (0.09)	0.35 (0.14)
	H (n=2130)[c]	-	-	-	-	-	-
Ampicillin	S (n=3429)	1.7 (0.004)	0.85 (0.54)	0.61 (0.09)	0.85 (0.50)	1.0 (0.95)	0.92 (0.73)
	H (n=2130)	0.59 (0.10)	0.51 (0.05)	0.31 (0.01)	0.24 (0.001)	0.27 (0.008)	0.42 (0.04)

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Spring 2004 n=661	Summer 2004 n=688	Autumn 2004 n=688	Winter 2005 n=929	Spring 2005 n=260
Cefoxitin	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Ceftiofur	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Ceftriaxone	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Chloramphenicol	S (n=3429)	0.57 (0.10)	0.88 (0.67)	0.67 (0.22)	0.34 (0.005)	0.45 (0.15)
	H (n=2130)[c]	-	-	-	-	-

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Summer 2005 n=337	Autumn 2005 n=235	Winter 2006 n=224	Spring 2006 n=290	Summer 2006 n=223	Autumn 2006 n=246
Cefoxitin	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Ceftiofur	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Ceftriaxone	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Chloramphenicol	S (n=3429)	0.60 (0.23)	0.36 (0.11)	0.94 (0.89)	0.40 (0.08)	0.93 (0.87)	0.52 (0.18)
	H (n=2130)[c]	-	-	-	-	-	-

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Spring 2004 n=661	Summer 2004 n=688	Autumn 2004 n=688	Winter 2005 n=929	Spring 2005 n=260
Ciprofloxacin	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Gentamicin	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-
Kanamycin	S (n=3429)	0.70 (0.11)	0.87 (0.51)	0.87 (0.51)	0.76 (0.18)	0.37 (0.02)
	H (n=2130)[c]	-	-	-	-	-
Nalidixic Acid	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)	0.46 (0.02)	0.46 (0.02)	0.22 (0.003)	0.85 (0.52)	0.20 (0.02)

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Summer 2005 n=337	Autumn 2005 n=235	Winter 2006 n=224	Spring 2006 n=290	Summer 2006 n=223	Autumn 2006 n=246
Ciprofloxacin	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Gentamicin	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)[c]	-	-	-	-	-	-
Kanamycin	S (n=3429)	1.2 (0.36)	0.96 (0.89)	0.74 (0.36)	1.4 (0.16)	0.83 (0.54)	0.92 (0.78)
	H (n=2130)[c]	-	-	-	-	-	-
Nalidixic Acid	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)	1.4 (0.28)	0.18 (0.03)	0.42 (0.12)	0.23 (0.03)	0.16 (0.05)	0.45 (0.16)

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Spring 2004 n=661	Summer 2004 n=688	Autumn 2004 n=688	Winter 2005 n=929	Spring 2005 n=260
Streptomycin	S (n=3429)	0.72 (0.03)	0.99 (0.96)	0.86 (0.31)	0.72 (0.02)	0.65 (0.05)
	H (n=2130)	0.76 (0.4)	1.1 (0.8)	1.1 (0.68)	1.4 (0.15)	0.29 (0.05)
Sulfisoxazole	S (n=3429)	1.4 (0.02)	2.2 (<0.001)	2.2 (<0.001)	0.68 (0.02)	1.0 (0.92)
	H (n=2130)	0.78 (0.39)	0.82 (0.47)	1.2 (0.52)	2.0 (0.003)	0.53 (0.14)
Tetracycline	S (n=3429)	1.0 (0.80)	1.7 (0.003)	1.8 (0.001)	1.2 (0.20)	3.1 (<0.001)
	H (n=2130)	0.85 (0.51)	1.4 (0.14)	1.2 (0.36)	1.5 (0.03)	0.51 (0.07)
Trimethoprim / Sulfamethoxazole	S (n=3429)[c]	-	-	-	-	-
	H (n=2130)	0.40 (0.02)	0.86 (0.64)	1.2 (0.55)	1.1 (0.73)	0.56 (0.25)

Table 14 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Summer 2005 n=337	Autumn 2005 n=235	Winter 2006 n=224	Spring 2006 n=290	Summer 2006 n=223	Autumn 2006 n=246
Streptomycin	S (n=3429)	0.95 (0.77)	0.75 (0.19)	0.38 (<0.001)	0.68 (0.05)	0.43 (0.001)	0.44 (<0.001)
	H (n=2130)	1.2 (0.67)	1.1 (0.81)	0.39 (0.13)	0.30 (0.06)	0.55 (0.29)	0.9 (0.82)
Sulfisoxazole	S (n=3429)	1.6 (0.009)	1.1 (0.67)	0.77 (0.28)	0.75 (0.20)	0.96 (0.86)	0.94 (0.77)
	H (n=2130)	0.90 (0.77)	13.9 (<0.001)	0.61 (0.29)	0.4 (0.06)	0.43 (0.12)	0.83 (0.67)
Tetracycline	S (n=3429)	1.2 (0.44)	1.2 (0.55)	1.1 (0.56)	1.1 (0.61)	1.1 (0.77)	1.0 (0.86)
	H (n=2130)	2.3 (0.001)	1.1 (0.72)	0.60 (0.19)	1.1 (0.80)	0.53 (0.13)	0.96 (0.90)
Trimethoprim / Sulfamethoxazole	S (n=3429)[c]	-	-	-	-	-	-
	H (n=2130)	1.4 (0.43)	1.1 (0.78)	0.77 (0.60)	0.23 (0.05)	0.48 (0.25)	0.88 (0.80)

Table 14 (Continued)

[a] Odds-ratios are presented comparing the odds of season resistant *E. coli* isolates for each antimicrobial agent to winter 2004 (referent season).
[b] *p*-values are adjusted for the dependence of season isolate response within each unit location by using the generalized estimating equation (GEE) statistic (STATA™ ver. 9.2, College Station, TX).
[c] Odds-ratios were not reported because the GEE model failed to converge to report the parameter estimates because of the absence of positive outcome (i.e., resistance) in at least one season (zero cell count).

Table 15

Phenotypic resistance of commensal *E. coli* isolates from human wastewater and swine fecal samples. Odds ratios of resistant *E. coli* isolates are presented and contrasted by host species and season-collapsed. Isolate comparisons are across all unit locations, human vocation cohorts and swine production groups.

		Sampled by season-collapsed (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b])		
Antimicrobial agent	Host Species (S=Swine, H=Human)	Spring	Summer	Autumn
Amikacin	S (n=3429)[c]	-	-	-
	H (n=2130)[c]	-	-	-
Amoxicillin /Clavulanic Acid	S (n=3429)	0.94 (0.849)	1.10 (0.765)	0.83 (0.624)
	H (n=2130)	1.86 (0.67)	1.1 (0.82)	1.38 (0.41)
Ampicillin	S (n=3429)	0.96 (0.741)	1.48 (0.001)	0.98 (0.863)
	H (n=2130)	0.62 (0.004)	0.66 (0.012)	0.93 (0.65)
Cefoxitin	S (n=3429)	1.11 (0.758)	1.16 (0.670)	0.85 (0.664)
	H (n=2130)	1.72 (0.137)	0.98 (0.96)	1.04 (0.93)
Ceftiofur	S (n=3429)	2.19 (0.014)	1.37 (0.354)	1.42 (0.306)
	H (n=2130)[c]	-	-	-
Ceftriaxone	S (n=3429)[c]	-	-	-
	H (n=2130)[c]	-	-	-

Table 15 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season-collapsed (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Spring	Summer	Autumn
Chloramphenicol	S (n=3429)	0.71 (0.202)	1.16 (0.529)	0.82 (0.459)
	H (n=2130)	0.41 (0.079)	0.41 (0.083)	0.96 (0.919)
Ciprofloxacin	S (n=3429)[c]	-	-	-
	H (n=2130)	0.35 (0.173)	0.36 (0.184)	0.39 (0.212)
Gentamicin	S (n=3429)	1.29 (0.583)	1.43 (0.431)	1.68 (0.257)
	H (n=2130)[c]	-	-	-
Kanamycin	S (n=3429)	0.93 (0.656)	1.08 (0.612)	1.01 (0.968)
	H (n=2130)	1.07 (0.88)	0.27 (0.087)	0.72 (0.525)
Nalidixic Acid	S (n=3429)[c]	-	-	-
	H (n=2130)	0.40 (0.003)	0.66 (0.069)	0.28 (0.001)
Streptomycin	S (n=3429)	0.89 (0.278)	1.10 (0.391)	0.92 (0.451)
	H (n=2130)	0.48 (0.002)	0.87 (0.485)	0.93 (0.727)
Sulfisoxazole	S (n=3429)	1.40 (0.004)	2.18 (<0.001)	1.95 (<0.001)
	H (n=2130)	0.45 (<0.001)	0.54 (0.001)	1.71 (<0.001)

Table 15 (Continued)

Antimicrobial agent	Host Species (S=Swine, H=Human)	Sampled by season-collapsed (n=5559 overall sample isolates) Odds ratio[a] of resistant *E. coli* (*p*-value[b]) Spring	Summer	Autumn
Tetracycline	S (n=3429)	1.14 (0.244)	1.27 (0.047)	1.29 (0.038)
	H (n=2130)	0.67 (0.013)	1.2 (0.32)	0.95 (0.725)
Trimethoprim / Sulfamethoxazole	S (n=3429)	1.01 (0.991)	2.32 (0.075)	3.03 (0.016)
	H (n=2130)	0.39 (0.001)	0.88 (0.563)	1.10 (0.661)

[a] Odds-ratios are presented comparing the odds of season resistant *E. coli* isolates for each antimicrobial agent to winter 2004 (referent season).
[b] *p*-values are adjusted for the dependence of season isolate response within each unit location by using the generalized estimating equation (GEE) statistic (STATA™ ver. 9.2, College Station, TX).
[c] Odds-ratios were not reported because the GEE model failed to converge to report the parameter estimates because of the absence of positive outcome (i.e., resistance) in at least one season (zero cell count).

4.3.2. Comparison of E. coli *resistance to multiple antimicrobial agents by swine production group*

The relative odds of multiple resistance did not differ significantly ($p > 0.05$). Table 13 provides the proportion of multi-resistant *E. coli* by swine production group for each antimicrobial agent.

4.3.3. Comparison of E. coli *resistance to multiple antimicrobial agents by human vocation cohort*

The relative odds of multiple resistance did not differ significantly ($p > 0.05$). Table 13 provides the proportion of multi-resistant *E. coli* by human vocation for each antimicrobial agent.

4.3.4. Comparison of E. coli *resistance to multiple antimicrobial agents by season*

The relative odds of multiple resistance did not differ significantly ($p > 0.05$) for both human and swine isolates. Tables 11 and 12 provide the proportion of multi-resistant *E. coli* by season and season-collapsed, respectively of human and swine isolates for each antimicrobial agent.

4.4. Multivariate analysis of risk factors for E. coli *resistance to multiple outcomes*

4.4.1. Cluster analysis approach

The six hierarchical agglomerative methods with a squared Euclidean distance were compared with cluster solutions of 10, 15, and 22 in order to identify the cluster fit that best described the binary data. The method with the highest R^2 that explained the highest variability and met our goal and criteria for AR phenotypic clustering was Ward's minimum variance at a 15 cluster solution and with $R^2 = 0.83$ (Table 16). Other

hierarchical methods were not able to identify homogenous clusters as well as Ward's method. For example, single, average, centroid and median linkage methods were unable to identify any useful cluster structure due to the fact that CCC values were all negative, suggesting that the data were too sparse to be grouped by those cluster algorithms. For these methods, most of the isolates were in one large cluster, and the rest were spread out over several clusters with 1-2 isolates per group. However, the complete-linkage method with a 25-cluster solution resulted in 12 well-partitioned clusters, while the remaining 13 clusters had only 1 to 3 isolates each.

The iterative partitioning k-means method was performed on both types of data with squared Euclidian distance proximity measure. Using the cluster solution (n = 15) that was suggested by Ward's minimum variance method, k-means produced 2 large clusters, and 13 small clusters. Ward's minimum variance method established well-defined, homogenous, and balanced clusters for our *E. coli* AR phenotypic data in their binary (i.e., resistant or susceptible) form, accounting for 83% of the variability (Fig. 6). The distribution of the *E. coli* isolates' AR phenotypes over the 15 clusters is shown in table on page 123. Per our objectives, there was no single resistance pattern (phenotype) found in one cluster that also was assigned to another cluster.

Forty-six (46) % of the isolates (233/504) were pan-susceptible and grouped in cluster A. Single and multiple resistant phenotypes ranged from 1 to 12 antibiotics. Phenotypes exhibiting single resistance to ampicillin and tetracycline were found in clusters D and E, respectively, accounting for 7% (36/504) of the isolates. Double resistance patterns existed in 28% (139/504) of the isolates and appeared in clusters

Table 16

Cluster generation history for 504 *E. coli* isolates using Ward's minimum variance.

Cluster solution	R^2 [a]	CCC [b]	Pseudo-F [c]	PST2 [d]
25	0.916	76.5	217	2.8
24	0.910	74.0	212	11.1
23	0.904	65.0	206	6.5
22	0.896	62.2	199	5.5
21	0.889	59.6	193	3.0
20	0.881	56.9	188	7.2
19	0.872	54.5	184	5.6
18	0.863	52.3	181	5.3
17	0.855	50.3	179	11.6
16	0.845	48.2	177	14.8
15	0.834	45.9	175	3.6
14	0.821	43.3	172	3.5
13	0.807	41.0	172	52.4
12	0.792	38.5	170	.[e]
11	0.775	36.1	170	.[e]
10	0.755	33.1	169	6.7
9	0.731	29.9	168	25.1
8	0.706	27.1	170	30.5
7	0.673	23.6	171	22.6
6	0.637	18.2	175	25.4
5	0.581	13.2	173	758
4	0.511	6.74	174	94.5
3	0.399	- 0.71	166	43.8
2	0.238	- 4.3	157	255
1	0.000	0.00	.	157

[a] squared multiple correlation, [b] cubic clustering criterion, [c] pseudo-F, and [d] pseudo-T^2 statistics.

[e] values of pseudo-T^2 were not reported because $J_e(1)$ (the sum of square error before splitting the data) was zero.

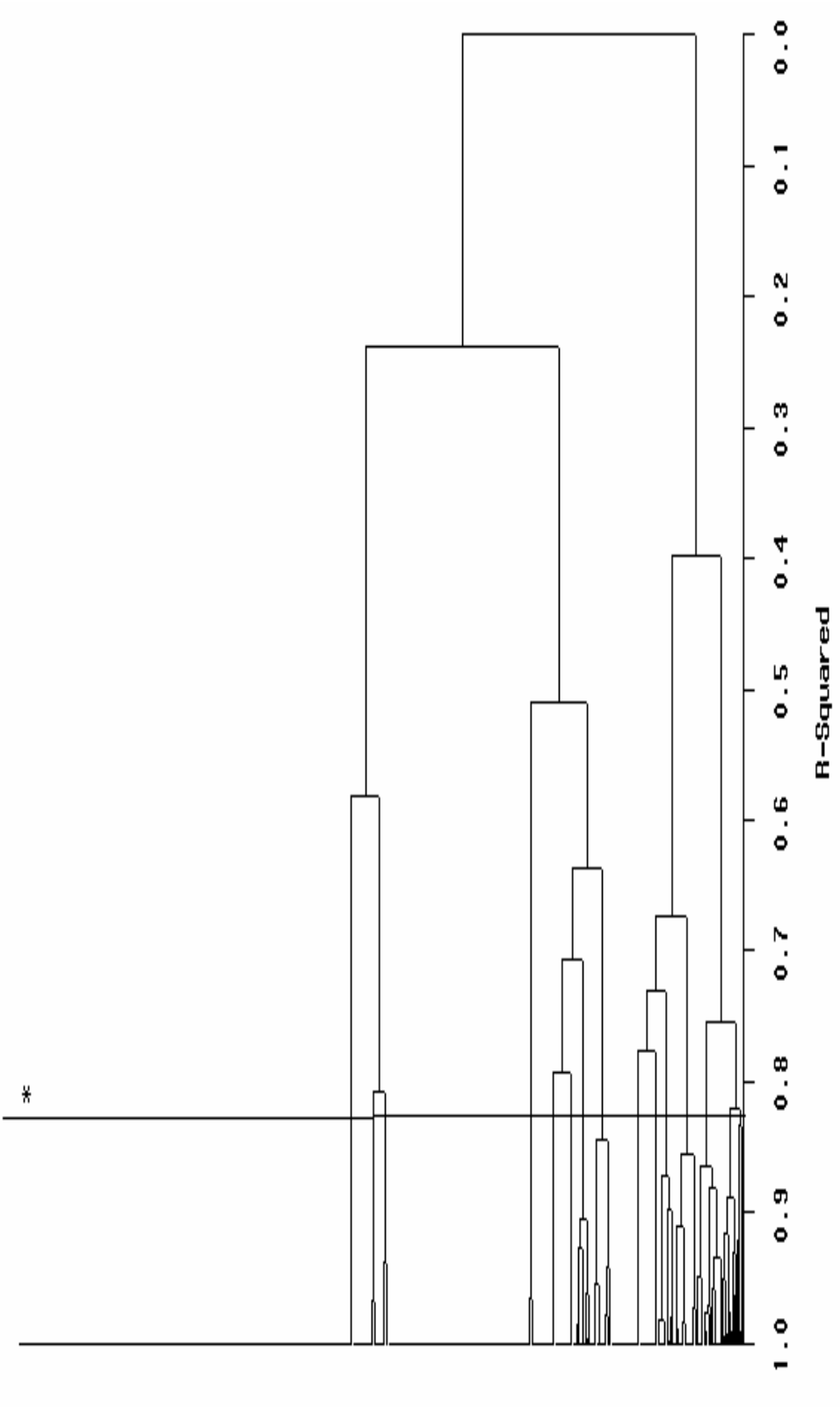

Fig. 6. A dendrogram showing different clusters for 504 *E. coli* AR phenotypic binary data using Ward's minimum variance method. R^2=1 when each isolate is a cluster by itself on the tree. The vertical line (*) where R^2=0.83 specifies a 15-cluster solution.

Table 17

AR binary phenotypic clusters for 504 *E. coli* isolates using Ward's minimum variance method. The table shows 15 clusters and the number of isolates per cluster. The grey-shaded boxes show the number of resistant isolates to each antimicrobial agent (NARMS 2001 panel).

	Antimicrobial agent[a]															No. of
Cluster	AUG	AMP	FOX	TIO	CRO	CEP	CHL	CIP	GEN	KAN	NAL	STR	SUL	TET	SXT	isolates
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	233
B	0	0	0	1	0	100	0	0	0	0	0	0	0	0	0	100
C	0	0	0	0	0	0	0	0	0	0	15	0	0	1	0	15
D	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	16
E	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	20
F	0	5	0	0	0	4	0	0	0	0	1	0	12	0	1	12
G	0	12	0	0	0	12	0	0	0	0	0	0	0	0	0	12
H	0	0	0	0	0	12	0	0	0	0	0	0	0	12	0	12
I	0	2	0	0	0	9	0	0	0	0	9	0	0	0	0	9
J	15	8	0	0	0	15	0	0	0	0	2	0	2	1	0	15
K	0	16	0	0	0	12	0	0	0	1	1	8	0	16	0	16
L	0	0	0	0	0	2	0	1	0	3	0	11	4	10	0	11
M	1	14	0	0	0	13	0	0	0	1	0	11	18	18	15	18
N	1	9	0	0	0	7	5	0	1	0	2	10	9	2	9	10
O	2	5	0	0	0	4	2	4	3	2	4	4	5	3	5	5

[a] amoxicillin/clavulanic acid (AUG), ampicillin (AMP), cefoxitin (FOX), ceftiofur (TIO), ceftriaxone (CRO), cephalothin (CEP), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SUL), tetracycline (TET), trimethoprim/ sulfamethoxazole (SXT).

B, G and H with resistance to cephalothin combined with ceftiofur, ampicillin, or tetracycline, and in cluster C: nalidixic acid with tetracycline. At least one of the 4 antibiotics: ampicillin, cephalothin, nalidixic acid, or tetracycline was found in one or more of the multiple resistance clusters (B – O).

We were unable to obtain meaningful cluster distributions that best described ordinal data (i.e., MIC), with or without standardization or transformation using the six hierarchical agglomerative and k-means approaches. Similar resistance patterns (phenotypes) that were found in one cluster were also found in one or more other clusters, which violated our objectives. Note that these results arose from analyses performed on the NARMS 2001 panels and preceded the samples described from the longitudinal analyses.

4.4.2. Multivariate model of correlated dependence

Seven antimicrobial agents were excluded from the multivariate analysis model because the GEE model failed to converge; that is, it failed to report the parameter estimates with all 15 antimicrobial agents included. Therefore, 8 antimicrobial agents were included in the final analysis (Table 18). The multivariate odds-ratios, adjusted for multiple binary outcomes (i.e., multivariate phenotypes), were increased relative to unadjusted odds-ratios among swine isolates when compared to human isolates. This was especially true for tetracycline (with the highest change (increase) in odds-ratios compare to other antimicrobial agents) (OR = 21.8 vs. 19.6). The adjusted versus unadjusted odds-ratios for the other antimicrobial agents are shown in Table 18.

Table 18

Multivariate resistance phenotypes of commensal *E. coli* isolates from human wastewater and swine fecal samples. The odds ratios and 95 % confidence intervals (CI) of *E. coli* isolates are presented and contrasted by host-species and human swine-workers as well as swine production groups. Isolate comparisons are across all unit locations and seasons.

Antimicrobial agent	Adjusted for dependence among binary outcomes?[b]	Odds ratios of multivariate resistant *E. coli* phenotypes (95% CI)[a]							
		Host species (S=Swine)[c]	Swine-worker isolates	Isolation boar isolates	Breeding boar isolates	Farrowing sows & piglet isolates	Breeding/gestation females isolates	Grower & finisher pig isolates	Nursery pig isolates
Amoxicillin /Clavulanic Acid	No	0.8 (0.44 - 1.48)	1.1 (0.65 - 1.85)	0.82 (0.61 - 1.1)	0.21 (0.04 - 1.17)	1.6 (0.76 - 3.4)	0.35 (0.07 - 1.77)	0.32 (0.22 - 0.46)	2.9 (0.80 - 10.3)
	Yes	0.72 (0.33 - 1.59)	0.96 (0.54 - 1.72)	0.85 (0.62 - 1.15)	0.12 (0.01 - 2.4)	1.3 (0.60 - 2.82)	0.23 (0.02 - 2.69)	0.31 (0.21 - 0.46)	2.4 (0.59 - 9.76)
Ampicillin	No	1.13 (0.71 - 1.82)	0.87 (0.60 - 1.26)	5.3 (4.56 - 6.27)	1.5 (0.87 - 2.47)	1.5 (1.22 - 1.95)	0.6 (0.37 - 1.02)	0.62 (0.47 - 0.83)	1.3 (0.90 - 2.01)
	Yes	1.21 (0.76 - 1.90)	0.86 (0.60 - 1.24)	4.9 (3.97 - 5.97)	1.6 (0.96 - 2.52)	1.6 (1.24 - 2.12)	0.65 (0.38 - 1.11)	0.63 (0.46 - 0.86)	1.4 (0.95 - 2.12)
Chloramphenicol	No	2.02 (1.09 - 3.75)	1.6 (0.73 - 3.61)	9.4 (5.27 - 16.81)	0.64 (0.06 - 6.46)	1.4 (0.35 - 5.63)	1.2 (0.24 - 5.83)	1.8 (0.68 - 4.70)	3.7 (0.96 - 14.12)
	Yes	2.04 (1.04 - 4.0)	1.4 (0.64 - 3.19)	8.4 (4.64 - 15.29)	0.66 (0.1 - 4.58)	1.4 (0.41 - 5.0)	1.1 (0.28 - 4.40)	1.9 (0.76 - 4.63)	3.6 (0.97 - 13.0)
Kanamycin	No	10.02 (5.56 - 18.06)	0.67 (0.32 - 1.41)	28.5 (18.23 - 44.56)	8.5 (5.0 - 14.54)	6.2 (3.34 - 11.65)	5.1 (1.98 - 13.40)	4.7 (2.7 - 8.03)	17.7 (6.82 - 45.94)
	Yes	9.28 (4.38 - 19.67)	0.67 (0.33 - 1.34)	33.6 (21.26 - 53.06)	7.4 (4.12 - 13.32)	5.3 (2.84 - 9.99)	4.7 (1.74 - 12.51)	4.3 (2.4 - 7.54)	16.3 (5.73 - 46.60)
Streptomycin	No	3.47 (2.68 - 4.48)	1.1 (0.71 - 1.65)	5.4 (4.3 - 6.9)	3.8 (2.64 - 5.61)	4.4 (3.43 - 5.56)	1.9 (1.26 - 2.78)	2.4 (1.88 - 3.16)	8.9 (6.53 - 12.20)
	Yes	3.43 (2.66 - 4.43)	1.1 (0.71 - 1.63)	5.7 (4.5 - 7.2)	3.7 (2.55 - 5.33)	4.2 (3.22 - 5.39)	1.8 (1.2 - 2.68)	2.5 (1.87 - 3.21)	8.6 (6.11 - 12.15)

Table 18 (Continued)

Antimicrobial agent	Adjusted for dependence among binary outcomes?[b]	Odds ratios of multivariate resistant *E. coli* phenotypes (95% CI)[a]							
		Host species (S=Swine)[c]	Swine-worker isolates	Isolation boar isolates	Breeding boar isolates	Farrowing sows & piglet isolates	Breeding/gestation females isolates	Grower & finisher pig isolates	Nursery pig isolates
Sulfisoxazole	No	1.99 (1.39 - 2.86)	0.72 (0.56 - 0.93)	6.0 (5.14 - 6.90)	0.9 (0.52 - 1.64)	1.6 (1.26 - 1.96)	0.8 (0.55 - 1.20)	1.3 (1.0 - 1.71)	4.1 (2.97 - 5.70)
	Yes	1.97 (1.35 - 2.87)	0.69 (0.53 - 0.91)	6.3 (5.4 - 7.24)	0.84 (0.44 - 1.60)	1.5 (1.12 - 1.80)	0.75 (0.49 - 1.15)	1.3 (0.99 - 1.72)	3.9 (2.79 - 5.31)
Tetracycline	No	19.56 (12.75 - 30.07)	1.3 (0.98 - 1.63)	202.3 (131.6 - 310.9)	28.1 (14.5 - 54.2)	15.3 (10.9 - 21.34)	15.7 (10.1 - 24.4)	22 (13.5 - 35.98)	30.7 (18.6 - 50.7)
	Yes	21.78 (14.22 - 33.36)	1.4 (1.13 - 1.69)	311.7 (261.6 - 371.4)	38.8 (17.65 - 84.75)	20.0 (13.97 - 28.54)	19.6 (12.54 - 30.64)	22.9 (13.9 - 27.78)	42.7 (24.83 - 73-27)
Trimethoprim / Sulfamethoxazole	No	0.15 (0.095 - 0.24)	0.97 (0.63 - 1.49)	0.16 (0.11 - 0.23)	0.26 (0.05 - 1.42)	0.16 (0.07 - 0.34)	0.1 (0.02 - 0.58)	0.08 (0.05 - 0.14)	0.23 (0.0003)
	Yes	0.11 (0.056 - 0.23)	0.94 (0.6 - 1.48)	0.27 (0.2 - 0.35)	0.20 (0.02 - 1.92)	0.11 (0.025 - 0.45)	0.08 (0.008 - 0.63)	0.08 (0.05 - 0.15)	0.18 (0.058 - 0.58)

[a] The antimicrobial agent odds-ratios (unadjusted versus adjusted for dependence among resistance phenotypes) and their 95 % CI are examined for: 1) host species (swine versus human (referent))[c], 2) human swine-workers, isolation boars, breeding boars, farrowing sows and piglets, grower-finisher pigs, Breeding/gestation females, and nursery pigs, compared to human non-worker cohort (referent).

[b] Adjustment for dependence among multivariate resistance phenotypes (multiple binary outcomes) within each unit location was conducted by a GEE statistic in a univariate and multivariate model using a SAS® macro (SAS® PROC GENMOD, SAS Institute, Inc., Cary, NC).

The adjusted odds-ratios of multiple binary outcomes (i.e., multivariate phenotypes) were increased significantly ($p < 0.05$) relative to unadjusted odds-ratios among swine-worker isolates as compared to swine non-worker isolates only for tetracycline (OR = 1.4 vs. 1.3). The changes in adjusted odds-ratios relative to the unadjusted odds-ratios (increased or decreased) were variable among swine-workers and swine production group isolates when compared to swine non-workers (Table 18, columns 4 – 10).

4.5. Genotypic analysis

Repeatable standard curves, within the acceptable range of slope (-3.1 – -3.6), or efficiency (90 – 110%) (Chico et al., 2006), were established for both the target gene (bla_{CMY-2}) and the reference gene (*rpoB*). Illustration of the bla_{CMY-2} and *rpoB* gene standard curves is shown in Figures 7 and 8.

The genotypic analysis of unknown community DNA samples remains incomplete (as of August 6, 2007) and is contingent upon resolving the problem of quantifying the *rpoB* gene, which is a single copy conserved genomic gene (Mollet et al., 1997; Dahllof et al., 2000). The gene quantities (copy numbers/µl) appeared to be highly variable (sometimes very low relative to the bla_{CMY-2} copy numbers) among the community DNA samples extracted from both human wastewater and swine fecal matter. This observation was based on the ratio of the *rpoB*: bla_{CMY-2} which ranged from 27,611:1 to 14:1 in swine community DNA samples and 1637:1 to 18:1 in human community DNA samples. Therefore, we assumed that *rpoB* copy numbers cannot possibly reflect what is expected to correspond to the number of bacterial cells in a community DNA sample.

The concentration of tested community DNA samples ranged from 5.0 to 35.5 ng/µl and from 6.0 to 40.0 ng/µl in human and swine samples, respectively.

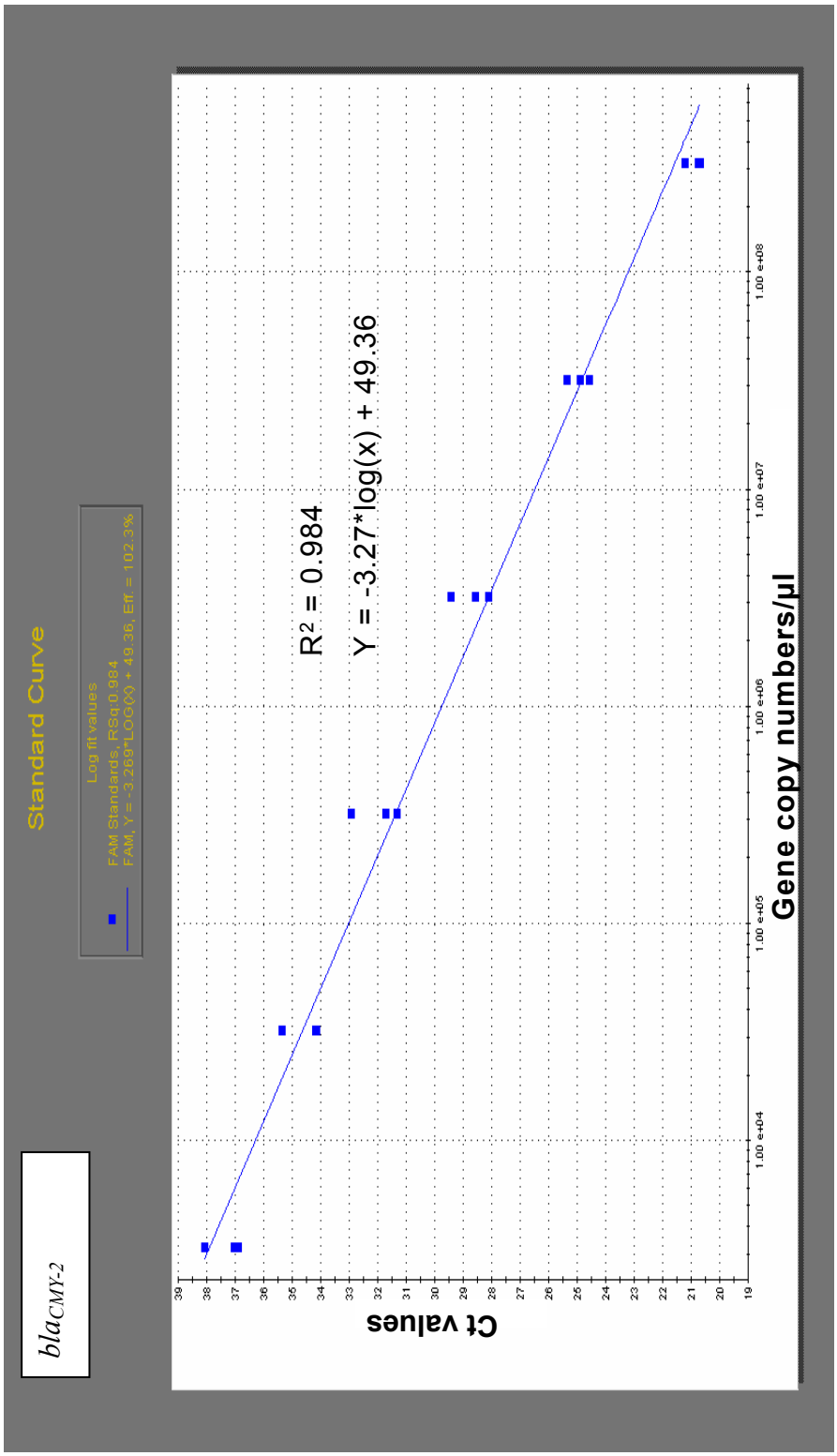

Fig. 7. The resulting standard curves generated by plotting the gene copy numbers per µl of target gene (bla_{CMY-2}) versus threshold cycle (Ct values).

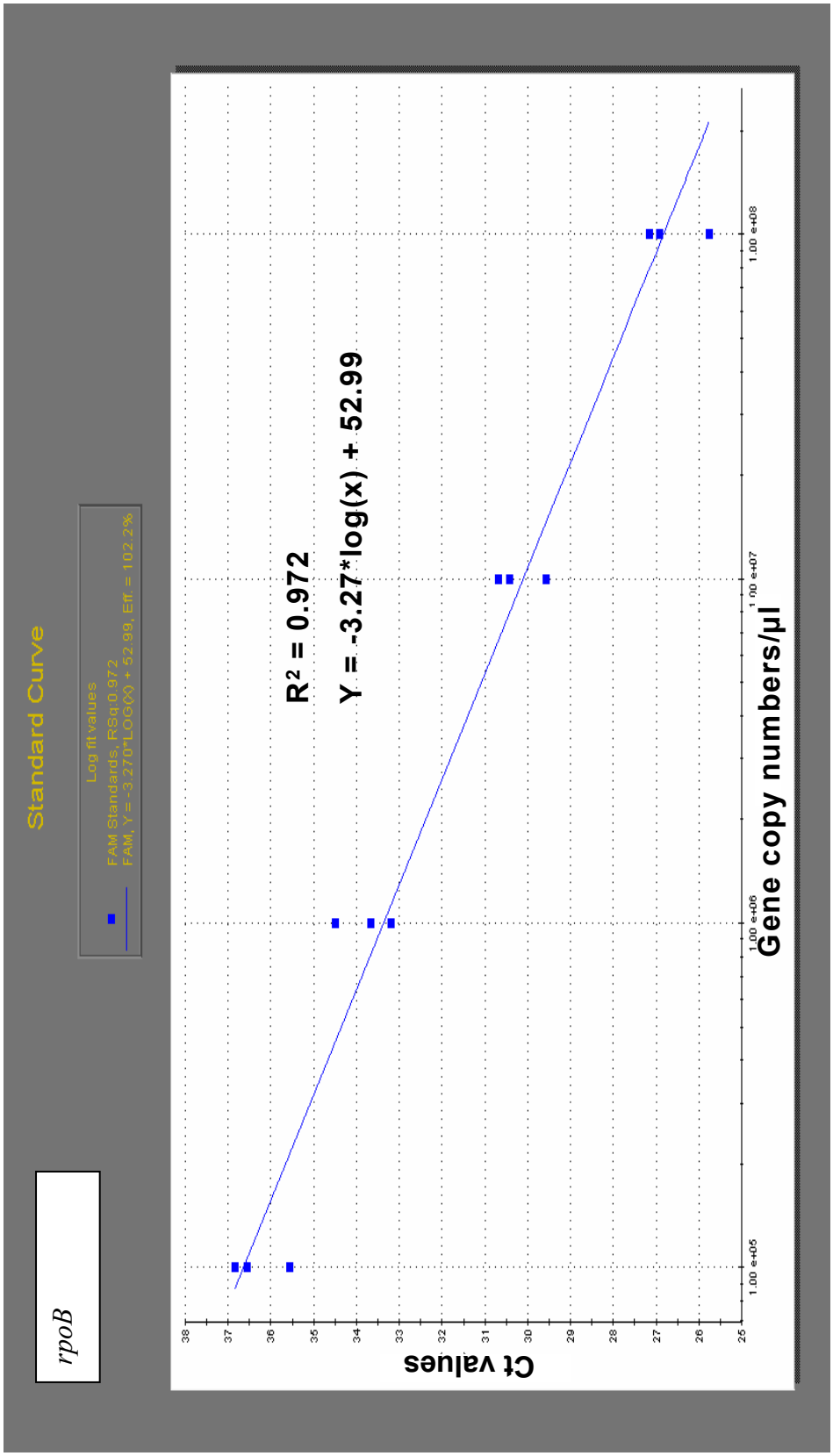

Fig. 8. The resulting standard curves generated by plotting the gene copy numbers per µl of reference gene (*rpoB*) versus threshold cycle (Ct values).

CHAPTER V

DISCUSSION

Our pilot cross-sectional study in 2003 – 2004 initially suggested that human occupational exposure to swine farms and slaughtered pigs was not associated with an increased risk of *E. coli* tetracycline resistance phenotype prevalence (Scott et al., 2005). However, cross-sectional studies lack a temporal component measuring the changes in the bacterial isolates resistance level over a long period of time. This present study is the first reported to longitudinally assess the risk of elevated prevalence of AR bacteria, at the group-level, possibly arising from animals to humans as a result of direct occupational exposure to animals (i.e., swine); especially when taking into account the challenges that other studies were faced with (discussed in Chapter II). To summarize the advantages of our system in brief, the human and swine populations had a very limited movement to and from the system, and occupational and food exposures were known. The human populations consisted of 1) swine-workers: exposed to swine rearing operations or slaughtered pigs, 2) non-workers that were not exposed to swine (there was no mixing between worker and non-worker populations), and 3) a non-worker and non-consumer group representing a negative control population that was sampled at several of the units. All the pigs from the swine operations were slaughtered and pork products were fed back to human (consumers) within the system. Pork trim were imported from outside the system and each lot was sampled to assess AR bacteria introduced to the system.

The target population is similar semi-closed human and swine populations systems in the US. We believe that the internal validity (to obtain unbiased estimates based on the study data and make unbiased inferences about the associations of interest in the target population) of our study is strong because of the uniqueness of the study design and populations as well as the nature of the study system. Also, the present study allows us to make inferences from the study population applicable to the target population. However, every study is prone to some types of bias. In particular, we could be underestimating the 'true' prevalence and distribution of AR bacteria in human and swine populations because of the biases associated with the phenotypic analysis of wastewater and fecal matter samples. These biases are related to: 1) the need for isolating the target organism, 2) missing unculturable bacterial cells that may carry resistance traits, 3) measuring the gene expression *in vitro*, 4) testing a colony out of a sample that contains millions of *E. coli* isolates, and 5) the added enrichment of the human wastewater sample that may favor sensitive strains over resistant strains.

The enrichment of the human wastewater samples and not the swine fecal samples could potentially cause a differential misclassification bias. This bias can occur when: 1) *E. coli* bacteria shed their resistant plasmids in the enrichment broth that affecst the level of resistant bacteria growth on CHROM agar for human samples, 2) higher numbers of sensitive bacteria growth in the broth compared to resistant ones; whereas, *E. coli* bacteria were directly streaked on CHROM agar from fecal samples. This could potentially bias the measure of association (OR) of human versus swine regarding resistance levels away from the null.

On the other hand, the external validity (ability to draw inferences to populations beyond the target population based on the study data) is questionable. This is because our study agri-food system model was semi-closed in nature where: 1) human movement was limited, and 2) the antimicrobial use in the human and swine populations was different from that of the general US population (personal communication with the human medical officer and the swine specialist veterinarian). Nonetheless, it is important not to sacrifice internal validity to gain strong external validity because it is worthless to extrapolate biased estimates (i.e., results) to the target population (Dohoo et al., 2003).

Our study design does not follow the classic characteristics of cohort studies; rather, it is more appropriately termed a longitudinal study, with repeated cross-sectional measurements of both exposure and outcome. In cohort studies, study subjects generally do not have the outcome of interest at the start of the follow-up period; therefore, incidence of the outcome in the cohorts defined by the exposure is compared during the follow-up period (Dohoo et al., 2003). Therefore, in our study, the freedom of the study subjects of the outcome (i.e., the prevalence of AR bacteria) cannot be determined. Also, prevalence was the measure of the outcome frequency and not incidence.

The sample collection and analysis was conducted at the group-level in this study. Hence, the interpretation of results and inferences were therefore also at the group-level, and not at the individual-level. Studies that draw inferences at the individual-level based on data collected at the group-level fall into what is called ecological fallacy (Dohoo et al., 2003), which was not the case in our study. The collection of group-level data was

preferred in our study because of: 1) the constraints on measuring resistance at the individual level due to: a) the ethical and security reasons related to sampling individual humans, and b) swine aggregate fecal samples are more representative of the large number of pens/pigs at different production stages than individual samples, 2) antimicrobial resistance is a population-based problem (both for bacteria and hosts), and not an individual one, and 3) the variability within groups (humans and swine) based on the exposure to swine is expected to be small relative to the variability among different groups. That is because of the management practices within swine groups are the same (personal communication with the swine specialist veterinarian), and also the living environment and food sources (standards) are virtually the same within the exposure groups (e.g., workers and non-workers) within units (personal communication with the wastewater facilities manager).

Assessing the risks of AR bacterial carriage due to human exposure (i.e., occupational) to animals and animal food products (i.e., contaminated food with AR bacteria) were evaluated in several cross-sectional studies (Aubry-Damon et al., 2004; Bates, et al., 1994; Nijsten et al., 1994; Nijsten et al., 1996b; Stobberingh et al., 1999; Van den Bogaard et al., 2001; Van den Bogaard et al., 2002). These studies suggested that the risk of presence of AR bacteria in pig farmers or slaughter-plant workers increased: 1) as a result of direct exposure to reared pigs or slaughtered pigs compared to suburban residents (Nijsten et al., 1994), 2) as a result of farmers' exposure to their pigs (Nijsten et al., 1996b), 3) in pig farmers (i.e., farmer exposure to pigs) compared to insurance company workers (Aubry-Damon et al., 2004), 4) as a result of exposure to

poultry through farming or working at poultry slaughter-plant compared to suburban residents (Stobberingh et al., 1999), and 5) in poultry farmers (as a result of farmers' exposure to poultry) compared to poultry slaughter-plant workers (Van den Bogaard et al., 2001; Van den Bogaard et al., 2002). However, these studies all faced the common challenges that accompany assessing the risk of transmission AR bacteria in a general population. The majority of these challenges were a result of the limited or lack of control over the study population dynamics (movement of humans and animals), follow-up of human cohorts or individual study subjects over time, and human travel and multiple sources of food products of animal origin that can potentially carry different resistant organisms and may introduce them to susceptible populations.

In our study, the swine *E. coli* isolates expressed greater levels of resistance to 7 individual antimicrobial agents, as compared to human isolates, with the highest level of resistance to tetracycline, followed by kanamycin, ceftiofur, gentamicin, streptomycin, and lowest for chloramphenicol as well as sulfisoxazole. The higher levels of resistance to these antimicrobial agents in swine fecal *E. coli* isolates compared to human wastewater isolates is likely associated with either past or current antimicrobial use at these and other swine farms (e.g., as ceftiofur sodium (injectable) and florfenicol (injectable)), and at a larger scale than in human medicine such for chlortetracycline (in feed) and neomycin (in water for nursery piglets). On the other hand, human isolates were at greater probability of expressing resistance to 3 individual agents, when compared to swine isolates, with the highest relative odds for ciprofloxacin, followed by trimethoprim/sulfamethoxazole, and lowest for nalidixic acid. Once again, this might be

explained by the use of these antimicrobial agents mainly in human medicine, and rarely, if ever, in the swine industry (especially in our study population). Nijsten et al. (1996b) reported similar findings to ours, where significantly ($p < 0.05$) higher levels of resistance among swine *E. coli* isolates were found for oxytetracycline, streptomycin, sulphamethoxazole and chloramphenicol, when compared to human isolates arising from pig farmers. However, none of the human isolates in their paper were at significantly ($p < 0.05$) higher levels of resistance when compared to swine, except for nalidixic acid, which exhibited very low, but slightly higher levels of resistance in humans (2%) than in swine (0%) (though not significantly ($p > 0.05$)). The authors explained this as being due to the larger amount of total antimicrobial given to pigs at 125 mg/kg a year versus 75 mg/kg consumed by humans. Furthermore, the very low number of isolates resistant to nalidixic acid and ciprofloxacin in one or both of the host-species does not permit any inferences to be made. Resistance to trimethoprim/sulfamethoxazole was not measured in their study and cannot be compared to our study. Campbell et al. (2005) found that *E. coli* isolates from swine fecal matter had a higher prevalence of class 1 integrons than those from human wastewater; however, human isolates were more likely to harbor an AR gene cassette in their integrons than swine isolates. That study was conducted in the same agri-food system as our study, but was limited in scope and scale.

Swine fecal *E. coli* isolates exhibiting multi-drug resistance were present at higher levels when compared to human isolates. Sixty-two percent of the human *E. coli* isolates were pan-susceptible, 20% were single-resistant, and 18% were resistant to 2 or more antimicrobial agents. In contrast, only 13% of the swine *E. coli* isolates were pan-

susceptible, 42% were single-resistant, and 45% were resistant to 2 or more antimicrobial agents. The higher levels of multi-drug resistance in the swine population, when compared to the human population, might be attributed to several factors: 1) prophylactic/subtherapeutic use of several antimicrobial agents in-feed at the swine farms, and 2) intensive farm management practices on swine farms that may facilitate the transmission, propagation, and maintenance of the AR bacterial populations. In the Nijsten et al. (1996b) study, 74% of *E. coli* isolates from pigs showed resistance to 2 or more antimicrobial agents, whereas 43% of isolates from pig farmers were resistant to 2 or more agents. In addition, those authors reported that 15% of pig isolates were pansusceptible versus only 34% of those from pig farmers. In another study, the highest multi-drug resistance was detected among *E. coli* isolates from swine, when compared to humans, and other domestic and wildlife isolates (Sayah et al., 2005).

The relative odds of resistance were significantly increased ($p < 0.05$) among swine-worker isolates for tetracycline (OR = 1.36) when compared to non-workers, with the highest prevalence (25.9%) in slaughter-plant workers, followed by swine-workers (23.1%), non-workers (17.6%), non-workers and non-consumers (14.3%), and influent mixture (22.8%). Ibekwe and Grieve (2003) reported a larger difference in the *E. coli* resistance prevalence for tetracycline between workers (pig farmers) (79%) compared to suburban (non-worker) residents (36%). Van den Bogaard et al., (2001) detected higher oxytetracycline resistance prevalence in *E. coli* isolates from turkey farmers (79%) and broiler farmers (61%) when compared to turkey slaughter-plant workers (55%) and broiler slaughter-plant workers (43%). Conversely, in our study, we detected slightly

higher tetracycline resistance prevalence in slaughter-plant workers when compared to swine-workers. Nevertheless, the OR measure of association was significantly higher for tetracycline resistance in swine workers (OR = 1.36, 95% CI: 0.41 – 1.57) compared to swine non-workers (referent), but was not significantly different in slaughter-plant workers (OR = 1.17, 95% CI: 0.54 – 2.54) compared to swine non-workers. This is likely because of the low number of isolates from the slaughter-plant workers (providing insufficient statistical power). Therefore, to detect a statistically significant difference when it truly exists between the slaughter-plant worker isolates and those from non-workers, a larger number of isolates from the former cohort would be needed (i.e., greater statistical power). Thus, at this time no concrete conclusions can be made regarding the occupational exposure to slaughtered pigs in relation to the *E. coli* resistance prevalence in our study system.

The relative odds of resistance did not differ significantly for *E. coli* AR isolates from pork consumers as compared to non-consumers. Once again, the number of isolates from the non-consumer cohort is probably too low to detect a statistically significant difference when it exists (low statistical power). Higher numbers of isolates are also needed from this cohort to definitively determine a difference. Therefore, no concrete conclusions can be made regarding the consumer status (i.e., consumption of pork) in relation to the *E. coli* resistance prevalence. In general, when speaking of dose (logs of bacteria), the risk of exposure to foodborne *E. coli* (e.g., pork contaminated with resistant bacteria) is much lower than the risk of occupational exposure to animals that shed AR bacteria (e.g., pigs in our study system). This is because: 1) the slaughter-plant

processing techniques are designed to reduce or eliminate contamination of pork with AR bacteria, specifically resistant *E. coli* and other enteric pathogens, 2) the slaughtered pigs are skinned, washed rigorously after evisceration, inspected by a 3rd party, meat parts contaminated with feces are disposed of, and pork is chilled immediately after processing (personal communication with the swine specialist veterinarian), 3) the imported pork trim had a very low *E. coli* prevalence (7.5%), and 4) food preparation (e.g., cooking) in kitchens within the units most often reduces or eliminates AR bacteria found on uncooked meat.

In general, the isolation (i.e., purchased) boars showed higher levels of resistance compared to swine-rearing and slaughtered pigs for the following antimicrobial agents: ampicillin, chloramphenicol, kanamycin, sulfisoxazole, and tetracycline. In contrast, nursery piglets showed higher levels of resistance than isolation boars, other swine-rearing and slaughtered pigs for the following antimicrobial agents: cefoxitin, ceftiofur, gentamicin, and streptomycin. The isolation (purchased) boars' higher resistance levels relative to the swine-rearing facilities and slaughter-plant pigs may be attributed to unknown, but likely higher historical antimicrobial use within the outside multiplier units. Furthermore, in our study population, nursery piglets received larger amounts of injectable antimicrobial agents compared to the other swine production groups (data not shown). Dunlop et al. (1998a) reported that the individual resistance prevalences among *E. coli* isolated from finisher pigs were tetracycline (71%), sulfisoxazole (38%), ampicillin (29%), and gentamicin (0.6%), all of which were similar in trend to the resistance levels among grower-to-finisher pig isolates in our study: tetracycline

(83.1%), sulfisoxazole (20.6%), ampicillin (11.2%), and gentamicin (0.8%). Mathew et al. (1998) found a higher prevalence of *E. coli* tetracycline resistance in grower pigs (98.8%) compared to nursery piglets (94.4%) and farrowing piglets (92%) in Tennessee commercial swine farms. However, they pooled their samples by groups of four, which biased the resistance prevalence upwards. Furthermore, gentamicin resistance in nursery piglets was higher (92.0%) when compared to grower pigs (86.0%) and farrowing piglets (73.9%). However, in our study, tetracycline resistance was higher in nursery piglets (91%), than grower-finisher (83.1%) and farrowing sows and piglets (82.4%). Moreover, a similar trend among swine production groups was observed with gentamicin resistance prevalences in our study (was higher in nursery piglets (8.7%), than grower-finisher (0.8%) and farrowing sows and piglets (1.2%)) but at much lower resistance prevalence compared to the Mathew et al. (1998) study. Those authors reported that the study commercial swine farms used oxytetracycline in-feed and injectable as well as gentamicin in-feed, whereas little or no gentamicin has been used in water and injectable in piglets, and none in feed, within the swine operation in the present study (personal communication with the swine specialist veterinarian). In contrast to our study, Docic and Bilkei (2003) have reported that oxytetracycline was higher in breeding sow *E. coli* isolates than from fattening (i.e., grower-finisher) pigs, suckling (i.e., farrowing) piglets, and weaning (i.e., nursery) piglets in herds that used antibiotics for treatment and growth promotion. Furthermore, those authors showed higher resistance levels in suckling piglets and weaning piglets to ampicillin, gentamicin

and sulfamethacin as compared to breeding sows and fattening pigs, which agrees with our study results (see Table 7).

In our study, imported pork trim samples had very low levels of AR *E. coli* isolates (7.5%) suggesting that a very small proportion of resistant bacteria was introduced from outside to the system through imported pork. To the best of our knowledge, there is no comparable literature data on AR bacteria level in pork trim or pork fat.

There was a high variability observed among seasonal (winter, spring, summer, and autumn) samples over the 3-year period for both human and swine; both for individual antimicrobial agent resistance, and multi-drug resistance phenotypes. Parveen et al. (2006) reported strong seasonal differences in AR prevalences for individual antimicrobial agents in *E. coli* isolates obtained from livestock (swine, dairy, poultry and beef) composite samples over a 1-year period. The seasonal variability was observed even within livestock operations that have very similar resistance phenotypes (Parveen et al., 2006). The source of the seasonal variability was not determined in their study. We have ongoing analyses to determine the likely source of the seasonal variability; whether it be attributed to seasonal differences in historical and concurrent antimicrobial use in swine and human populations; or else other risk factors related to management and selective bacterial survival at different times of year.

We assessed the mean number of *E. coli* colonies exhibiting distinctly different resistance phenotypes in samples collected during a single month (February 2005) for both host species. The mean numbers were 2.25 and 1.98 for human wastewater and swine fecal samples, respectively, and the median was 2 for both. This was similar to

the findings in the Berge et al. (2003) study. Those authors reported a mean of 1.8 phenotypes per 5 *E. coli* colonies from dairy calf fecal samples with a total of 5366 isolates evaluated. With a mean of less than 2.3, the additional resources of performing 5 isolates per sample, and the greater interest in assessing resistance level longitudinally over the 3-year period, we elected instead to assess 1 isolate per sample to test for antimicrobial susceptibility.

Adjusting for the dependence in the multiple binary outcomes (i.e., multivariate phenotypes) using the SAS macro had a variable effect on the odds-ratio values (i.e., 3 – 53% increase or decrease) and their confidence intervals, when compared to unadjusted odds-ratios and confidence intervals. In general, there was not a dramatic change in the odds-ratios direction (e.g., < 1 to >1 or vice versa) when adjusted for multiple binary outcomes. However, the confidence interval for swine-workers did not include 1 (null value) when adjusted for multiple binary outcomes, indicating that the odds-ratio for tetracycline resistance became statistically significant when compared to non-workers. The changes in adjusted odds-ratios relative to the unadjusted odds-ratios were variable among swine-workers and swine production group isolates when compared to non-workers (see Table 18, columns 4 – 10). Furthermore, the adjusted odds-ratios of multiple binary outcomes were increased relative to unadjusted odds-ratios among swine isolates when compared to human isolates for tetracycline (highest change in odds-ratios compare to other antimicrobial agents). Seven antimicrobial agents were excluded from the multivariate model because the positive outcome (i.e., resistance) was exceedingly rare for both human and swine isolates, and as such did not fit the error distribution

appropriately for asymptotic estimates. Exact methods (e.g., Fisher's) might have worked better, but the SAS macro did not permit such an analysis, and software are lacking at this time to deal with multivariate dependent outcomes that are binary. Indeed, there is little or no published work that adjusts for the dependence among multiple binary AR outcome data. Adjusting for the dependency among the multiple binary outcomes (i.e., phenotypes) is needed because of the existing correlation among resistant phenotypes. For instance, an isolate that is resistant to one antimicrobial agent (e.g., ceftriaxone) is more likely to be resistance to another agent (e.g., ceftiofur) (Dunne et al., 2000). We used an exchangeable correlation structure in the SAS macro to adjust for clustering (i.e., dependency) among phenotypes and within unit location. The exchangeable correlation structure is used mainly when the same correlation is expected to exist among all pairs of observations (i.e., phenotypes) within the specified cluster (i.e., unit).

Cluster analysis techniques are descriptive, non-analytical, statistical methods. The goal of using this analytic approach was to describe the homogeneous groups present among multiple AR phenotypes based on proximity measures and cluster algorithms that minimized within-cluster variability and maximized between-cluster variability. It is important to examine the recovered clusters to determine whether they have an isolate phenotype that is present in one cluster and ensure that it is not also present in another cluster. There is no “best” cluster technique that works for all types of data (Milligan, 1981). Therefore, several cluster techniques (i.e., algorithms) should be applied to recover the best cluster structure in a dataset. Furthermore, different statistical packages

may produce different cluster solutions, probably due to the difference in their cluster algorithms. Thus, we recommend using different statistical packages to carry out the analysis. In this analysis, as well in the analysis described Berge et al. (2003), Ward's minimum variance with squared Euclidian distance produced well separated homogenous clusters. Those authors used disk diffusion method to assess the isolates' antimicrobial susceptibility; as a result, this approach produced continuous measurements. In his Monte Carlo simulations, evaluating several cluster analysis approaches, Milligan (1981) supported our finding that Ward's minimum is the best method to produce well separated clusters on binary and continuous data.

We applied the cluster algorithms on both ordinal (MIC) and binary AR data in order to assess the ability of those algorithms to recover structured clusters as well as to determine if they were as useful as when applied to continuous data (Berge et al., 2003). Cluster algorithms were unable to group MIC values (i.e., ordinal data) into well-separated clusters. We believe that the nature of these ordinal data (i.e., with 2-fold differences in antimicrobial agent concentrations per dilution, and variable numbers of dilutions per antimicrobial on the NARMS panels) caused instability in the cluster algorithms which resulted in non-homogenous clusters that did not represent the data properly. Also, not all antimicrobial agents had multiple cutpoints and our sample size was small.

The 504 *E. coli* isolates in the human wastewater data set contained 57 uniquely different resistance patterns. These patterns ranged from pansusceptible through single and multiple resistance of up to 12 antimicrobial agents. Theoretically, in an *E. coli*

isolate data set of infinite size, when assessing phenotypic resistance to 15 antimicrobial agents (and assuming a completely random process), there could be a maximum of 2^{15} = 32,768 possible unique phenotypes. Obviously, biological features (e.g., actual existence of resistance mechanisms, shared mechanisms for multiple antimicrobial agents of the same class, samples size) restrict the possibilities to a much lower number. Other factors may also reduce the number of possible phenotypes. For example, resistance genes often are suggested to be genetically linked in bacterial isolates (Mazel and Davies, 1999). Bacterial exposure to an antimicrobial agent may cause the organism to express resistance to multiple antibiotics that could be genetically related. Also, resistance may persist due to the genetic linkage of several AR genes, providing for their continued existence even as antimicrobial selection pressures change (Salyers et al., 1997). The clusters we obtained represent "groupings" among the resistance phenotypes that might point to the possibility of AR genetic linkage in the bacterial isolates (i.e., genotype). Poole et al. (2005) found that 46% of the VRE bacteria isolated from human wastewater samples at 4 units with swine operations in our study system, were clonal based on the PFGE analysis. This points to the possibility of clustering among AR bacteria isolated from different locations.

Several issues related to cluster analysis of multiple AR resistant phenotypes should be discussed in conjunction with this type of analysis. First, one should consider whether the resulting clusters reflected the underlying biological distribution of the phenotypes (i.e., biologically meaningful clusters). For instance, some of the resistant phenotypes that are known to be genetically linked (e.g., ceftiofur and ceftriaxone) were

grouped in one cluster reflecting the underlying genotypic linkage. However, other phenotypes (e.g., tetracycline and nalidixic acid) found in one cluster may not actually be genetically linked. Second, the repeatability of the cluster analysis results, when cluster techniques are applied to different data sets, should be assessed. That is, to say, whether the resulting clusters from those data sets will be similar or different. Repeatability may be assessed using bootstrapping technique (re-sampling with replacement from the original data set); that is, using a random sampling of a subset from the original data set, then performing cluster analysis on those sampled data sets, or using an entirely different data set, and then comparing the results of the analysis. Third, cluster analysis techniques initially assume that resistance phenotypes are considered to be independent. However, due to the correlation among resistant phenotypes, the assumption of initial independence is not likely to hold. Fourth, sorting the resistance clusters in ascending order on an ordinal scale low-to-high resistance clusters, may be misleading. This is an issue where, for example, more than one single-resistance cluster or more than one cluster with resistance to 2 antimicrobial agents are obtained by the analysis. Cluster analysis was performed on AR phenotypes on a continuous scale by Berge et al. (2003). However, several of these issues addressed above were not discussed by the authors; such as the repeatability of the cluster methods across datasets and the assumption of independence among phenotypes.

Broth microdilution and disk diffusion procedures are two common *in vitro* methods used to test bacterial isolate susceptibility to one or more antimicrobial agents at different concentrations. We chose the microdilution approach over the disk diffusion

method to test our isolates because of: 1) the reproducibility of the results due to the antibiotic panel manufacturing process assuring precise repeatable concentrations, 2) the expediency of having prepared panels available when required, 3) the combined generation of semi-quantitative (i.e., MIC values) and interpreted qualitative data (susceptible vs. resistant), 4) the availability of a computerized automated recording and reporting system that reduces intra- and inter-operator variability, and 5) ability to compare with NARMS results (Jorgensen and Ferraro, 1998). Semi-quantitative results are important in order to monitor small shifts in susceptibility at the population level. Therefore, MIC values are more precise measures that can reflect slight changes in susceptibility compared to the categorical data. This represents the first application of cluster techniques among non-continuous AR data. Many antimicrobial resistance studies lack the use of MIC values in their analyses due to the difficulties that arise from the nature of these types of data. However, at this point the question of how to group MIC data into meaningful clusters remains unresolved.

The marginal (i.e., population-averaged) GEE model was used to assess the relationship between the individual or multi-drug resistance phenotypes and the risk factors at the group-level, assuming this relation is the same for all subjects within clusters. Subject-specific (i.e., random effect) models assume that the relationship between the outcome and the predictors (i.e., risk factors) differs between subjects within clusters (Carriere and Bouyer, 2002; Dohoo et al., 2003). In our study, the samples were collected at the group-level for the human and swine populations. The GLLAMM models have the advantage of adjusting for multi-level hierarchical

clustering (i.e., dependency) in the data (Rabe-Hesketh et al., 2004). This type of hierarchical clustering exists in our data where host-species/farms/pens are nested within unit. The GEE models have major drawbacks in that it can only adjust for 1-level of clustering. Also, the within-cluster variability can not be estimated using GEE (Hanley et al., 2003). It has been suggested that alternating logistic regression (ALR) can adjust for multi-level clustering in the data such as dependency within unit, and dependency in farms/pens nested within unit using a population-averaged model (Carey et al., 1993). Alternating logistic regression is a marginal model that adjusts for multi-level clustered binary data by alternating between generalized estimating equations and logistic regression models to estimate the odds-ratios across and within clusters (Carey et al., 1993). However, the ALR model failed to converge when applied to our data (data not shown). That was likely due to the data structure where multiple *E. coli* bacteria isolated from multiple locations within unit were measured repeatedly at each time point (season).

Our qPCR methodology has focused primarily, but not strictly limited to, quantifying the bla_{CMY2} gene in total community DNA samples extracted from human wastewater and swine fecal matter. This gene is known to code for resistance to ceftiofur (an antimicrobial agent used in animal agriculture) and possibly to other 3rd generation cephalosporins such as ceftriaxone, which is commonly used in human medicine (Tragesser et al., 2006). The bla_{CMY2} gene is the most frequently described plasmid-encoded AmpC cephalosporinase (Bauernfeind et al., 1998). Also, it is the most prevalent cephalosporin gene and has been found in many areas of the world

(Philippon et al., 2002). This gene is mostly found to reside on bacterial plasmids, integrons, and transposons (Archambault et al., 2006; Winokur et al., 2001) and has been detected in both human and animal (e.g., swine) *E. coli* isolates (Alcaine et al, 2005). It has been suggested that the bla_{CMY-2} plasmid gene disseminates rapidly among different bacterial species in addition to the possible transmission of those bacteria from animals to human (Winokur et al., 2001).

Our chosen reference gene (*rpoB*) exists in a single copy per bacterial cell overcoming the inherent heterogeneity in copy/cell problem of 16S rDNA (Mollet et al., 1997; Dahllof et al., 2000). Standardization (i.e., normalization) of the target gene copy numbers to the reference gene copy numbers is crucial in this type of quantitative PCR analysis. This is because of the variability in the amount of bacterial- and non-bacterial-DNA present in the total community DNA samples (e.g., human cells, plants, algae). Thus, quantifying the bacterial cell copy numbers (i.e., background), which gives rise to the target gene, is necessary for standardization.

The high variability in the *rpoB* gene copy numbers that was reported in our study may be attributed to the community DNA extraction efficiency and/or to the quantification methodology of the genomic-conserved sequence in total community DNA samples using the SYBR® Green assay. Further research is needed to enhance the extraction efficiency to yield more bacterial DNA, concentrate the total community post extraction, perhaps use immunomagnetic beads coated with anti-*E. coli* to capture this organism, reduce PCR inhibitors in community DNA samples, and to optimize the PCR

parameters (e.g., primer sequence and concentration, and design) to enhance gene recovery.

There have been no published studies that quantified the absolute copy numbers of a target gene in total community DNA and standardized that to copy numbers of an appropriate house-keeping (i.e., reference) gene. Several studies have quantified either the number of rRNA genes in community DNA samples, based on the number of specific bacterial isolates (e.g., *E. coli* CFU or cells/g or ml) in the original raw sample, or the number of gene(s) in community DNA samples, with or without standardization (Khan et al., 2007; Ibekwe and Grieve, 2003; Smith et al., 2004; Castillo et al., 2006). Although Smith et al. (2004) have reported the log copy numbers for 3 tetracycline genes in feedlot lagoon community DNA, the authors did not standardize the target genes properly; rather, they used the volatile suspended solids (VSS) method, for standardizing the sample-to-sample variations. Moreover, the number of rRNA genes (e.g., 16S rDNA) based on the number *E. coli* bacterial isolates is not a proper method to use to standardize a target gene in community DNA because of the biases associated with it. For example, if this method was applied to our samples, the biases would be associated with 1) isolating *E. coli* from wastewater and fecal samples compared to community DNA, 2) the need for enrichment of wastewater before culturing, and 3) that the bla_{CMY2} gene exists in different bacterial species and not only in *E. coli* bacteria.

Molecular methods have the advantage of: 1) assessing the AR gene copies present (not simply the expression) even from dead and unculturable bacterial cells, 2) they are rapid and reliable in assessing resistance genes, and 3) there is no need to isolate the

bacteria when, for instance; total community DNA is used. Our future research will focus on quantifying and tracking longitudinally unique genotypes (e.g., bla_{CMY2}) in the total community DNA extracted from wastewater and fecal matter samples to better assess the AR transmission dynamic in human and swine populations, and relation to associated antimicrobial use in each species.

CHAPTER VI

CONCLUSIONS

This is the first longitudinal study conducted to assess the risk of transmission of AR bacteria due to human occupational exposure to swine or antimicrobials in feed in a multi-site vertically integrated agri-food system. The study design and sample collection strategy surpassed the existing related AR research that addressed the risk of resistant bacterial transmission to humans as a result of direct contact with animals. That is because: 1) our study populations had limited movement to and from the system (semi-closed agri-food system), 2) the study subjects (humans and pigs) exposure to other subjects within the study area was limited, and 3) pork was produced, processed, and consumed within the system with some pork trim imports. Our previous pilot cross-sectional study suggested that occupational exposure to swine was not associated with *E. coli* tetracycline resistance phenotype prevalence. However, in this longitudinal study (over the 3-year period) occupational exposure to swine-rearing appeared to be associated only with tetracycline resistance when compared to non-workers. These findings might be attributed to the high tetracycline resistance prevalence in the swine production groups that the workers were exposed to, as compared to the other antimicrobial agents resistance prevalences. Or, the exposure of the swine-workers to the agent itself (i.e., chlortetracycline in feed) through ingestion or inhaling the dust from the medicated feed may be to blame. Thus, this may indicate that occupational exposure to swine rearing facilities could be a risk for AR bacteria carriage by humans.

In general, 1) the swine *E. coli* isolates across all units were at higher level of resistance than humans, 2) the swine production group resistance isolates differed significantly with highest levels in purchased boars, breeding boars and nursery piglets. Adjusting for the dependence within multivariate phenotypes using the multivariate model of correlated dependence had an elevating effect on the odds-ratios and their confidence intervals before adjustment. Seasonal effect was highly variable over the 3-year study period. We have ongoing analyses to evaluate the relationship between AR seasonal variability and the historical use of antimicrobial agents in both host species.

In the future, the use of molecular epidemiology; that is utilization of molecular biology principles and techniques in epidemiologic research to better understand disease determinants and distribution at the exposure and outcome level, is needed. It is imperative to be able to track unique resistance gene(s) over time to obtain better quantitative data in order to enhance our understanding of those factors impacting the distribution of AR bacteria and ultimately to better assess AR bacteria transmission in the study system.

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APPENDICES

Table A1

Number of human wastewater samples (planned, received, received-labeled properly, cultured, samples that grew on chrome agar plate, at least one isolate retained and tested for antimicrobial susceptibility per plate, and total isolates retained and tested for antimicrobial susceptibility) by unit and month-year.

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
February	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	7	7	4	7
	Cultured samples	4	4	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	3	3	3	7	5	7	7	3	4	5
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	3	3	7	5	7	7	3	4	5
	Total isolates retained and tested for antimicrobial susceptibility	3	3	3	7	5	7	7	3	4	5
March	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	3	7	7	7	7	5	4	7
	Received-labeled samples	4	4	3	7	7	7	7	5	4	0
	Cultured samples	4	4	3	7	7	7	7	5	4	7
	Samples grew on chrome agar plate	3	1	2	4	6	5	5	4	4	0
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	1	2	4	6	5	5	4	4	0
	Total isolates retained and tested for antimicrobial susceptibility	3	1	2	4	6	5	5	4	4	0
April	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	5	4	7
	Received-labeled samples	4	4	4	7	7	7	7	5	4	0
	Cultured samples	4	4	4	7	7	7	7	5	4	7
	Samples grew on chrome agar plate	4	3	2	6	5	6	7	4	3	0
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	3	2	6	5	6	7	4	3	0
	Total isolates retained and tested for antimicrobial susceptibility	4	3	2	6	5	6	7	4	3	0

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
February	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	6	4	7	7	5	6	7	7	111
	Received-labeled samples	4	6	4	7	6	5	6	7	7	110
	Cultured samples	4	6	4	7	6	5	6	7	7	110
	Samples grew on chrome agar plate	1	5	4	6	6	4	11	7	6	97
	At least one isolate retained and tested for antimicrobial susceptibility per plate	1	5	4	6	6	4	11	7	6	97
	Total isolates retained and tested for antimicrobial susceptibility	1	5	4	6	6	4	11	7	6	97
March	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	6	4	7	7	5	7	7	7	109
	Received-labeled samples	4	5	4	7	6	5	6	7	6	98
	Cultured samples	4	6	4	7	7	5	7	7	7	109
	Samples grew on chrome agar plate	3	3	1	3	3	1	7	7	3	65
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	3	1	3	3	1	7	7	3	65
	Total isolates retained and tested for antimicrobial susceptibility	3	3	1	3	3	1	7	7	3	65
April	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	7	4	7	6	5	7	7	7	110
	Received-labeled samples	4	7	4	7	5	5	6	7	7	101
	Cultured samples	4	7	4	7	6	5	7	7	7	110
	Samples grew on chrome agar plate	3	7	4	7	4	5	6	6	5	87
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	7	4	7	4	5	6	6	5	87
	Total isolates retained and tested for antimicrobial susceptibility	3	7	4	7	4	5	6	6	5	87

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
May	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	5	4	7
	Received-labeled samples	4	4	4	7	7	7	7	5	4	7
	Cultured samples	4	4	4	7	7	7	7	5	4	7
	Samples grew on chrome agar plate	4	4	3	7	4	6	7	2	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	4	3	7	4	6	7	2	4	7
	Total isolates retained and tested for antimicrobial susceptibility	4	4	3	7	4	6	7	2	4	7
June	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	3	4	7	7	7	7	7	4	7
	Received-labeled samples	4	3	4	7	7	7	7	7	4	7
	Cultured samples	4	3	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	4	3	2	6	7	4	7	7	4	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	3	2	6	7	4	7	7	4	6
	Total isolates retained and tested for antimicrobial susceptibility	4	3	2	6	7	4	7	7	4	6
July	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	6	7	7	4	7
	Received-labeled samples	4	4	4	7	7	6	0	7	4	7
	Cultured samples	4	3	4	5	5	5	0	7	3	3
	Samples grew on chrome agar plate	4	3	4	5	5	5	0	7	3	3
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	3	4	5	5	5	0	7	3	3
	Total isolates retained and tested for antimicrobial susceptibility	4	3	4	5	5	5	0	7	3	3

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	7	4	7	4	5	7	7	7	108
	Received-labeled samples	4	7	4	7	4	5	6	7	7	107
	Cultured samples	4	7	4	7	4	5	7	7	7	108
	Samples grew on chrome agar plate	4	6	4	6	6	4	4	5	6	93
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	6	4	6	6	4	4	5	6	93
	Total isolates retained and tested for antimicrobial susceptibility	4	6	4	6	6	4	4	5	6	93
June	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	6	4	1	6	5	7	7	7	104
	Received-labeled samples	4	6	4	1	6	5	6	7	7	103
	Cultured samples	4	6	4	1	6	5	7	7	7	104
	Samples grew on chrome agar plate	4	6	4	1	7	4	5	6	6	93
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	6	4	1	7	4	5	6	6	93
	Total isolates retained and tested for antimicrobial susceptibility	4	6	4	1	7	4	5	6	6	93
July	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	7	4	1	0	5	6	6	0	90
	Received-labeled samples	4	7	4	1	0	5	5	6	0	82
	Cultured samples	3	6	4	1	0	4	5	5	0	67
	Samples grew on chrome agar plate	3	6	4	1	0	4	5	5	0	67
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	6	4	1	0	4	5	5	0	67
	Total isolates retained and tested for antimicrobial susceptibility	3	6	4	1	0	4	5	5	0	67

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
August	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	6	7	4	7
	Cultured samples	4	4	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	4	4	4	5	4	7	6	6	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	4	4	5	4	7	6	6	4	7
	Total isolates retained and tested for antimicrobial susceptibility	4	4	4	5	4	7	6	6	4	7
September	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	7	7	4	7
	Cultured samples	4	4	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	3	2	3	6	6	7	7	7	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	2	3	6	6	7	7	7	4	7
	Total isolates retained and tested for antimicrobial susceptibility	3	2	3	6	6	7	7	7	4	7
October	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	5	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	7	7	4	7
	Cultured samples	4	5	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	4	4	3	6	6	5	7	7	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	4	3	6	6	5	7	7	4	7
	Total isolates retained and tested for antimicrobial susceptibility	4	4	3	6	6	5	7	7	4	7

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
August 2004	Planned samples	4	7	4	7	7	5	7	7	7	113
	Received samples	3	5	4	6	4	5	6	7	6	104
	Received-labeled samples	3	5	4	6	4	5	5	7	6	102
	Cultured samples	3	5	4	6	4	5	6	7	6	104
	Samples grew on chrome agar plate	3	5	4	5	7	5	3	7	2	92
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	5	4	5	7	5	3	7	2	92
	Total isolates retained and tested for antimicrobial susceptibility	3	5	4	5	7	5	3	7	2	92
September 2004	Planned samples	4	7	4	7	7	5	7	7	7	113
	Received samples	4	7	4	6	7	5	5	7	7	110
	Received-labeled samples	4	7	4	6	7	5	4	7	7	109
	Cultured samples	4	7	4	6	7	5	5	7	7	110
	Samples grew on chrome agar plate	4	7	4	4	7	5	5	6	6	100
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	7	4	4	7	5	5	6	6	100
	Total isolates retained and tested for antimicrobial susceptibility	4	7	4	4	7	5	5	6	6	100
October 2004	Planned samples	4	7	4	7	7	5	7	7	7	113
	Received samples	4	7	4	6	4	5	5	7	6	107
	Received-labeled samples	4	7	4	6	4	5	5	7	6	106
	Cultured samples	4	7	4	6	4	5	5	7	6	107
	Samples grew on chrome agar plate	4	1	4	5	6	4	5	7	5	94
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	1	4	5	6	4	5	7	5	94
	Total isolates retained and tested for antimicrobial susceptibility	4	1	4	5	6	4	5	7	5	94

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
November	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	7	7	7	7	4	5
	Received-labeled samples	4	4	4	7	7	7	7	7	4	5
	Cultured samples	4	4	4	7	7	7	7	7	4	5
	Samples grew on chrome agar plate	2	3	2	7	6	5	3	6	3	5
	At least one isolate retained and tested for antimicrobial susceptibility per plate	2	3	2	7	6	5	3	6	3	5
	Total isolates retained and tested for antimicrobial susceptibility	2	3	2	7	6	5	3	6	3	5
December	Planned samples	4	4	4	7	7	7	7	7	4	7
2004	Received samples	4	4	4	7	0	7	7	7	4	7
	Received-labeled samples	4	4	4	7	0	7	7	7	4	7
	Cultured samples	4	4	4	7	0	7	7	7	4	7
	Samples grew on chrome agar plate	4	4	4	5	0	5	7	2	2	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	4	4	5	0	5	7	2	2	7
	Total isolates retained and tested for antimicrobial susceptibility	4	4	4	5	0	5	7	2	2	7
January	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	7	7	4	7
	Cultured samples	4	4	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	4	1	2	2	7	6	7	6	4	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	1	2	2	7	6	7	6	4	6
	Total isolates retained and tested for antimicrobial susceptibility	4	1	2	2	7	6	7	6	4	6

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
November	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	7	4	6	6	5	5	7	7	107
	Received-labeled samples	4	7	4	6	6	5	4	6	7	105
	Cultured samples	4	7	4	6	6	5	5	7	7	107
	Samples grew on chrome agar plate	4	6	3	6	5	5	2	4	4	81
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	6	3	6	5	5	2	4	4	81
	Total isolates retained and tested for antimicrobial susceptibility	4	6	3	6	5	5	2	4	4	81
December	Planned samples	4	7	4	7	7	5	7	7	7	113
2004	Received samples	4	6	4	6	4	5	5	7	7	99
	Received-labeled samples	4	6	4	6	4	5	5	7	7	99
	Cultured samples	4	6	4	6	4	5	5	7	7	99
	Samples grew on chrome agar plate	4	5	4	6	7	5	5	6	7	89
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	5	4	6	7	5	5	6	7	89
	Total isolates retained and tested for antimicrobial susceptibility	4	5	4	6	7	5	5	6	7	89
January	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	5	4	7	4	5	6	7	6	106
	Received-labeled samples	4	5	4	7	4	5	0	7	6	100
	Cultured samples	4	5	4	7	4	5	6	7	6	106
	Samples grew on chrome agar plate	4	5	1	7	7	4	0	6	6	85
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	5	1	7	7	4	0	6	6	85
	Total isolates retained and tested for antimicrobial susceptibility	4	5	1	7	7	4	0	6	6	85

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
February	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	3	7	7	7	7	7	4	7
	Received-labeled samples	4	4	3	7	7	7	6	7	4	7
	Cultured samples	4	4	3	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	4	4	3	7	7	7	6	7	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	4	3	4	7	7	6	4	4	7
	Total isolates retained and tested for antimicrobial susceptibility	8	14	12	13	34	34	23	12	13	27
March	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	7	7	6	4	7
	Received-labeled samples	4	4	4	7	7	7	7	6	2	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
April	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	6	7	7	4	7
	Received-labeled samples	0	4	4	7	0	6	7	7	4	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
February	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	3	7	4	7	7	10	5	7	7	114
	Received-labeled samples	3	7	4	7	7	10	5	7	7	113
	Cultured samples	3	7	4	7	7	10	5	7	7	114
	Samples grew on chrome agar plate	1	7	4	7	7	8	5	7	7	109
	At least one isolate retained and tested for antimicrobial susceptibility per plate	1	7	3	6	7	9	4	7	4	97
	Total isolates retained and tested for antimicrobial susceptibility	1	28	10	26	29	31	16	20	15	366
March	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	6	4	6	4	5	7	7	7	107
	Received-labeled samples	4	6	4	6	4	5	5	7	7	103
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
April	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	7	4	6	7	5	7	7	7	111
	Received-labeled samples	4	0	4	6	7	5	5	7	7	91
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
May	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	7	7	7	4	6
	Received-labeled samples	0	4	4	7	7	7	7	7	4	6
	Cultured samples	4	4	4	7	7	7	7	7	4	6
	Samples grew on chrome agar plate	0	4	3	5	6	7	7	7	4	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	0	4	3	5	6	7	7	7	4	6
	Total isolates retained and tested for antimicrobial susceptibility	0	4	3	5	6	7	7	7	4	6
June	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	6	7	7	7	4	7
	Received-labeled samples	4	3	4	7	6	7	7	7	4	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
July	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	0	4	7	7	6	7	5	4	7
	Received-labeled samples	4	0	4	7	7	6	7	5	4	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	14	4	0	7	5	6	7	7	111
	Received-labeled samples	4	7	4	0	7	5	4	7	7	98
	Cultured samples	4	14	4	0	7	5	6	7	7	111
	Samples grew on chrome agar plate	4	7	4	0	7	5	4	7	7	94
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	7	4	0	7	5	4	7	7	94
	Total isolates retained and tested for antimicrobial susceptibility	4	7	4	0	7	5	4	7	7	94
June	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	6	4	6	7	5	7	7	7	110
	Received-labeled samples	4	6	4	6	7	5	5	7	7	107
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
July	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	7	4	7	7	5	7	7	0	99
	Received-labeled samples	4	7	4	7	7	5	7	7	0	99
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
August	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	14	7	7	4	7
	Received-labeled samples	4	0	4	7	7	12	7	7	4	7
	Cultured samples	4	4	4	7	7	14	7	7	4	7
	Samples grew on chrome agar plate	4	0	3	7	6	12	7	5	4	7
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	0	3	7	6	12	7	5	4	7
	Total isolates retained and tested for antimicrobial susceptibility	4	0	3	7	6	12	7	5	4	7
September	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	3	4	7	7	7	7	7	4	7
	Received-labeled samples	4	0	4	7	7	7	7	7	4	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
October	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	0	4	3	7	7	7	6	7	3	6
	Received-labeled samples	0	0	3	7	7	7	6	7	3	6
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
August	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	6	4	6	6	5	7	7	6	116
	Received-labeled samples	4	6	4	6	6	5	7	7	6	110
	Cultured samples	4	6	4	6	6	5	7	7	6	116
	Samples grew on chrome agar plate	4	0	4	5	6	5	7	7	5	98
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	0	4	5	6	5	7	7	5	98
	Total isolates retained and tested for antimicrobial susceptibility	4	0	4	5	6	5	7	7	5	98
September	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	5	7	4	6	6	5	7	7	7	111
	Received-labeled samples	4	7	4	6	6	5	7	7	7	107
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
October	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	7	4	6	6	5	7	7	7	103
	Received-labeled samples	4	7	4	6	6	5	7	7	7	99
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
November	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	0	4	7	7	7	7	7	4	7
	Cultured samples	4	4	4	7	7	7	7	7	4	7
	Samples grew on chrome agar plate	3	0	3	6	5	6	7	6	4	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	3	0	3	6	5	6	7	6	4	6
	Total isolates retained and tested for antimicrobial susceptibility	3	0	3	6	5	6	7	6	4	6
December	Planned samples	4	4	4	7	7	7	7	7	4	7
2005	Received samples	4	3	3	7	7	7	7	7	4	7
	Received-labeled samples	4	3	3	7	7	7	7	7	4	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
January	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	0	4	4	7	7	7	7	7	0	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
November	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	7	4	6	6	5	7	7	7	111
	Received-labeled samples	4	7	4	6	6	5	7	7	7	107
	Cultured samples	4	7	4	6	6	5	7	7	7	111
	Samples grew on chrome agar plate	4	7	3	6	6	5	7	6	7	97
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	7	3	6	6	5	7	6	7	97
	Total isolates retained and tested for antimicrobial susceptibility	4	7	3	6	6	5	7	6	7	97
December	Planned samples	4	7	4	7	7	5	7	7	7	113
2005	Received samples	4	7	4	6	6	5	7	7	7	109
	Received-labeled samples	0	4	4	6	6	5	7	7	7	102
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
January	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	6	5	7	7	7	112
	Received-labeled samples	4	7	4	7	0	5	7	7	7	98
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
February	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	0	7	8	4	0
	Received-labeled samples	0	0	4	7	7	0	7	7	4	0
	Cultured samples	4	4	4	7	7	0	7	8	4	0
	Samples grew on chrome agar plate	0	0	3	4	3	0	7	7	3	0
	At least one isolate retained and tested for antimicrobial susceptibility per plate	0	0	3	4	3	0	7	7	3	0
	Total isolates retained and tested for antimicrobial susceptibility	0	0	3	4	3	0	7	7	3	0
March	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	6	7	4	7
	Received-labeled samples	0	3	4	7	7	7	6	7	0	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
April	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	0	4	4	7	7	7	5	0	0	0
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
February	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	0	5	7	7	7	93
	Received-labeled samples	4	7	4	7	0	5	7	7	7	84
	Cultured samples	4	7	4	7	0	5	7	7	7	93
	Samples grew on chrome agar plate	4	6	3	7	0	5	6	7	6	71
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	6	3	7	0	5	6	7	6	71
	Total isolates retained and tested for antimicrobial susceptibility	4	6	3	7	0	5	6	7	6	71
March	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	0	7	4	7	6	5	7	7	7	107
	Received-labeled samples	0	7	4	5	6	5	7	7	6	95
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
April	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	0	7	4	7	6	5	7	7	7	108
	Received-labeled samples	0	7	4	7	6	5	7	7	6	83
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
May	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	0	7	4	7
	Received-labeled samples	0	4	4	7	7	7	0	7	4	7
	Cultured samples	4	4	4	7	7	7	0	7	4	7
	Samples grew on chrome agar plate	0	4	2	7	7	6	0	7	3	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	0	4	2	7	7	6	0	7	3	6
	Total isolates retained and tested for antimicrobial susceptibility	0	4	2	7	7	6	0	7	3	6
June	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	0	7	4	7
	Received-labeled samples	4	4	4	7	7	7	0	0	0	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
July	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	0	7	0	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	8	4	4	7	6	5	7	7	7	106
	Received-labeled samples	8	4	4	7	6	5	7	7	7	102
	Cultured samples	8	4	4	7	6	5	7	7	7	106
	Samples grew on chrome agar plate	8	4	3	6	5	5	7	7	7	94
	At least one isolate retained and tested for antimicrobial susceptibility per plate	8	4	3	6	5	5	7	7	7	94
	Total isolates retained and tested for antimicrobial susceptibility	8	4	3	6	5	5	7	7	7	94
June	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	6	5	7	7	7	105
	Received-labeled samples	4	7	4	3	6	5	7	7	7	90
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
July	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	6	5	7	7	7	112
	Received-labeled samples	4	7	4	7	6	0	7	7	7	96
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
August	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	0	7	7	0	0	0	0
	Received-labeled samples	4	4	4	0	7	7	0	0	0	0
	Cultured samples	4	4	4	0	7	7	0	0	0	0
	Samples grew on chrome agar plate	4	4	4	0	6	7	0	0	0	0
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	4	4	0	6	7	0	0	0	0
	Total isolates retained and tested for antimicrobial susceptibility	4	4	4	0	6	7	0	0	0	0
September	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	4	4	7	7	7	7	7	4	7
	Received-labeled samples	4	4	4	7	7	7	0	7	0	7
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
October	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	4	0	4	7	7	7	7	7	4	0
	Received-labeled samples	0	0	4	7	7	7	0	7	4	0
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
August	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	8	7	4	7	0	5	7	7	7	78
	Received-labeled samples	8	7	4	7	0	0	7	7	7	73
	Cultured samples	8	7	4	7	0	5	7	7	7	78
	Samples grew on chrome agar plate	8	7	4	5	0	0	6	6	3	64
	At least one isolate retained and tested for antimicrobial susceptibility per plate	8	7	4	5	0	0	6	6	3	64
	Total isolates retained and tested for antimicrobial susceptibility	8	7	4	5	0	0	6	6	3	64
September	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	0	5	7	7	7	106
	Received-labeled samples	4	7	4	7	0	0	7	7	7	90
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
October	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	0	7	8	7	11	5	7	7	7	106
	Received-labeled samples	0	7	8	7	11	0	7	7	7	90
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
November	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	0	4	4	0	7	7	0	7	4	0
	Received-labeled samples	0	4	4	0	7	7	0	7	0	0
	Cultured samples	0	4	4	0	7	7	0	7	4	0
	Samples grew on chrome agar plate	0	3	3	0	7	2	0	5	0	0
	At least one isolate retained and tested for antimicrobial susceptibility per plate	0	3	3	0	7	2	0	5	0	0
	Total isolates retained and tested for antimicrobial susceptibility	0	3	3	0	7	2	0	5	0	0
December	Planned samples	4	4	4	7	7	7	7	7	4	7
2006	Received samples	0	0	4	0	7	7	0	7	4	0
	Received-labeled samples	0	0	4	0	7	7	0	7	4	0
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
January	Planned samples	4	4	4	7	7	7	7	7	4	7
2007	Received samples	0	0	4	0	7	7	0	7	4	0
	Received-labeled samples	0	0	4	0	7	7	0	7	4	0
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
November	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	8	0	4	7	6	5	7	7	6	83
	Received-labeled samples	4	0	4	7	6	5	7	7	6	75
	Cultured samples	8	0	4	7	6	5	7	7	6	83
	Samples grew on chrome agar plate	4	0	4	6	3	5	5	4	5	56
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	0	4	6	3	5	5	4	5	56
	Total isolates retained and tested for antimicrobial susceptibility	4	0	4	6	3	5	5	4	5	56
December	Planned samples	4	7	4	7	7	5	7	7	7	113
2006	Received samples	4	7	4	7	0	5	7	7	6	76
	Received-labeled samples	4	7	4	7	0	5	7	7	6	76
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	-
January	Planned samples	4	7	4	7	7	5	7	7	7	113
2007	Received samples	4	7	4	7	0	5	7	7	6	76
	Received-labeled samples	4	7	4	7	0	5	7	7	6	76
	Cultured samples	-	-	-	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-	-	-	

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
Total by year											
2004	Planned samples	48	48	48	84	84	84	84	84	48	84
	Received samples	48	48	47	84	77	83	84	78	48	82
	Received-labeled samples	48	47	47	84	77	83	76	78	48	68
	Cultured samples	48	47	47	82	75	82	77	78	47	78
	Samples grew on chrome agar plate	43	35	34	66	61	68	70	61	43	60
	At least one isolate retained and tested for antimicrobial susceptibility per plate	43	35	34	66	61	68	70	61	43	60
	Total isolates retained and tested for antimicrobial susceptibility	43	36	35	66	60	68	77	67	47	70
2005	Planned samples	48	48	48	84	84	84	84	84	48	84
	Received samples	44	42	45	84	83	89	83	81	47	82
	Received-labeled samples	32	26	45	84	76	87	82	81	41	82
	Cultured samples	16	16	15	28	28	35	28	28	16	27
	Samples grew on chrome agar plate	11	8	12	25	24	32	27	25	16	26
	At least one isolate retained and tested for antimicrobial susceptibility per plate	10	8	12	22	24	32	27	22	16	26
	Total isolates retained and tested for antimicrobial susceptibility	19	24	17	31	51	61	44	60	25	46
2006	Planned samples	48	48	48	84	84	84	84	84	48	84
	Received samples	36	36	48	56	84	77	41	78	44	42
	Received-labeled samples	16	31	48	56	84	77	18	63	20	35
	Cultured samples	12	16	16	14	28	21	7	22	12	7
	Samples grew on chrome agar plate	4	11	12	11	23	15	7	19	6	6
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	11	12	11	23	15	7	19	6	6
	Total isolates retained and tested for antimicrobial susceptibility	12	15	12	11	23	15	13	19	13	6

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
Total by year											
2004	Planned samples	48	84	48	84	84	60	84	84	84	1356
	Received samples	47	76	48	67	59	60	72	83	74	1265
	Received-labeled samples	47	75	48	67	56	60	58	82	73	1222
	Cultured samples	46	75	48	67	58	59	71	82	74	1241
	Samples grew on chrome agar plate	41	62	41	57	65	50	58	72	56	1043
	At least one isolate retained and tested for antimicrobial susceptibility per plate	41	62	41	57	65	50	58	72	56	1043
	Total isolates retained and tested for antimicrobial susceptibility	41	62	41	59	66	51	75	72	57	1093
2005	Planned samples	48	84	48	84	84	60	84	84	84	1356
	Received samples	48	88	48	69	75	65	81	84	76	1314
	Received-labeled samples	43	71	48	69	69	65	73	84	76	1234
	Cultured samples	15	34	16	19	26	25	25	28	27	452
	Samples grew on chrome agar plate	13	21	15	18	26	23	23	27	26	398
	At least one isolate retained and tested for antimicrobial susceptibility per plate	13	21	14	17	26	24	22	27	23	386
	Total isolates retained and tested for antimicrobial susceptibility	13	49	21	37	52	47	46	40	34	717
2006	Planned samples	48	84	48	84	84	60	84	84	84	1356
	Received samples	48	74	52	84	47	60	84	84	81	1156
	Received-labeled samples	44	74	52	78	47	40	84	84	79	1030
	Cultured samples	28	18	16	28	12	20	28	28	27	360
	Samples grew on chrome agar plate	24	17	14	24	8	15	24	24	21	285
	At least one isolate retained and tested for antimicrobial susceptibility per plate	24	17	14	24	8	15	24	24	21	285
	Total isolates retained and tested for antimicrobial susceptibility	28	17	15	24	8	20	24	24	21	320

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-1	H-intake	HU-3	HU-4	HU-5	HU-6	HU-7	HU-8	HU-9	HU-10
Proportions by year											
2004	Received/ planned samples	100.00	100.00	97.92	100.00	91.67	98.81	100.00	92.86	100.00	97.62
	Received-labeled samples/proposed samples	100.00	97.92	97.92	100.00	91.67	98.81	90.48	92.86	100.00	80.95
	Cultured samples/received-labeled samples	100.00	100.00	100.00	97.62	97.40	98.80	101.32	100.00	97.92	114.71
	Samples grew on chrome agar/cultured samples	89.58	74.47	72.34	80.49	81.33	82.93	90.91	78.21	91.49	76.92
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	89.58	74.47	72.34	80.49	81.33	82.93	90.91	78.21	91.49	76.92
2005	Received/ planned samples	91.67	87.50	93.75	100.00	98.81	105.95	98.81	96.43	97.92	97.62
	Received-labeled samples/proposed samples	66.67	54.17	93.75	100.00	90.48	103.57	97.62	96.43	85.42	97.62
	Cultured samples/received-labeled samples	50.00	61.54	33.33	33.33	36.84	40.23	34.15	34.57	39.02	32.93
	Samples grew on chrome agar/cultured samples	68.75	50.00	80.00	89.29	85.71	91.43	96.43	89.29	100.00	96.30
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	62.50	50.00	80.00	78.57	85.71	91.43	96.43	78.57	100.00	96.30
2006	Received/ planned samples	75.00	86.11	100.00	100.00	100.00	100.00	43.90	80.77	45.45	83.33
	Received-labeled samples/proposed samples	33.33	44.44	33.33	25.00	33.33	27.27	17.07	28.21	27.27	16.67
	Cultured samples/received-labeled samples	75.00	68.75	75.00	78.57	82.14	71.43	100.00	86.36	50.00	85.71
	Samples grew on chrome agar/cultured samples	33.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	33.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table A1 (Continued)

		Human wastewater samples									
	Number of:	HU-11	HU-12	HU-13	H-Slaugh	HU-15	HU-16	HU-17	HU-18	HU-19 (S-Isolation)	Total
Proportions by year											
2004	Received/ Planned samples	97.92	90.48	100.00	79.76	70.24	100.00	85.71	98.81	88.10	93.29
	Received-labeled samples/proposed samples	97.92	89.29	100.00	79.76	66.67	100.00	69.05	97.62	86.90	90.12
	Cultured samples/received-labeled samples	97.87	100.00	100.00	100.00	103.57	98.33	122.41	100.00	101.37	101.55
	Samples grew on chrome agar/cultured samples	89.13	82.67	85.42	85.07	112.07	84.75	81.69	87.80	75.68	84.05
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	89.13	82.67	85.42	85.07	112.07	84.75	81.69	87.80	75.68	84.05
2005	Received/proposed samples	100.00	104.76	100.00	82.14	89.29	108.33	96.43	100.00	90.48	96.90
	Received-labeled samples/ planned samples	89.58	84.52	100.00	82.14	82.14	108.33	86.90	100.00	90.48	91.00
	Cultured samples/received-labeled samples	34.88	47.89	33.33	27.54	37.68	38.46	34.25	33.33	35.53	36.63
	Samples grew on chrome agar/cultured samples	86.67	61.76	93.75	94.74	100.00	92.00	92.00	96.43	96.30	88.05
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	86.67	61.76	87.50	89.47	100.00	96.00	88.00	96.43	85.19	85.40
2006	Received/proposed samples	91.67	100.00	100.00	92.86	100.00	66.67	100.00	100.00	97.53	89.10
	Received-labeled samples/ planned samples	58.33	24.32	30.77	33.33	25.53	33.33	33.33	33.33	33.33	31.14
	Cultured samples/received-labeled samples	85.71	94.44	87.50	85.71	66.67	75.00	85.71	85.71	77.78	79.17
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	At least one isolate retained and tested for antimicrobial susceptibility per plate/ cultured samples	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table A2
Number of swine fecal samples (received, cultured, grew on chrome agar plate, at least one isolate retained and tested for antimicrobial susceptibility per plate, and total isolates retained and tested for antimicrobial susceptibility) by unit and month-year.

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
January	Received samples	17	5	19	5	19	18	19
2004	Cultured samples	17	5	19	5	19	18	19
	Samples grew on chrome agar plate	17	5	19	5	18	18	18
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	5	19	5	18	18	18
	Total isolates retained and tested for antimicrobial susceptibility	17	5	19	5	18	18	18
February	Received samples	17	5	19	5	20	18	18
2004	Cultured samples	17	5	19	5	20	18	18
	Samples grew on chrome agar plate	17	5	19	5	18	18	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	5	19	5	18	18	17
	Total isolates retained and tested for antimicrobial susceptibility	17	5	19	5	18	18	17
March	Received samples	19	4	18	5	20	17	18
2004	Cultured samples	19	4	17	5	18	16	16
	Samples grew on chrome agar plate	19	4	17	5	18	16	16
	At least one isolate retained and tested for antimicrobial susceptibility per plate	19	4	17	5	18	16	16
	Total isolates retained and tested for antimicrobial susceptibility	19	4	17	5	18	16	16
April	Received samples	18	6	17	7	18	17	17
2004	Cultured samples	15	6	16	6	15	16	16
	Samples grew on chrome agar plate	15	6	16	6	15	16	16
	At least one isolate retained and tested for antimicrobial susceptibility per plate	15	6	16	6	15	16	16
	Total isolates retained and tested for antimicrobial susceptibility	15	6	16	6	15	16	16

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Intake)	Total
January	Received samples	7	6	7	8	5	33	66
2004	Cultured samples	7	6	7	8	5	33	66
	Samples grew on chrome agar plate	7	5	7	8	5	31	63
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	5	7	8	5	31	63
	Total isolates retained and tested for antimicrobial susceptibility	7	5	7	8	5	31	63
February	Received samples	5	12	5	8	6	6	42
2004	Cultured samples	5	12	5	8	6	6	42
	Samples grew on chrome agar plate	5	3	5	8	6	5	32
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	3	5	8	6	5	32
	Total isolates retained and tested for antimicrobial susceptibility	5	3	5	8	6	5	32
March	Received samples	5	9	7	5	6	2	34
2004	Cultured samples	5	9	6	5	5	2	32
	Samples grew on chrome agar plate	5	6	6	5	5	2	29
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	6	6	5	5	2	29
	Total isolates retained and tested for antimicrobial susceptibility	5	6	6	5	5	2	29
April	Received samples	6	19	7	6	6	5	49
2004	Cultured samples	5	19	7	6	4	4	45
	Samples grew on chrome agar plate	5	7	7	6	4	4	33
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	7	7	6	4	4	33
	Total isolates retained and tested for antimicrobial susceptibility	5	7	7	6	4	4	33

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
May	Received samples	18	6	18	6	23	19	18
2004	Cultured samples	17	6	17	6	21	19	15
	Samples grew on chrome agar plate	17	6	17	6	21	19	15
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	6	17	6	21	19	15
	Total isolates retained and tested for antimicrobial susceptibility	17	6	17	6	21	19	15
June	Received samples	17	6	17	6	21	17	18
2004	Cultured samples	17	6	17	6	21	16	17
	Samples grew on chrome agar plate	17	6	17	6	21	16	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	6	17	6	21	16	17
	Total isolates retained and tested for antimicrobial susceptibility	17	6	17	6	21	16	17
July	Received samples	18	6	17	7	20	17	18
2004	Cultured samples	18	6	17	7	18	16	17
	Samples grew on chrome agar plate	18	6	17	7	18	16	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	18	6	17	7	18	16	17
	Total isolates retained and tested for antimicrobial susceptibility	18	6	17	7	18	16	17
August	Received samples	18	5	17	7	21	17	17
2004	Cultured samples	16	5	14	7	20	15	14
	Samples grew on chrome agar plate	16	5	14	7	20	15	14
	At least one isolate retained and tested for antimicrobial susceptibility per plate	16	5	14	7	20	15	14
	Total isolates retained and tested for antimicrobial susceptibility	16	5	14	7	20	15	14

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Received samples	7	10	7	6	6	4	40
2004	Cultured samples	7	10	7	6	6	4	40
	Samples grew on chrome agar plate	7	5	7	6	6	4	35
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	5	7	6	6	4	35
	Total isolates retained and tested for antimicrobial susceptibility	7	5	7	6	6	4	35
June	Received samples	7	11	7	5	8	6	44
2004	Cultured samples	7	11	7	5	8	6	44
	Samples grew on chrome agar plate	7	6	7	5	8	6	39
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	6	7	5	8	6	39
	Total isolates retained and tested for antimicrobial susceptibility	7	6	7	5	8	6	39
July	Received samples	7	5	6	5	5	6	34
2004	Cultured samples	7	5	6	5	4	6	33
	Samples grew on chrome agar plate	7	0	6	5	4	6	28
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	0	6	5	4	6	28
	Total isolates retained and tested for antimicrobial susceptibility	7	0	6	5	4	6	28
August	Received samples	8	11	5	6	7	5	42
2004	Cultured samples	6	11	3	6	5	4	35
	Samples grew on chrome agar plate	6	5	3	6	5	4	29
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	5	3	6	5	4	29
	Total isolates retained and tested for antimicrobial susceptibility	6	5	3	6	5	4	29

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
September	Received samples	18	5	16	8	20	17	17
2004	Cultured samples	17	5	16	8	20	16	17
	Samples grew on chrome agar plate	17	5	16	8	20	16	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	5	16	8	20	16	17
	Total isolates retained and tested for antimicrobial susceptibility	17	5	16	8	20	16	17
October	Received samples	17	5	16	6	19	17	18
2004	Cultured samples	16	5	14	6	19	16	18
	Samples grew on chrome agar plate	16	5	14	6	19	16	18
	At least one isolate retained and tested for antimicrobial susceptibility per plate	16	5	14	6	19	16	18
	Total isolates retained and tested for antimicrobial susceptibility	16	5	14	6	19	16	18
November	Received samples	18	5	15	6	19	16	17
2004	Cultured samples	17	5	15	6	17	15	17
	Samples grew on chrome agar plate	17	5	15	6	17	15	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	5	15	6	17	15	17
	Total isolates retained and tested for antimicrobial susceptibility	17	5	15	6	17	15	17
December	Received samples	17	6	15	7	20	17	17
2004	Cultured samples	17	6	14	7	20	16	17
	Samples grew on chrome agar plate	17	6	14	7	20	16	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	6	14	7	20	16	17
	Total isolates retained and tested for antimicrobial susceptibility	17	6	14	7	20	16	17

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
September	Received samples	7	16	5	6	8	39	81
2004	Cultured samples	7	16	5	6	8	31	73
	Samples grew on chrome agar plate	7	16	5	6	8	31	73
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	16	5	6	8	31	73
	Total isolates retained and tested for antimicrobial susceptibility	7	16	5	6	8	31	73
October	Received samples	8	17	6	5	6	2	44
2004	Cultured samples	8	17	6	5	6	2	44
	Samples grew on chrome agar plate	8	7	6	5	6	2	34
	At least one isolate retained and tested for antimicrobial susceptibility per plate	8	7	6	5	6	2	34
	Total isolates retained and tested for antimicrobial susceptibility	8	7	6	5	6	2	34
November	Received samples	6	15	6	5	7	1	40
2004	Cultured samples	6	15	6	4	7	0	38
	Samples grew on chrome agar plate	6	8	6	4	7	0	31
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	8	6	4	7	0	31
	Total isolates retained and tested for antimicrobial susceptibility	6	8	6	4	7	0	31
December	Received samples	6	9	6	5	8	5	39
2004	Cultured samples	6	9	6	5	7	5	38
	Samples grew on chrome agar plate	6	6	6	5	7	5	35
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	6	6	5	7	5	35
	Total isolates retained and tested for antimicrobial susceptibility	6	6	6	5	7	5	35

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
January	Received samples	17	6	15	7	20	15	17
2005	Cultured samples	17	6	14	7	19	13	13
	Samples grew on chrome agar plate	17	6	14	7	19	13	13
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	6	14	7	19	13	13
	Total isolates retained and tested for antimicrobial susceptibility	17	6	14	7	19	13	13
February	Received samples	19	7	15	8	19	16	17
2005	Cultured samples	19	7	15	8	19	16	17
	Samples grew on chrome agar plate	19	7	15	8	19	16	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	19	7	15	8	19	16	17
	Total isolates retained and tested for antimicrobial susceptibility	80	28	63	35	82	58	69
March	Received samples	17	6	15	6	21	15	17
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
April	Received samples	18	4	15	8	21	15	17
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
January	Received samples	7	13	6	2	7	37	72
2005	Cultured samples	6	13	5	2	7	33	66
	Samples grew on chrome agar plate	6	4	5	2	7	33	57
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	4	5	2	7	33	57
	Total isolates retained and tested for antimicrobial susceptibility	6	4	5	2	7	33	57
February	Received samples	7	9	6	5	8	2	37
2005	Cultured samples	7	9	6	5	8	2	37
	Samples grew on chrome agar plate	6	2	6	5	8	1	28
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	2	6	5	8	1	28
	Total isolates retained and tested for antimicrobial susceptibility	28	7	25	15	31	5	111
March	Received samples	7	10	6	5	8	0	36
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
April	Received samples	7	12	5	6	8	30	68
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
May	Received samples	22	6	22	6	25	21	22
2005	Cultured samples	22	6	22	6	25	21	22
	Samples grew on chrome agar plate	22	6	22	6	25	21	22
	At least one isolate retained and tested for antimicrobial susceptibility per plate	22	6	22	6	25	21	22
	Total isolates retained and tested for antimicrobial susceptibility	22	6	22	6	25	21	22
June	Received samples	22	5	20	6	25	21	22
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
July	Received samples	16	6	15	3	23	18	18
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
August	Received samples	16	5	15	4	20	17	17
2005	Cultured samples	16	5	15	4	20	17	17
	Samples grew on chrome agar plate	16	5	15	4	20	17	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	16	5	15	4	20	17	17
	Total isolates retained and tested for antimicrobial susceptibility	16	5	15	4	20	17	17

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Received samples	7	17	6	6	8	3	47
2005	Cultured samples	7	17	6	6	8	3	47
	Samples grew on chrome agar plate	7	17	6	6	8	3	47
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	4	6	6	8	3	34
	Total isolates retained and tested for antimicrobial susceptibility	7	4	6	6	8	3	34
June	Received samples	7	16	7	4	6	2	42
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
July	Received samples	5	7	5	5	6	2	30
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
August	Received samples	7	6	7	3	7	28	58
2005	Cultured samples	7	6	7	3	7	28	58
	Samples grew on chrome agar plate	7	6	7	3	7	28	58
	At least one isolate retained and tested for antimicrobial susceptibility per plate	7	6	7	3	7	112	142
	Total isolates retained and tested for antimicrobial susceptibility	7	6	7	3	7	112	142

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
September	Received samples	16	5	14	5	22	16	17
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
October	Received samples	16	7	14	5	26	17	18
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
November	Received samples	22	5	14	6	23	18	23
2005	Cultured samples	22	5	14	6	23	18	23
	Samples grew on chrome agar plate	22	5	14	6	23	18	23
	At least one isolate retained and tested for antimicrobial susceptibility per plate	22	4	14	6	23	18	23
	Total isolates retained and tested for antimicrobial susceptibility	22	4	14	6	23	18	23
December	Received samples	20	6	14	6	25	19	17
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
September	Received samples	5	9	6	5	4	31	60
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
October	Received samples	5	14	6	4	6	3	38
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
November	Received samples	5	16	5	6	5	3	40
2005	Cultured samples	5	16	5	6	5	3	40
	Samples grew on chrome agar plate	5	16	5	6	5	3	40
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	3	5	6	5	3	27
	Total isolates retained and tested for antimicrobial susceptibility	5	3	5	6	5	3	27
December	Received samples	7	11	6	6	6	3	39
2005	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
January	Received samples	20	6	15	7	26	19	18
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
February	Received samples	22	6	21	7	26	19	21
2006	Cultured samples	22	6	21	7	26	19	21
	Samples grew on chrome agar plate	22	6	21	7	26	19	21
	At least one isolate retained and tested for antimicrobial susceptibility per plate	22	5	21	7	26	19	20
	Total isolates retained and tested for antimicrobial susceptibility	22	5	21	7	26	19	20
March	Received samples	23	6	20	6	25	18	21
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
April	Received samples	20	6	20	6	26	19	21
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
January	Received samples	7	19	6	4	7	58	101
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
February	Received samples	5	14	5	5	5	5	39
2006	Cultured samples	5	14	5	5	5	5	39
	Samples grew on chrome agar plate	5	14	5	5	5	5	39
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	0	5	5	5	5	25
	Total isolates retained and tested for antimicrobial susceptibility	5	0	5	5	5	5	25
March	Received samples	7	15	6	3	6	2	39
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
April	Received samples	5	10	6	4	6	3	34
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
May	Received samples	19	5	18	5	26	20	20
2006	Cultured samples	19	5	18	5	26	20	20
	Samples grew on chrome agar plate	19	5	18	5	26	20	20
	At least one isolate retained and tested for antimicrobial susceptibility per plate	17	5	18	5	26	20	20
	Total isolates retained and tested for antimicrobial susceptibility	17	5	18	5	26	20	20
June	Received samples	16	6	20	6	26	19	23
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
July	Received samples	21	6	16	6	28	17	21
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
August	Received samples	16	6	15	4	22	17	18
2006	Cultured samples	16	6	15	4	22	17	18
	Samples grew on chrome agar plate	16	6	15	4	22	17	18
	At least one isolate retained and tested for antimicrobial susceptibility per plate	16	6	15	4	20	17	18
	Total isolates retained and tested for antimicrobial susceptibility	16	6	15	4	20	17	18

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
May	Received samples	7	16	6	5	4	52	90
2006	Cultured samples	7	16	6	5	4	52	90
	Samples grew on chrome agar plate	7	16	6	5	4	52	90
	At least one isolate retained and tested for antimicrobial susceptibility per plate	6	7	6	5	4	52	80
	Total isolates retained and tested for antimicrobial susceptibility	6	7	6	5	4	52	80
June	Received samples	6	8	5	6	6	3	34
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
July	Received samples	5	16	5	5	6	24	61
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
August	Received samples	5	14	5	4	5	2	35
2006	Cultured samples	5	14	5	4	5	2	35
	Samples grew on chrome agar plate	5	14	5	4	5	2	35
	At least one isolate retained and tested for antimicrobial susceptibility per plate	5	4	5	4	5	2	25
	Total isolates retained and tested for antimicrobial susceptibility	5	4	5	4	5	2	25

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
September	Received samples	16	6	15	4	24	17	18
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
October	Received samples	15	5	14	5	23	15	17
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
November	Received samples	16	6	14	6	24	15	17
2006	Cultured samples	16	6	14	6	24	15	17
	Samples grew on chrome agar plate	16	6	14	6	24	15	17
	At least one isolate retained and tested for antimicrobial susceptibility per plate	16	6	14	6	24	15	17
	Total isolates retained and tested for antimicrobial susceptibility	16	6	14	6	24	15	17
December	Received samples	20	6	20	4	30	16	22
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
September	Received samples	7	14	5	6	4	3	39
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-
October	Received samples	6	11	5	5	6	32	65
2006	Cultured samples	-	-	-	-	-	32	32
	Samples grew on chrome agar plate	-	-	-	-	-	29	29
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	29	29
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	29	29
November	Received samples	4	16	3	5	4	32	64
2006	Cultured samples	4	16	3	5	4	32	64
	Samples grew on chrome agar plate	4	16	3	5	4	32	64
	At least one isolate retained and tested for antimicrobial susceptibility per plate	4	6	3	5	4	22	44
	Total isolates retained and tested for antimicrobial susceptibility	4	6	3	5	4	62	84
December	Received samples	5	11	3	6	5	0	30
2006	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
January	Received samples	21	5	20	6	29	20	22
2007	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Intake)	Total
January	Received samples	3	14	5	6	5	3	36
2007	Cultured samples	-	-	-	-	-	-	-
	Samples grew on chrome agar plate	-	-	-	-	-	-	-
	At least one isolate retained and tested for antimicrobial susceptibility per plate	-	-	-	-	-	-	-
	Total isolates retained and tested for antimicrobial susceptibility	-	-	-	-	-	-	-

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
Total by year								
2004	Received samples	229	70	219	82	260	222	229
	Cultured samples	220	70	209	81	247	210	214
	Samples grew on chrome agar plate	220	70	209	81	244	210	212
	At least one isolate retained and tested for antimicrobial susceptibility per plate	220	70	209	81	244	210	212
	Total isolates retained and tested for antimicrobial susceptibility	220	70	209	81	244	210	212
2005	Received samples	224	68	188	70	276	212	223
	Cultured samples	79	23	66	24	87	72	79
	Samples grew on chrome agar plate	79	23	66	24	87	72	79
	At least one isolate retained and tested for antimicrobial susceptibility per plate	79	22	66	24	87	72	79
	Total isolates retained and tested for antimicrobial susceptibility	140	43	114	51	149	111	131
2006	Received samples	225	69	213	65	309	212	241
	Cultured samples	73	23	68	22	98	71	76
	Samples grew on chrome agar plate	73	23	68	22	98	71	76
	At least one isolate retained and tested for antimicrobial susceptibility per plate	71	22	68	22	96	71	75
	Total isolates retained and tested for antimicrobial susceptibility	71	22	68	22	96	71	75

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
Total by year								
2004	Received samples	86	153	80	72	85	151	1938
	Cultured samples	82	153	76	71	78	136	1847
	Samples grew on chrome agar plate	82	78	76	71	78	133	1764
	At least one isolate retained and tested for antimicrobial susceptibility per plate	82	78	76	71	78	133	1764
	Total isolates retained and tested for antimicrobial susceptibility	82	78	76	71	78	133	1764
2005	Received samples	76	146	71	59	79	165	1857
	Cultured samples	26	48	24	20	28	36	612
	Samples grew on chrome agar plate	25	41	24	20	28	35	603
	At least one isolate retained and tested for antimicrobial susceptibility per plate	25	15	24	20	28	119	660
	Total isolates retained and tested for antimicrobial susceptibility	47	20	43	30	51	123	1053
2006	Received samples	65	159	59	60	62	161	1900
	Cultured samples	21	60	19	19	18	123	691
	Samples grew on chrome agar plate	21	60	19	19	18	120	688
	At least one isolate retained and tested for antimicrobial susceptibility per plate	20	17	19	19	18	110	628
	Total isolates retained and tested for antimicrobial susceptibility	20	17	19	19	18	150	668

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-1	HU-4	HU-5	HU-6	HU-7	HU-8	HU-10
Proportions by year								
2004	Cultured samples/received samples	96.07	100.00	95.43	98.78	95.00	94.59	93.45
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	98.79	100.00	99.07
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	100.00	100.00	100.00	100.00	98.79	100.00	99.07
2005	Cultured samples/received samples	35.27	33.82	35.11	34.29	31.52	33.96	35.43
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	100.00	95.65	100.00	100.00	100.00	100.00	100.00
2006	Cultured samples/received samples	32.44	33.33	31.92	33.85	31.72	33.49	31.54
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	97.26	95.65	100.00	100.00	97.96	100.00	98.68

Table A2 (Continued)

		Swine fecal matter samples						
	Number of	HU-11	H-Slaugh	HU-15	HU-17	HU-18	HU-19 (S-Isolation)	Total
Proportions by year								
2004	Cultured samples/received samples	95.35	100.00	95.00	98.61	91.76	90.07	95.30
	Samples grew on chrome agar/cultured samples	100.00	50.98	100.00	100.00	100.00	97.79	95.51
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	100.00	50.98	100.00	100.00	100.00	97.79	95.51
2005	Cultured samples/received samples	34.21	32.88	33.80	33.90	35.44	21.82	32.96
	Samples grew on chrome agar/cultured samples	96.15	85.42	100.00	100.00	100.00	97.22	98.53
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	96.15	31.25	100.00	100.00	100.00	330.56	107.84
2006	Cultured samples/received samples	32.31	37.74	32.20	31.67	29.03	76.40	36.37
	Samples grew on chrome agar/cultured samples	100.00	100.00	100.00	100.00	100.00	97.56	99.57
	At least one isolate retained and tested for antimicrobial susceptibility per plate/cultured samples	95.24	28.33	100.00	100.00	100.00	89.43	90.88

VITA

Walid Qasim Mohammad Alali
Department of Veterinary Integrative Biosciences
College of Veterinary Medicine and Biomedical Sciences
Texas A&M University
4458 TAMU
College Station, TX, USA 77843-4458

EDUCATION

2007 Doctor of Philosophy in Biomedical Sciences, Texas A&M University

2003 Master of Science in Epidemiology, Kansas State University

2000 Doctor of Veterinary Medicine, Jordan University of Science and Technology

PROFESSIONAL EXPERIENCE

2003 – 2007 Graduate Research Assistant, Texas A&M University, College Station, TX

2001 – 2003 Graduate Research Assistant, Kansas State University, Manhattan, KS

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Alali, W.Q., Sargeant, J.M., Nagaraja T.G., DeBey B.M, 2004. Effect of antibiotics on fecal shedding of *Escherichia coli* O157:H7 in pre-weaned calves. J. Anim. Sci. 82, 2148-52.

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